ELSEVIER

Teaser This review describes preclinical and clinical findings of anticancer effects of histone deacetylase inhibitors (HDACi) in breast cancer.

Emerging role of histone deacetylase inhibitors as anti-breast-cancer agents

Meran Keshawa Ediriweera, Kamani Hemamala Tennekoon and Sameera Ranganath Samarakoon

Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, 90, Cumaratunga Munidasa Mawatha, Colombo 03, Sri Lanka

Breast cancer (BC) remains the most frequently diagnosed cancer in women. A balance in the opposing actions of histone acetyltransferases (HATs) and histone deacetylases (HDACs) is necessary for epigenetic regulation of gene expression. Impairment in the balance between the actions of HATs and HDACs has been reported in the development of BC. By targeting histone and several non-histone proteins, histone deacetylase inhibitors (HDACi) can maintain the cellular acetylation profile and reverse the function of several proteins responsible for BC development. Preclinical and clinical data show that HDACi can evoke different anticancer mechanisms in distinct BC types.

Introduction

Despite the significant developments in diagnostic methods and novel therapies, breast cancer (BC) still ranks as the second-leading cause of cancer-related deaths among women [1]. Family history, gene mutations, various reproductive factors, obesity and prolonged exposure to endogenous and exogenous estrogen have been identified as major causes of developing BC [2]. Based on the receptor status [estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER2)] and molecular and/or genetic characterization, five major subtypes of breast tumors have been identified: luminal A (ER/PR-positive/HER2-negative), luminal B (ER/PR-positive and HER2-positive or negative), triple-negative (ER/PR/HER2-negative), HER2-positive and claudin-low (ER/PR/HER2-negative and low expression of claudin 3, 4 and 7, E-cadherin and occludin) [3,4]. Treatment plans for BC are mainly determined based on clinicopathological features and receptor status of tumors. Although chemotherapy, radiotherapy, hormone therapy and targeted therapies have shown considerable therapeutic success, drug resistance has been reported as one of the major challenges in BC treatments [5]. Apart from these therapies, epigenetic therapy is an emerging field that targets the epigenome of cancer cells. Histone deacetylase inhibitors (HDACi) and demethylation agents have been identified as the representative drug candidates with the ability to target the epigenome of cancer cells. Although

Corresponding author: Ediriweera, M.K. (mk.ediriweera@gmail.com)

Meran Keshawa Ediriweera was awarded his PhD in medicinal chemistry and molecular biology from the Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB), University of Colombo, Sri Lanka. He is currently a postdoctoral research scientist at the IBMBB. His main research focus is on the identification and characterization of novel bioactive compounds against breast cancer. He has co-authored several publications in reputed journals related to natural products and drug discovery.

Kamani H. Tennekoon is Professor of Molecular Life Sciences at the Institute of Biochemistry Molecular Biology and Biotechnology (IBMBB), University of Colombo, Sri Lanka. She is a medical graduate from the University of Colombo, obtained her PhD in reproductive endocrinology from the university of Sheffield, UK, and postdoctoral training in molecular biology from the University of Uppsala, Sweden. Her research between 1986 and 2002 mostly focused on reproductive endocrinology, antidiabetic and anti-galactagogue medicinal plants. Later she expanded her research to include cancer, more specifically cancer genetics, biomarkers in cancer and discovery of anticancer compounds from medicinal and endemic plants in Sri Lanka. She also has a keen interest in DNA identification and continues to work on reproductive and developmental biology.

Sameera R. Samarakoon is a science graduate from the University of Kelaniya, Sri Lanka. He holds an MSc in molecular life sciences from the Institute of Biochemistry Molecular Biology and Biotechnology (IBMBB), University of Colombo, Sri Lanka, and he was awarded his PhD in medicinal chemistry from the IBMBB. He also completed a post-graduate diploma in toxicology from the faculty of medicine, University of Colombo, Sir Lanka. He is currently working as a lecturer (cell biology) at the IBMBB. His research interests include cancer stem cell research, anticancer drug development and computer-based drug design. He has published his research findings in many peer-reviewed journals, supervised many MSc students and is currently supervising several PhD students. He has obtained many awards including presidential awards for his research.

https://doi.org/10.1016/j.drudis.2019.02.003

the clinical use of these drug candidates has been limited in hematological malignancies [6], their use in the treatment of BC has not yet been explicated.

Epigenetic modifications

The term epigenetics has been defined as heritable changes acquired in gene functions that cannot be assigned to variations in the sequence of DNA. DNA methylation and histone modification have been identified as the two major processes responsible for such epigenetic changes in gene functions [7]. Histones are found in almost all the eukaryotic cells and they are rich in arginine and lysine. In eukaryotic cells, five major types (H1 or H5, H2A, H2B, H3 and H4) of histones have been identified [8]. Histones H2A, H2B, H3 and H4 are considered as core histones involved in the formation of histone octamer, whereas histones H1 or H5 are identified as linker histones. For the regulation of gene expression (transcriptional activation/inactivation), tails of histones H3 and H4 are modified post-translationally by acetylation, methylation, sumoylation, ubiquitylation or phosphorylation by organizing DNA into active euchromatin (open chromatin) [8] (Fig. 1). Given that post-translational modifications tightly regulate gene expression and structure of chromatin, it is not surprising that any aberration in post-translational events is linked with the development of a cancer. Among the post-translational histone modifications, histone acetylation is a well-studied event that is known to be controlled by the balanced action of histone acetyltransferases (HATs) and HDACs (Fig. 2). Acetylation of ε-NH_2 groups on lysine residues by HATs is generally associated with chromatin relaxation and transcriptional activation [8]. Notably, some human HATs serve as transcriptional co-activators (for example: P300/CBP, PCAF and SRC1) that can interact with specific binding proteins in chromatin [9]. HDACs counteract the function of HATs by deacetylating histone lysine residues and inducing transcriptional repression by condensing chromatin [8].

Intriguing evidence illustrates that epigenetic and genetic events are not separate processes in cancer; these two separate events interlink together and play a prominent part in tumorigenesis. Although epigenetic alterations could lead to genetic changes, genetic abnormalities in epigenetic regulators have been reported to cause modifications in the epigenome [10,11]. Genetic mutations associated with some epigenetic regulators have been identified and these genetic mutations apparently cause epigenetic alteration, including nucleosome positioning, histone modifications and DNA methylation [12]. These alterations in the epigenome can result in genetic instability and abnormal expression of genes, which can lead to the development of a cancer. Several genetic abnormalities [germline or somatic mutations (deletions, amplifications, duplications and translocations)] associated with human epigenetic genes in distinct types of cancer have been detected [13]. Gene *NCOA3* (nuclear receptor coactivator-3, also known as *AIB-1*) encodes a transcriptional coactivator protein, which can act as a histone acetyltransferase [14]. It can also interact with nuclear receptors and induce transcriptional events [14]. CAG/CAA repeat length polymorphism in *NCOA3* has been found to increase the risk of developing BC in carriers with *BRCA1* and *BRCA2* mutations [15,16]. In addition, two single nucleotide polymorphisms (SNPs) in *NCOA3* [rs2076546 (T960T) and rs2230782 (Q586H)] have been reported in BC [17]. Germline mutations in HATs, *EP300* [G1830A (GGC > AGC)] [18] and *EP300/CREBBP* (ten SNPs) [19] have also been detected in BC. Truncating mutations in *EP300* [20] and amplification mutations in *NCOA3* [21] have also been identified in BC. Apart from germline or somatic mutations changes in genes encoding for HATs, several germline or somatic mutations in genes encoding for HDACs have also been found. Germline variants in *HDAC2 and HDAC5* (nine SNPs and haplotypes) were detected in a study conducted by Cebrian *et al.* [19]. Somatic changes in *HDAC4* (overexpression and splice-site/missense mutations) [22] and *SIRT1* (overexpression) [23] have also been identified in BC samples.

HDACs and BC

So far, 18 different HDACs have been identified and they have been classified into four different classes (class I, II, III and IV) based on their homology [6,24]. Class I HDACs comprise HDAC1, 2, 3 and 8, and they are exclusively present in the nucleus. Class II HDACs are subdivided into IIa (HDAC4, 5, 7 and 9) and IIb (HDAC6 and 8) and their expression can shift between the nucleus and cytoplasm [6,24]. Class III HDACs, called sirtuins (sirtuin 1–7) require NAD^+ as a co-factor for their deacetylation activity [6]. HDAC11 is the only member of class IV HDACs. Class I, II and IV HDACs are also known as Zn^{2+}-dependent HDACs because Zn^{2+} ions are essential to stabilize their catalytic centers [25]. Because histone acetylation is tightly controlled by the opposing actions of HATs and HDACs, it is clear that any impairment in the balance between the expression of HATs and HDACs can result in the development of a cancer. In BC, although expression of all the members of the HDAC family has not been fully described, expression of HDAC1, 2, 3, 4 and 6 has been described to some extent. Suzuki *et al.* [26] described alterations in histone acetylation from mammary epithelial cells to ductal carcinoma to be caused by reduced expression of HDAC1, 2 and 6 during tumor development. A significant correlation among the expression of HDAC1/3 levels and PRs and ERs and HDAC1 as an independent prognosis marker for disease free survival was described by Krusche *et al.* [27]. Hu *et al.* [28] reported that ER- and PR-positive BC cells possess deletion or loss of the HDAC2 gene locus. Strong association between HDAC2/3 and aggressive BC tumor types has been reported in a study conducted by Müller *et al.* [29]. Özdağ *et al.* [30] showed overexpression of HDAC4 in BC cells compared with lung and colon cancer cells. HDAC6 regulation was identified to be controlled through the ER signaling in hormone-receptor-positive BC cells [31]. Zhang *et al.* [32] demonstrated increased expression of HDAC6 mRNA levels in ER- and PR-positive BC tumors. In the same study, prognostic significance of HDAC6 in BC remains controversial, showing that high levels of HDAC6 expression are associated with better disease-free survival but not overall survival. Furthermore, a potential role of HDAC6 in anchorage-independent growth of BC cells was reported in a study conducted by Lee *et al.* [33]. Silencing of HDAC6 expression by siRNA was found to cause inhibition of cell migration and invasion of BC tumor cells, suggesting that inhibition of HDAC6 expression can block metastasis [34]. A study conducted by Ververis and Karagiannis demonstrated increased expression of class I HDACs compared with class II HDACs in BC tissues [35]. In conclusion, it is clear that aberrant expression of HDACs plays a significant part in BC development and tumor progression.

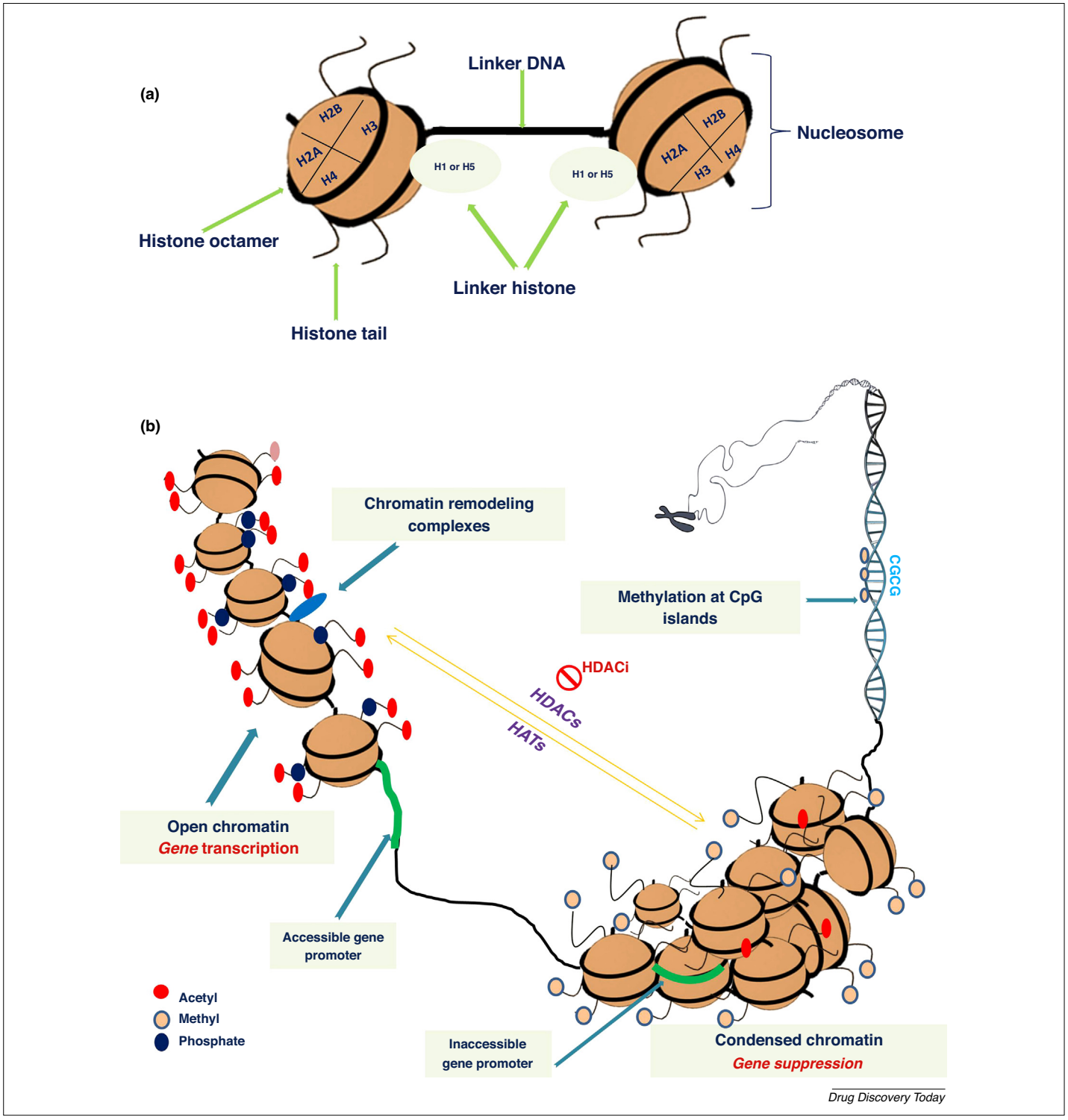


FIGURE 1

Arrangement of histone octamer in nucleosome **(a)** and activation of gene transcription or suppression through epigenetic regulation of structure of chromatin **(b)**. (a) A nucleosome comprises 150 bp DNA wrapped around a histone octamer made up of two copies of four types of core histones (H2A, H2B, H3 and H4). Histones H1 or H5 are identified as linker histones. (b) Tails of histones are modified post-translationally by acetylation, methylation, sumoylation, ubiquitylation or phosphorylation to regulate gene expression (transcriptional activation or inactivation) by organizing DNA into active chromatin (open chromatin). Among histone modifications, histone acetylation is a well-studied event regulated by balanced action of histone acetyltransferases (HATs) and histone deacetylases (HDACs). HDACs counteract the function of HATs by deacetylating histone lysine residues and they induce transcriptional repression by condensing chromatin. By targeting aberrantly expressed HDACs, histone deacetylase inhibitors (HDACi) can correct aberrant acetylation profile of some cancer pathway proteins.

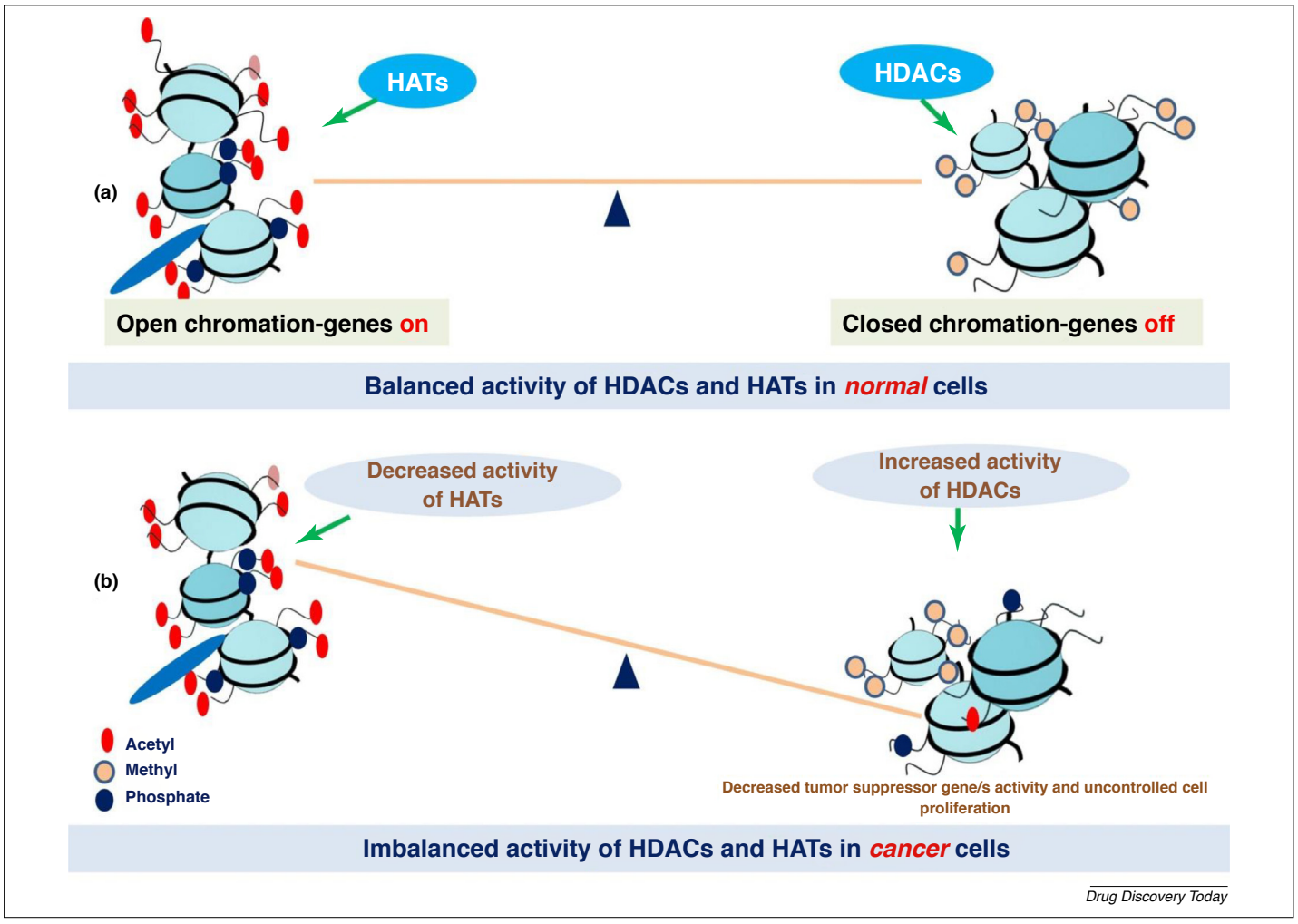


FIGURE 2

Opposing actions of histone acetyltransferases (HATs) and histone deacetylases (HDACs) in normal and cancer cells. **(a)** Balanced HAT/HDAC activity ensures normal cell function. **(b)** Dysregulation of balance in the action of HATs and HDACs is seen in many forms of cancer.

HDACi

In a quest for the discovery of cancer therapeutics, several HDACi have been identified as promising small-molecule therapeutics. Because a disrupted acetylation profile is associated with several cancer types owing to altered HDACs expression, targeting HDACs in cancer by HDACi has been identified as an effective treatment modality. It has been reported that HDACi can reestablish the aberrant acetylation profile of histone and some nonhistone proteins and regulate the function of several oncogenes and tumor suppressor genes, allowing cancer cells to undergo cell cycle arrest, apoptosis, modulate immune response and inhibition of angiogenesis [6,36,37]. Particularly, because HDACs are necessary for the functioning of key genes associated with growth and survival of cancer cells, which is not usually seen in normal cells, targeting HDACs with HDACi provides additional therapeutic efficacy in cancer cells [6,36,37]. Several natural or chemically synthesized HDACi targeting all classes of HDACs (pan inhibitors) or specific HDACs have been identified (Table 1). Based on chemical structures, HDACi can be classified into hydroxamic acids known as hydroxamates, short-chain fatty acids, benzamides, cyclic tetrapeptides and sirtuin inhibitors [36,37] (Table 1).

Influence of HDACi on miRNA

RNA is known to be a messenger molecule, communicating genetic information from genes for translation and translational regulation. Recently, the noncoding part of RNA gained much attention because the noncoding RNA was found to play a prominent part in several diseases and major cellular processes such as translation, DNA replication, RNA splicing and gene regulation [38]. microRNA (miRNA) is a group of small noncoding RNA (nonmessenger RNA) widely found in eukaryotic cells. It has been reported that miRNA is involved in gene activation, suppression and regulation [39,40]. Of note, miRNA has an ability to control gene expression via a post-transcriptional gene regulatory mechanism, including degradation of mRNA and translational inhibition [39,40]. It has also been reported that a single miRNA molecule can act as the main controller of several gene regulatory pathways [39,40]. By contrast, distinct miRNA can work together to regulate a single gene. Because of these characteristics, miRNA is reported to play a major part in gene regulation, controlling major cellular pathways vital for normal cellular physiology as well as tumorigenesis [39,40]. Several studies have identified differentially expressed miRNA in BC with unique expression profiles associated with some BC subtypes. For example, overexpression of miR-135b,

TABLE 1

Classification, chemical structures and specificity of some selected HDACi

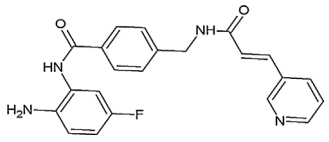
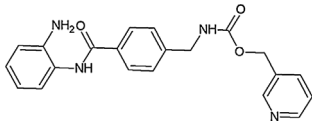
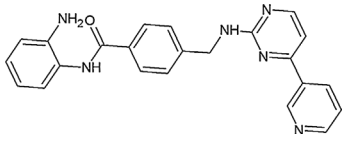
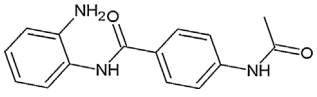
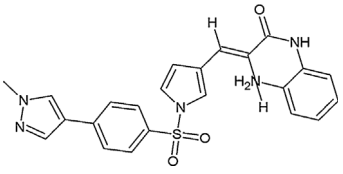
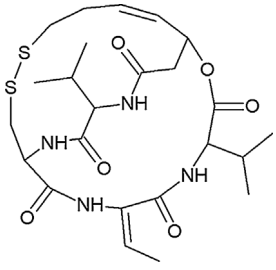
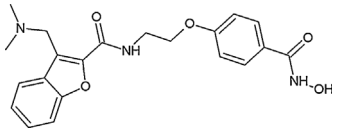
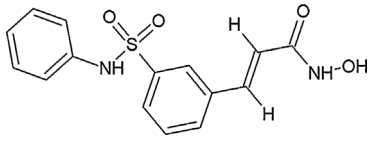
HDACi class	Examples	Chemical structure	Specificity
Benzamides	Chidamide (CS055/HBI-8000)		Class I and IIb
	Entinostat (MS-275)		Class I and III
	Mocetinostat (MGCD0103)		Class I and IV
	Tacedinaline (CI-994)		Class I
	4SC202		Class I
Cyclic peptides	Romidepsin (Depsipeptide/FK228)		Class I
Hydroxamic acids	Abexinostat (PCI-24781)		Pan inhibitor
	Belinostat (Beleodaq/PXD101)		Pan inhibitor

TABLE 1 (*Continued*)

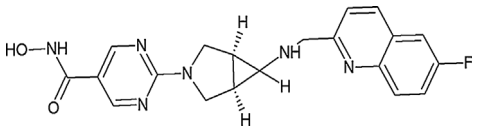
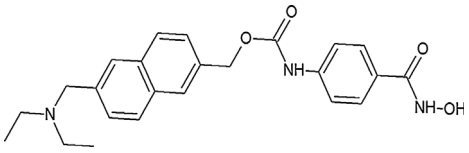
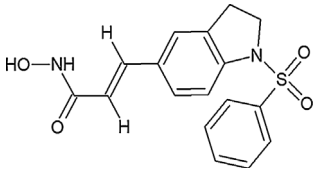
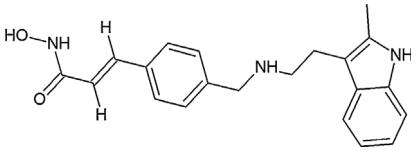
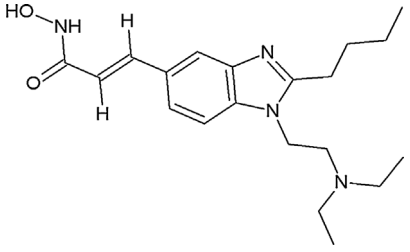
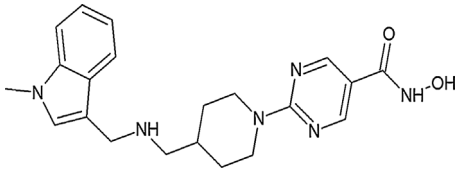
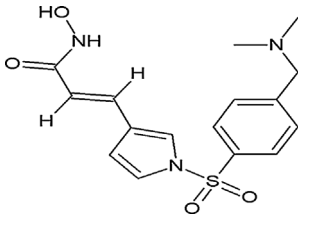
HDACi class	Examples	Chemical structure	Specificity
	CHR-3996		Class I
	Givinostat (ITF2357)		Pan inhibitor
	MPT0E028		Class I and IIb
	Panobinostat (LBH-589)		Pan inhibitor
	Pracinostat (SB939)		Class I, II and IV
	Quisinostat (JNJ-26481585)		Pan inhibitor
	Resminostat (4SC-201)		Pan inhibitor

TABLE 1 (*Continued*)

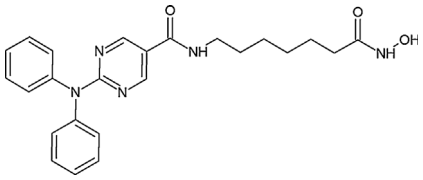
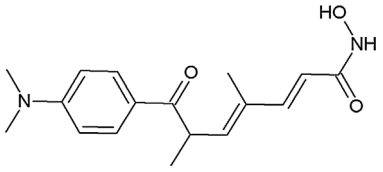
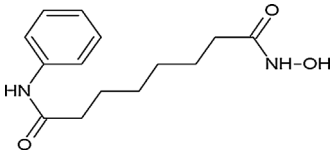
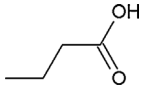
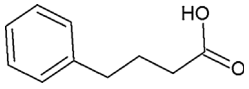
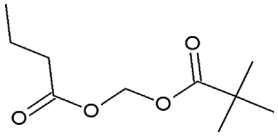
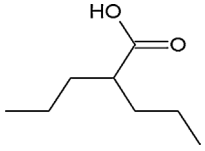
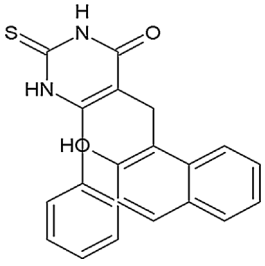
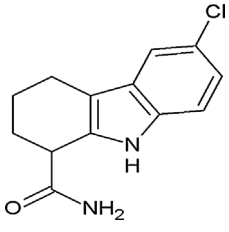
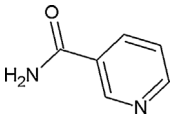
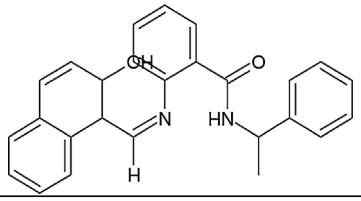
HDACi class	Examples	Chemical structure	Specificity
	Ricolinostat (ACY-1215)		Class II
	Trichostatin A		Pan inhibitor
	Vorinostat (SAHA)		Pan inhibitor
Aliphatic fatty acids	Butyric acid		Class I and II
	Phenylbutyric acid		Class I and II
	Pivanex (AN-9)		Class I
	Valproic acid (VPA)		Class I and IIa
Sirtuin inhibitors	Cambinol		SIRT 1 and 2
	EX-527		SIRT 1 and 2

TABLE 1 (*Continued*)

HDACi class	Examples	Chemical structure	Specificity
	Nicotinamide		Class III
	Sirtinol		SIRT 1 and 2

miR-190, miR-217 and miR-218 was found to be associated with ER-positive tumors [41], whereas miR-377, miR-520, miR-527 and miR-520f-520C were found to be PR-specific [41]. Furthermore, overexpressed miR-21, miR-210 and miR-221 have been detected in triple-negative BC [42].

Of considerable interest, a few miRNAs have been identified as oncogenic or tumor suppressors or to play an important part in therapy resistance [39,40]. Oncogenic miRNAs are often overexpressed in cancer and cause inactivation of tumor suppressor genes responsible for apoptosis and different stages of cancer (proliferation, invasion and migration) [39–41]. In the miR-10 family, oncogenic miR-10a and miR-10b are highly overexpressed in BC [43]. miR-21 is another miRNA that has been found to link with BC progression and metastasis [44]. Moreover, miR-17-5p is overexpressed in triple-negative BC, but not in ER-positive BC [45]. Tumor suppressor miRNA can inhibit the function of oncogenes involved in apoptosis and different stages of cancer [39–41]. Low expression of tumor suppressor let-7 family miRNA has been detected in BC tumor-initiating cells [46]. Several tumor-suppressive miRNAs such as members of the miR-200 family, miR-205 and miR-145, are downregulated in BC [47]. Restoration of the dysregulated miRNA profile has been proven to inhibit cancer progression, allowing potential therapeutic targets by anticancer drugs. By reversing the aberrantly expressed acetylation profile, HDACi can effectively reactivate tumor-suppressor genes or tumor-suppressor miRNA and inhibit the function of oncogenic miRNA [48]. A study by Cho *et al.* [49] reported that sodium butyrate (NAB) can downregulate tumor-suppressive miR-31 in MDA-MB-231 triple-negative BC cells. Increased acetylation of H3 and H4 has also been observed in NAB-treated MDA-MB-231 cells. Induction apoptosis through upregulation of miR-125a-5p in NAB-treated MDA-MB-231 BC cells and R2N1d BC stem cells was reported by Hsieh *et al.* [50]. Upregulation of miR-200a in MDA-MB-231 BC cells exposed to vorinostat was reported in a study conducted by Eades *et al.* [51]. Downregulation of oncogenic miR-17-92 cluster and increased H3 acetylation were reported in vorinostat-treated triple-negative (MDA-MB-231) and ER-positive (MCF-7) BC cells [52]. Significant upregulation of a series of tumor-suppressive miRNA and downregulation of metastatic miRNA in trichostatin A (TSA)-treated apoptotic-resistant MCF-7TN-R BC cells were reported in a study conducted by Rhodes *et al.* [53]. Tu *et al.*[54] reported that TSA can downregulate oncogenic miR-7 and increase α-tubulin acetylation in MDA-MB-231 BC cells. MS-275 has been reported to induce apoptosis in MDA-MB-231 and BT474 BC cells by increasing the expression of miR-125a, miR-125b and miR-205 and concomitant downregulation of erbB2/erbB3 signaling [55]. Combined treatment of short-chain aliphatic acid sodium phenylbutyrate (NaPBA) and azacitidine increased the expression of tumor suppressor miR-126 in MCF-7 BC cells along with increased acetylation of H3 [56].

In conclusion, chromatin-modifying drugs have the potential to restore aberrantly expressed tumor suppressor or oncogenic miRNA in distinct types of cancer. Because miRNA expression symbolizes potential biomarkers in a large number of cancers, evaluation of miRNA expression might be suitable to assess drug response, mainly for HDACi-based anticancer therapies. Furthermore, because many oncogenic miRNAs are associated with chemotherapy resistance, their expression levels could also be useful to determine drug resistance. Given the prominent role of miRNA in tumor development and progression, addition of miRNA expression profiling to HDACi-based clinical trials will be helpful to understand underlying mechanisms associated with tumor-suppressor or oncogenic miRNA expressions and enhance therapeutic responses.

Preclinical investigations of HDACi

Preclinical evidence indicates that HDACi exert diverse anticancer effects in different cancer cell lines and cancer xenograft models, by targeting multiple cancer pathways, as a single drug candidate or in combination with other anticancer drugs. Notably, combinatorial treatment of HDACi with chemotherapeutic agents has significantly increased the therapeutic efficacy of HDACi by reducing drug resistance and re-sensitizing cancer cells to chemotherapeutic agents. Although several preclinical studies have explored the effects of HDACi in several cancer types, the following section will provide evidence on preclinical efficacy of some selected HDACi in *in vitro* and *in vivo* BC cell models.

Hydroxamic acids (hydroxamates)

Among the hydroxamic acids, vorinostat, TSA, belinostat, panobinostat, givinostat, resminostat, abexinostat and quisinostat are common examples for pan-HDACi, whereas ricolinostat, pracinostat and CHR-3996 are selective hydroxamates. Among these, effects of vorinostat, TSA, panobinostat and belinostat have widely

been investigated in distinct BC cell models and clinical studies (Table 2).

Vorinostat

Vorinostat was the first FDA-approved HDACi for the treatment of cutaneous T cell lymphoma (CTCL) [6]. A recent study indicated that vorinostat can inhibit the proliferation of triple-negative BC cells (MDA-MB-231) by upregulating tumor suppressor miR-200c expression via targeting CRK-like proto-oncogene (CRKL) [57]. Findings of this study revealed that targeting the HDAC expression profile/miR-200c/CRKL signaling axis by HDACi is an important component for inhibiting the growth of BC cells and this axis could be used as a new diagnostic marker and a therapeutic target for HDACi therapy. In another study, vorinostat inhibited the growth, proliferation and invasive ability of inflammatory BC cells SUM190 and SUM149 and caused translocation of E-cadherin from the plasma membrane to the cytoplasm [58]. Lee *et al.* [59] demonstrated that vorinostat could inhibit the proliferation of TAMR/MCF-7 BC cells (tamoxifen-resistant) through the induction of apoptosis and autophagic cell death. Autophagic cell death in TAMR/MCF-7 cells exposed to vorinostat was confirmed by analyzing autophagic cell death markers (beclin-1 and LC3-II) and morphological changes in TAMR/MCF-7 cells stained with acridine orange. Vorinostat also caused reduction in tumor cell growth in mice with TAMR/MCF-7 tumors. Palmieri *et al.* [60] showed that vorinostat could significantly prevent brain metastasis (62% large metastasis compared with untreated groups) colonization in a mouse model for triple-negative BC and can induce dsDNA breaks. In another study [61], vorinostat was found to induce cell differentiation regardless of ER status in SKBR-3 and MDA-MB-468 BC cells and exert cytotoxic and apoptotic effects in MCF-7 BC cells. Low concentrations of vorinostat (1.25 and 2.5 μM) caused MCF-7 cell cycle arrest at G1 phase, whereas at higher concentrations (>5 μM) cell cycle arrest was mainly G2-M phase. Increased acetylation (H3) was also reported in MCF-7 cells exposed to vorinostat. Fiskus *et al.* [62] demonstrated that vorinostat can activate heat shock protein (hsp)90 hyperacetylation, reduce binding of hsp90 to ERα, induce polyubiquitylation and decrease ERα expression in ERα-positive BC cells. Furthermore, vorinostat abrogated expression of estrogen response elements activated by 17β-estradiol, reduced the expression of hsp90 client proteins and sensitized hormone-receptor-positive BC cells to tamoxifen. Combined treatment of vorinostat with simvastatin (a cholesterol-lowering drug) caused induction of apoptosis through the interruption of Rab7 prenylation and inhibition of fusion of autophagosome-lysosome in triple-negative BC cells. Combined treatment of vorinostat and simvastatin also demonstrated apoptotic effects and inhibited Rab7 prenylation in xenografted mice, suggesting that Rab7 is a potential drug target for vorinostat and simvastatin combination [63]. In another study, combined treatment of vorinostat and letrozole (aromatase inhibitor) resulted in inhibition of proliferation of MCF-7 BC cells, induction of apoptosis and inhibition of differentiation of peripheral blood mononuclear cells (PBMCs) into osteoclasts, suggesting this combination can be used to minimize the risk of developing osteoporosis in BC patients [64]. According to a study reported by Bali *et al.* [65], treatment of vorinostat caused upregulation of p21 (WAF1) and p27 (KIP1), induction of apoptosis and increased H3 and H4 acetylation in SKBR-3 and MCF-7 cells. Furthermore, increased hsp90 acetylation and decreased p-AKT and ERK1/2 levels were also observed in SKBR-3 and MCF-7 BC cells exposed to vorinostat. In the same study, co-treatment of vorinostat with docetaxel or trastuzumab was found to induce cell cycle arrest and apoptosis in SKBR-3 and BT-474 BC cells by attenuating AKT signaling. Vorinostat combined with ionizing radiation (IR) was reported to increase the therapeutic efficacy in MCF-7, MDA-MB-231 and 4T1 BC cells by inducing apoptosis and inhibiting cell migration and invasion. Enhanced radiosensitizing effects of vorinostat were also evident with no detectable toxicity. Additionally, vorinostat was found to inhibit migratory and invasive ability of 4T1 BC cells. Results of the *in vivo* experiments in the same study demonstrated that vorinostat could inhibit metastasis of 4T1-luc cells [66]. Immunomodulation by enhancing T-cell-mediated lysis by vorinostat in triple-negative BC cells (MDA-MB-231) has been reported in a recent study conducted by Gameiro *et al.* [67]. Marchion *et al.* [68] reported that vorinostat can sensitize BC cells to epirubicin (topoisomerase II inhibitor) and cause decondensation of chromatin and accumulation of epirubicin in the nucleus with subsequent DNA damage in BC cells. Vorinostat-induced histone hyperacetylation was identified as an early event (4 h), whereas chromatin decondensation was identified as a late process (48 h) in BC cells treated with vorinostat. In BC, PD-L1 expression is basically seen in triple-negative and $Her2^{+}$ types [69,70]. Increased expression of PD-L1 correlated with increased tumor-infiltrating lymphocytes, which are successively linked with stronger immune response and better survival [69,70]. Moreover, low human leukocyte antigen-DR isotype (HLA-DR) expression has been found to link with increased metastasis in BC [71]. A study reported by Terranova-Barberio *et al.* [72] showed that treatment of vorinostat caused upregulation of PD-L1 and HLA-DR expression in triple-negative BC cells. Moreover, co-treatment of vorinostat along with an immune checkpoint blockade (PD-1/CTLA-4) can promote tumor apoptosis and regression in a 4T1 triple-negative BC tumor model. An ongoing Phase II study also investigates epigenetic-immunomodulatory effects of combination therapy containing vorinostat, tamoxifen and pembrolizumab in BC patients with ER-positive tumors (Table 3).

TSA

TSA is a naturally derived HDACi [37]. Inhibition of ERα-dependent transcription by TSA in MCF-7 BC cells was reported in a study conducted by Alao *et al.* [73]. Additionally, TSA caused degradation of cyclin D1 (ubiquitin dependent) by arresting MCF-7 cell cycle at G1-S-phase. By contrast, although cyclin D1 degradation was enhanced in ERα-negative BC cells (MDA-MB-231) exposed to TSA, cyclin D1 transcription was not affected by TSA. In another investigation, interestingly, TSA was found to sensitize the action of tamoxifen in triple-negative BC cells by modulating the transcriptional activity of ERβ in ERα-negative BC cells, making hormone-receptor-negative BC cells responsive to tamoxifen [74]. Another study conducted by Fan *et al.* [75] illustrated combined treatment of TSA and 5-aza-2′-deoxycytidine (a DNMT inhibitor) can enhance the sensitivity of tamoxifen and re-express functional ERα in MDA-MB-231 triple-negative BC cells, making ERα-negative BC cells responsive to endocrine therapy. A recent study conducted by Chen *et al.* [76] demonstrated that co-

TABLE 2

Summary of completed clinical trials of HDACi in BC patients

Drug regimen/plan	Type/design of study	Number of BC patients	Major clinical findings/ response	Refs
1. 400 mg of vorinostat (PO) on day 1 (fasted) followed by a high fat diet on days 5 and 28. 2. 400 mg of vorinostat (q.d) on days 7–28 and on day 28 vorinostat was fed with a high-fat diet	Phase I	4 BC patients	1 patient maintained SD for >15 months	[116]
200, 300 and 400 mg of vorinostat (PO b.i.d) for 14 days of a 21-day cycle	Open-label Phase II	3 refractory BC patients	A durable response was observed in only 1 patient	[117]
200 mg of vorinostat (PO b.i.d) for 14 days of a 21-day cycle	Phase II (first BC-specific trial)	14 BC patients	4 had SD and 8.5 months of median progression-free survival were observed in 4 patients	[118]
15–160 mg/kg/day VPA followed by 100 mg/m^2 of epirubicin on day 3 of 21 days cycle	A proof-of-principle (POP) phase I	10 BC patients	1 had SD at 15 mg/kg of VPA, 1 had PR at 45 mg/g of VPA, 1 had PR at 60 mg/kg of VPA and 2 had SD at 75 mg/kg of VPA	[121]
120 mg/kg/day of VPA (loading dose) followed by 60 mg/kg of VPA given every 12 h for 5 doses followed by 500 mg/m^2 of 5-fluorouracil (FEC100), 100 mg/m^2 epirubicin and 500 mg/m^2 cyclophosphamide	BC-specific cohort Phase II expansion study	15 BC patients	Objective tumor response was observed in 9 out of 14 BC patients at the dose expansion after median of 6 cycles	[122]
Vorinostat at 400–1000 mg/day on days 1–3 followed by 20 mg/m^2 of doxorubicin (day 3 of 4 weeks)	Phase I	5 BC patients	800 mg/day of vorinostat was found to be the MTD and one BC patient had PaR	[123]
500 mg of famethazine, followed by 182 or 83 mg of hydralazine. Patents then received magnesium valproate tablets (30 mg/kg t.i.d.) from day 7 until the final day of fourth chemotherapy cycle (doxorubicin 60 mg/m^2 and cyclophosphamide 600 mg/m^2)	Phase II	16 BC patients	5 patients had CR, 3 had SD and 8 showed PaR	[124]
200 or 300 mg of vorinostat (OP, b.i.d.) on days 1–3, 8–10 and 15–17 followed by paclitaxel (90 mg/m^2 on days 2, 9 and 16) and bevacizumab (10 mg/kg on days 2 and 16 for every 28 days	Phase I/II	54 BC patients	24 out of 44 patients showed an objective response at recommended Phase II dose of vorinostat (300 mg). 16 patients showed SD (>24 weeks)	[125]
Patients received neoadjuvant treatments along with 400 mg of vorinostst PO q.d. vorinostat (PO q.d. on days 1–3 of 7 days) or placebo)] for 12 weeks	Phase I/II	62 BC patients	A significant association was evident among tissue CMI levels and CR in the group received vorinostat [overall response 0.44 (0.20, 0.93), $P = 0.03$] was observed	[126]
400 mg of vorinostat (daily) for 3 of 4 weeks with 20 mg of daily tamoxifen	Phase II	43 BC patients	8 out of 43 patients showed PaR and 9 out of 43 patients showed SD (>24 weeks)	[127]
BC patients progressed on an AI received 5 mg of entinostat weekly in 28-day cycles	Phase II	27 BC patients	1 patient showed PaR and another patient showed SD for >6 months	[128]
64 patients (EE group) received 25 mg of exemestane daily with 5 mg of entinostat (PO) 1 per week and 66 patients (EP group) received exemestane (25 mg PO q.d) and placebo	Phase II	130 BC patients	Improved PFS in the EE compared with the EP and improved overall survival was seen in the EE compared to the EP	[129]
Patients received 2 dose levels of panobinostat and letrozole [level 1: 20 mg of panobinostat (PO 3 times weekly) plus 2.5 mg of letrozole (PO q.d) and level 2: 30 mg of panobinostat (PO three times weekly) plus 2.5 mg of letrozole (PO q.d)] in 43 cycles	Phase I	12 BC patients	2 patients had PaR, whereas 5 patients showed SD belonged to the level 2	[131]
40 mg/m^2 of 5-azacitidine (s.c. on days 1–5 and 8–10) and 7 mg of entinostat (PO) on days 3 and 10 of 28 repeated cycles	Phase II	40 BC patients	Examination of biopsies revealed upregulation of 33 genes related to immunomodulatory pathways	[137]
200 mg of vorinostat (PO b.i.d) and 6 mg/kg of trastuzumab (i.v.) of a 21-day cycle	Phase I/II	3–6 patients in Phase I 10 patients in Phase II	Phase II was terminated owing to low efficacy of combined therapy	[138]

Abbreviations: b.i.d., twice daily; CR, complete response; MTD, maximum tolerated dose; OR, overall response; PaR, partial response; PFS, progression-free survival; PO, oral; q.d., daily; SD, stable disease; t.i.d., three times daily; i.v., intravenously.

TABLE 3

Summary of ongoing clinical trials of HDACi in BC patients

Title of the trial/study	Responsible party	Drug regimen/plan	Trial identifier	Refs
A Phase I/II study to evaluate the efficacy of combined therapy containing cisplatin plus romidepsin and nivolumab in patients with TNBC associated with *BRCA* mutation	University of Kansas Medical Center, USA	Romidepsin (8, 10 and 12 mg/m^2 i.v.) on days 2 and 9 of 21-day cycle and cisplatin (75 mg/m^2) on day 1 of 21-day cycle Romidepsin on days 2 and 9 of 21-day cycle, cisplatin (75 mg/m^2) on day of 21-day cycle and 360 mg of nivolumab on day 1 of 21 days cycle	NCT02393794	[143]
A Phase I/II study of atezolizumab and entinostat in patients with TNBC	Syndax Pharmaceuticals, USA	Entinostat (i.v.) at RPD2 in combination with atezolizumab or placebo along with atezolizumab (description about doses not available)	NCT02708680	[144]
A Phase III trial of exemestane and entinostat in hormone-receptor-positive BC patients	National Cancer Institute (NCI), USA	Exemestane on days 1–28 (PO. q.d) and entinostat on days 1, 8, 15 and 22 (PO) every 28 days (description about doses is not available)	NCT02115282	[130]
A Phase I study to evaluate the efficacy of combined therapy containing entinostat, nivolumab and ipilimumab in advanced HER2-negative BC patients	National Cancer Institute (NCI), USA	Entinostat (PO) on days 7 and 14 followed by nivolumab (i.v. for 60 min) on day 1 followed by every 2 weeks and ipilimumab (i.v. for 90 min) on day 1 followed by every 6 weeks in every 28 days (description about doses is not available)	NCT02453620	[145]
A Phase I study of entinostat with capecitabine in patients with high risk of developing BC following neoadjuvant chemotherapy	University of Virginia, USA	1. Entinostat (3 mg/week) and capecitabine (800 mg/m^2 b.i.d for two weeks) 2. Entinostat (5 mg/week) and capecitabine (800 mg/m^2 b.i.d for 2 weeks) 3. Entinostat (3 mg/week) and capecitabine (1000 mg/m^2 b.i.d for 2 weeks) 4. Entinostat (5 mg/week) and capecitabine (1000 mg/m^2 b.i.d for two weeks)	NCT03473639	[146]
A Phase II study to investigate epigenetic-immune modification in ER-positive BC patients with vorinostat, tamoxifen and pembrolizumab	University of California, San Francisco, USA	1. Tamoxifen (20 mg PO), vorinostat (400 mg for 5 days PO) and pembrolizumab (200 mg i.v. every 3 weeks)	NCT02395627	[147]

Abbreviations: b.i.d., twice per day; PO, oral; q.d., daily; t.i.d.: three times per day; i.v., intravenously; RPD2, recommended Phase II dose.

treatment of TSA with BEZ235 (PI3K/mTOR/AKT signaling inhibitor) can mediate significant antitumor effects and induce apoptosis in MCF-7, T47D and MDA-MB-231 BC cells. Reduction in the growth of MDA-MB-231 tumors in mouse xenograft models by co-treatment of TSA with BEZ235 was also reported in the same study. Chatterjee *et al*. [77] reported that TSA and CG-1521 (hydroxamate-based HDACi) can induce apoptosis and cause cell cycle arrest in SUM149PT and SUM190PT inflammatory BC cell lines. TSA and CG-1521 caused an increase in the levels of acetylated α-tubulin with different morphological effects, with CG-1521 blocking the formation of mitotic spindle and TSA increasing cell size in SUM149PT cells. In SUM190PT cells, TSA increased the acetylation of tubulin, whereas CG-1521 caused no increase in the acetylated α-tubulin levels without altering morphology of cells.

Panobinostat

Panobinostat is a pan-HDAC inhibitor approved by the FDA for the treatment of peripheral T cell lymphoma (PTCL). Preclinical evidence shows that panobinostat can significantly increase histone acetylation, cell cycle arrest and induce apoptosis in BC cells. A study conducted by Tate *et al*. [78] has demonstrated that panobinostat can inhibit proliferation and induce histone acetylation in MDA-MB157, MDA-MB-231, MDA-MB-468 and BT-549 triple-negative BC cell lines. Furthermore, G2/M cell cycle arrest and induction of apoptosis were evident in panobinostat-treated BC cells. Gene expression analysis in panobinostat-treated BC cells showed that panobinostat can upregulate tumor suppressor genes in MDA-MB-231 (*CDH1*) and MDA-MB-468 (*CDKN1A, SPRR1B* and *THBS1*) cells. Panobinostat also inhibited proliferation of MDA-MB-231 and BT-549 tumor formation *in vivo*. According to a recent study, panobinostat and trastuzumab co-treatment induced natural killer (NK)-cell-mediated anticancer effects in trastuzumab-resistant HER2$^+$ tumors, highlighting that a panobinostat and trastuzumab combination can be used as an anti-HER2-based treatment strategy [79]. Inhibition of the mesenchymal phenotype in a patient-derived triple-negative BC model (claudin-low) by panobinostat was reported in a recent study [80]. Zhou *et al*. [81] reported that panobinostat can also re-express silenced ERα gene in triple-negative BC cells by reestablishing heterochromatin-associated proteins without promoter hypermethylation. Reestablishment of silenced ERα gene triggered tamoxifen sensitivity in triple-negative BC cells. Furthermore, a study [82] has demonstrated that the combined treatment of panobinostat and letrozole synergistically inhibited aromatase expression in hormone-responsive BC cells, indicating that combination therapy comprising panobinostat and letrozole is an ideal approach to target hormone-receptor-positive/aromatase-positive BC cells.

Belinostat

Belinostat is another FDA-approved drug used for the treatment of PTCL. Recently, Hsu *et al*. [83] reported that belinostat significantly reduced the growth of and induced apoptosis in MDA-MB-231, SKBR-3 and MCF-7 BC cell lines via a caspase-dependent manner. Cell cycle arrest (G1 and S phase) in belinostat-treated BC cells was

confirmed by assessing p21, CDK4, CDK6 and CDK 2 protein expression in the same study. Belinostat also caused strong acetylation of histone proteins at H3K18, H3K56 and H4K16 sites in MDA-MB-231 and SKBR-3 cells. In addition, belinostat or vorinostat in combination with olaparib (an FDA-approved anticancer drug) were found to inhibit the proliferation of a triple-negative BC cell line panel (eight cell lines) and tumor growth in triple-negative BC xenografts. A strong synergistic effect was observed in *BRCA-1* mutated BC cells, whereas the drug combination exerted a weaker synergism in *PTEN*-deficient BC cells. Exposure of *BRCA-1*-mutated HCC-1937 BC cells caused significant reduction in DNA repair biomarkers [84]. Androutsopoulos and Spandidos [85] demonstrated inhibition of cell proliferation, potent HDAC inhibition and upregulation of acetylated tubulin levels in belinostat-treated MCF-7 BC cells.

Short-chain fatty acids

In *in vitro* and *in vivo* BC models, the short-chain fatty acid HDACi, valproic acid, butyric acid and phenylbutyric acid, affect a wide range of biological functions including HDAC inhibition, growth arrest and antiproliferative, apoptotic and radiosensitizing effects [6,36].

Valproic acid

Valproic acid (VPA) is a short-chain fatty acid derived from valeric acid [6,37]. Recently, Tian *et al*. [86] reported that VPA and hydroxyurea (a ribonucleotide reductase inhibitor) can synergistically inhibit the growth of MCF-7 BC cells through blocking the action of replication protein A2 (RPA2) and promoting the *Rad51*-mediated homologous recombination DNA repair pathway. A study conducted by Mawatari *et al*. [87] found that VPA could inhibit the growth of HER2-positive BC cells (SKBR-3) by upregulating p21 WAF1 expression. VPA also caused induction of apoptosis and acetylation of histone H3 in SKBR-3 cells by disrupting of the activity of hsp90 through hyperacetylation of hsp70. Hao *et al*. [88] demonstrated that VPA could induce apoptosis in A549 BC cells and suppress the expression of *H19* oncogene by inhibiting the activity of HDAC1 and changing the methylation status of *H19* through upregulation of enzyme DNA methyltransferase 1 (DNMT1) expression. VPA has been reported to induce radiosensitization and cause dysfunction of BRCA1-Rad51-mediated homologous recombination and Ku80-mediated nonhomologous end-joining DNA repair pathways in MCF-7 BC cells [89]. Aztopal *et al*. [90] showed that VPA could reduce the growth of and induce apoptosis in BC stem cells that are reported to accompany increased acetylation of histones H3. Co-treatment with VPA, 5-aza-2′-deoxycytidine (methyltransferase inhibitor) and all-trans retinoic acid (ATRA) has been reported to reactivate the transcription of RA receptor β2 (*RARβ2*) tumor suppressor gene in BC cells [91].

NAB

NAB is the sodium salt of the butyric acid found abundantly in milk fat, fruits and vegetables [92]. NAB can specifically target class I HDACs and has been shown to exert anticancer effects in several *in vitro* and *in vivo* BC models. A recent study demonstrated that NAB can induce apoptosis through oxidative stress in MCF-7 and MDA-MB-468 BC cell lines [93]. Louis *et al*. [94] have also proven same effects of NAB in MCF-7 BC cells. Apoptosis-inducing ligand (TRAIL), anti-Fas agonist antibody and NAB have synergistically induced apoptosis through the activation of death receptors in MCF-7 BC cells [95]. Chopin *et al*. [96] have also shown that interaction of proliferating cell nuclear antigen (PCNA) with P21waf1$^{/cip1}$ is essential for the induction of apoptosis in MCF-7 BC cells. Moreover, NAB has been reported to induce apoptosis in MCF-BC cells in a *p53*-independent manner by modulating Fas signaling [97].

Benzamides

Entinostat

Entinostat is a synthetic class I HDACi [98]. Schech *et al*. [99] reported that entinostat in combination with ATRA can inhibit the growth of a tumor-initiating cell population (TIC) in aromatase inhibitor (AI)-resistant BC cells by targeting the HER2 pathway. Another study conducted by Schech *et al*. [100] demonstrated that entinostat significantly reduced TICs from triple-negative BC cells. Furthermore, entinostat treatment reduced a CD44(high)/CD24(low) cancer stem cell population, aldehyde dehydrogenase 1 (ALDH1) levels and the expression of TIC markers (Oct-4, Bmi-1 and Nanog). Induction of apoptosis through FOXO3-associated Bim1 expression by combined treatment of entinostat and lapatinib (a tyrosine kinase inhibitor used for the treatment of HER2-positive BC) in HER2-positive BC was confirmed by Lee *et al*. [101]. Furthermore, entinostat was found to sensitize HER2-positive BC cells (trastuzumab/lapatinib resistance) to trastuzumab/lapatinib treatment, rationalizing the use of entinostat/lapatinib and trastuzumab combination for HER2-positive BC patients. Treatment of entinostat with AI letrozole was found to activate ERα by sensitizing cancer cells to letrozole in *in vitro* and *in vivo* ER-negative BC cell models, suggesting that entinostat and letrozole combination could be used to treat BC with ERα-negative tumors by restoring letrozole responsiveness [102]. Shah *et al*. [103] reported that entinostat can reverse epithelial–mesenchymal transition (EMT) by suppressing the binding of transcription factors Snail and Twist to the E-cadherin promoter in triple-negative BC cells. Additionally, entinostat suppressed cell migration and production of tubulin-based microtentacles, illustrating entinostat can attenuate EMT and suppress metastasis. SK7041 is a hybrid molecule synthesized from TSA and entinostat (MS-275). The hybrid molecule SK7041 is reported to have enhanced physicochemical properties to TSA. In a study [104], SK7041 was shown to inhibit the cell proliferation and induce apoptosis in MDA-MB-231, MCF-7 and SKBR-3 BC cells. SK7041 was also found to induce histone acetylation (H3 and H4) and cause G2-M and G1 cell cycle arrest in MDA-MB-231 BC cells. Some ongoing clinical trials test the combined effects of entinostat with other drugs in BC patients (Table 3).

Mocetinostat

Mocetinostat is a synthetic benzamide that can specifically inhibit class I and IV HDACs. In a preclinical study, combined treatment of mocetinostat with JQ1 [bromodomain and extra-C-terminal (BET) inhibitor] was shown to inhibit the growth of MDA-MB-231 triple-negative BC cells and upregulate ubiquitin-specific protease superfamily (USP17) genes, suggesting the combined treatment of mocetinostat and JQ1 has a strong inhibitory effect on the Ras/MAPK signaling pathway. Moreover, inhibition of deacetyla-

tion of histone H3 and H4 (except BT549 cells) by mocetinostat in MDA-MB-231, BT549, MCF7 and T47D BC cell lines has also been reported in the same study [105]. A recent study conducted by Witt *et al.* [106] has shown that HDAC1 and HDAC7 are overexpressed in BC stem-like cells (BPLER) compared with non-stem cells. In addition, authors have reported that expression of HDAC1 and HDAC7 are required to maintain stem cell characteristics in BC stem-like cells and targeting overexpressed HDAC1 and HDAC7 with mocetinostat can evoke significant antiproliferative effects in stem-like cells. Tacedinaline and 4SC-202 are the other well-known examples for benzamides with selective class I HDAC inhibitory effects [36]. However, preclinical studies describing the mechanisms of action of tacedinaline and 4SC-202 in distinct BC cell models are limited.

Cyclic tetrapeptides

Cyclic tetrapeptides are natural compounds usually found in fungi and marine bacteria [6,36,37]. Romidepsin is one of the selective class I HDACi approved by the FDA for the treatment of patients with CTCL. Robertson *et al.* [107] demonstrated induction of apoptosis, increased acetylation of H3 and expression of p21WAF1 in romidepsin-treated SUM149 inflammatory BC cells. In addition, romidepsin triggered demolition of tumor emboli and architecture of lymphatic vessels. Combined treatment of romidepsin and paclitaxel effectively inhibited the growth of primary and metastatic tumors. In another investigation, romidepsin and oncogenic H-Ras synergistically activated the ERK pathway, resulting in activation of Nox-1 and reactive oxygen species (ROS), leading to apoptosis in H-Ras-transfected MCF-10A mammary cells [108]. Combined treatment of romidepsin and chloroquine has significantly reduced the colony formation ability and induced apoptosis in BC stem cells. Combination treatment also caused a significant reduction in the $CD44^{+}/CD24^{-}$ BC stem cell population *in vivo* [109]. A Phase I/II study to evaluate the efficacy of combination therapy containing cisplatin plus romidepsin and nivolumab in TNBC patients associated with BRCA mutation is currently ongoing (Table 3).

Sirtuin inhibitors

Sirtuins are NAD^{+}-dependent class III HDACs and/or ADP ribosylases and, so far, seven members of the sirtuin family (SIRT1–7) have been identified [110]. In mammals, sirtuins are found in different subcellular locations, such as the nucleus (SIRT1, 2, 6 and 7), mitochondria (SIRT3, 4 and 5) and cytoplasm (SIRT1 and 2) and they are reported to be involved in the regulation of cell survival, metabolism, aging and genomic stability [110]. Growing evidence has demonstrated that sirtuins not only play a prominent part as tumor suppressors but are also responsible for tumor development [110]. For example, SIRT1 has been involved in the induction of apoptosis in cancer cells by deacetylating transcription factors such as hypoxia-inducible factor (HIF)-1α and nuclear factor (NF)-κB involved in tumor development, whereas, by contrast, the oncogenic role of SIRT1 has also been demonstrated in several cancers [111]. Therefore, identification of sirtuin activators and inhibitors would be helpful for cancer therapeutics development. Preclinical efficacy of sirtuin inhibitors such as sirtinol, cambinol, EX-527 and suramin has been investigated in several cancer cell models. Wang *et al.* [112] reported that sirtinol can induce apoptotic and autophagic cell death by inhibiting the expression of SIRT1/2 expression in MCF-7 BC cells. Autophagic cell death in sirtinol-treated MCF-7 BC cells was further confirmed by pre-treating MCF-7 cells with the autophagic inhibitor 3-methyladenine, in which pre-treatment of 3-methyladenine in MCF-7 cells demonstrated an increase in apoptotic cell number over autophagic cell death. In another study, sirtinol was shown to induce cell cycle arrest in the G0/G1 phase in MDA-MB-231 and MCF-7 cells, and particularly apoptosis in MDA-MB-231 cells through down-regulation of Bcl-2 [113]. Induction of a senescence-like growth arrest by attenuating the Ras/MAPK signaling pathway in sirtinol-treated MCF-7 BC cells was reported by Ota *et al.* [114]. Combination treatment of suramin and DNA methyltransferase inhibitors can trigger cytotoxic effects in highly invasive BC cells via re-expression and activation of PKD1. Additionally, combination treatment of suramin and DNA methyltransferase inhibitors was proven to inhibit the invasiveness of triple-negative BC tumors *in vivo* [115].

Clinical findings of HDACi in BC

Evaluation of clinical efficacy of HDACi has been largely limited to hematological malignancies, with promising therapeutic outcomes in leukemia, multiple myeloma and lymphoma. It is not very clear why HDACi show disappointing results in solid tumors and are clinically effective in hematological cancers. Poor pharmacokinetic properties and short half-life of HDACi have been identified as two major causes for poor therapeutic outcome of HDACi in solid tumors. In early BC clinical studies, vorinostat has been investigated as a single agent. However, overall clinical efficacy of HDACi as single agents in solid tumors has not been always positive. Rubin *et al.* [116] carried out a Phase I study to evaluate safety, tolerability, pharmacokinetics of single and multiple doses of vorinostat and effects of a diet rich in fat on pharmacokinetics of vorinostat in patients with advanced cancer including BC. Results of the study demonstrated that that one (stage IV BC) of four BC patients who received vorinostat [400 mg on days 1 (fasted) and 5 (fed)] and fed with a high fat meal maintained stable disease (SD) for >15 months. An open-label Phase II trial conducted by Vansteenkiste *et al.* [117] in patients with different solid tumors, including patients with refractory BC, evaluated effects of vorinostat as a single agent. Patients who participated in this study received three doses (200, 300 and 400 mg) of vorinostat twice daily (b.i.d) for 14 days of a 21-day cycle. A durable response was observed in only one patient who received prior treatments. The first BC-specific Phase II clinical study carried out by Luu *et al.* [118] evaluated the effects of vorinostat alone in confirmed stage IV metastatic BC patients who received adjuvant therapy, hormone therapy or two lines of prior chemotherapy. Patients received 200 mg of vorinostat b.i.d for 14 days of a 21-day cycle. The study was terminated with 14 patients when no response evaluation criteria in solid tumors (RECIST) were observed. However, a clinical benefit was observed in four patients with SD and 8.5 months of median progression-free survival.

Combination therapy: HDACi with chemotherapy

Low plasma concentration of vorinostat (1–2 μM) has been reported in clinical studies and, at these concentrations, no

significant induction of apoptosis was observed in preclinical BC models [119,120]. Therefore, to obtain more-meaningful clinical results by improving the synergistic efficacy, combination of HDACi with other chemotherapeutic agents has been considered in clinical studies. Several preclinical trials also showed that combined treatment of HDACi with other drugs can evoke enhanced biological effects over a single agent [64,65,72,86]. Munster *et al.* [121] conducted a proof-of-principle (POP) Phase I study to determine toxicity, safety and maximum-tolerated dose of combination treatment of VPA and epirubicin in solid tumors. Forty-four patients enrolled in the study (ten BC patients) were treated with 15–160 mg/kg/day VPA followed by 100 mg/m^2 of epirubicin on day 3 of 3-week cycles. Among BC patients, one patient with SD at 15 mg/kg of VPA, one patient with partial response (PaR) at 45 mg/g of VPA, one patient with PaR at 60 mg/kg of VPA and two patients with SD at 75 mg/kg of VPA were reported after treatments. In the BC specific cohort expansion study conducted by the same group [122], 15 BC patients received 120 mg/kg/day of VPA (loading dose) followed by 60 mg/kg given every 12 h for 5 doses followed by 500 mg/m^2 of 5-fluorouracil (FEC100), 100 mg/m^2 epirubicin and 500 mg/m^2 cyclophosphamide. An objective response was observed in nine out of 14 evaluable BC patients (64%) at the dose expansion after a median of six cycles. A strong correlation between histone acetylation and HDAC2 expression was also observed in PBMCs and tumor samples. Munster *et al.* [123] have conducted another study to evaluate the combined effects of vorinostat and doxorubicin in patients with solid tumors including BC. Of the 32 patients included in the study, five patients had BC. Before enrolment, patients received systemic therapies (median of two, ranging 0–6) and vorinostat at 400–1000 mg/day on days 1–3 followed by 20 mg/m^{-2} doxorubicin (day 3 of 4 weeks). Results showed that 800 mg/day of vorinostat was the maximal tolerated dose (MTD) and one BC patient reported PaR. A single-arm Phase II interventional trial with 16 BC patents was conducted by Arce *et al.* [124]. In this study patients were first given 500 mg of famethazine (to evaluate their acetylator phenotype), followed by 182 mg (for rapid-acetylators) or 83 mg (for slow acetylators) of hydralazine. Patents then received magnesium valproate tablets (30 mg/Kb t.i.d) from day 7 until the final day of the fourth chemotherapy cycle (doxorubicin 60 mg/m^2 and cyclophosphamide 600 mg/m^2). Of the 16 patients, five patients (31%) had a complete response, three (20%) patients had SD and eight (50%) showed PaR. Microarray analysis demonstrated that genes *NDUFA13* and *DAPPER*, which are associated with apoptosis, were upregulated following therapy. A Phase I/II study with 54 metastasis BC patients with no prior chemotherapy has been conducted to evaluate safety and efficacy of vorinostat in combination with bevacizumab and paclitaxel [125]. BC patients received 200 or 300 mg vorinostat (OP, b.i.d) on days 1–3, 8–10 and 15–17 followed by paclitaxel (90 mg/m^2 on days 2, 9 and 16) and bevacizumab (10 mg/kg on days 2 and 16 for every 28 days). Twenty-four out of 44 patients (55%) showed an objective response at recommended Phase II dose of vorinostat (300 mg). Vorinostat administration also caused an increase in acetylation profiles of hsp90 and tubulin in tumors. In the TBCRC008 trial, Connolly *et al.* [126] investigated changes in serum and tissue methylation profiles as predictive markers in 62 HER2-negative BC patients progressing neoadjuvant chemotherapy. Patients received neoadjuvant treatments [carboplatin (AUC weekly) and 100 mg/m^2 of paclitaxel weekly plus 400 mg of vorinostat (PO q.d on days 1–3 of 7 days) or placebo] for 12 weeks and DNA from the blood and core biopsy samples was extracted after 15 days of the first treatment (D15). Methylation profiles of ten genes were analyzed by quantitative multiplex methylation-specific PCR and results were presented as cumulative methylation index (CMI). According to the findings, a significant association was evident among tissue CMI levels and complete response (free from viable cancer in the breast and axilla) in the group receiving vorinostat [overall response 0.44 (0.20, 0.93), $P = 0.03$] was observed.

HDACi with hormone therapy

Reversal of tamoxifen resistance, downregulation of the expression of ERα in ER-positive BC and re-expression of ERα in triple-negative BC cells by some HDACi provided a strong rational for the clinical investigation of potential role of HDACi in hormone therapy [62,75,81]. Munster *et al.* [127] conducted a Phase II study to investigate the clinical efficacy of vorinostat on the reversal of tamoxifen or AI resistance in 43 ER/PR-positive BC patients who progressed on endocrine therapy and received three regimens of chemotherapy. Patients received 400 mg of vorinostat (daily) for 3 of 4 weeks along with 20 mg of tamoxifen daily. Results of the study demonstrated that eight out of 43 patients (19%) showed PaR according to RECIST and nine out of 43 patients (21%) showed SD (>24 weeks) at a clinical benefit rate. Hyperacetylation of histones and increased expression of HDAC2 were also observed in patient PMBCs. Wardley *et al.* [128] conducted a Phase II study to investigate whether combined treatment of entinostat with AI can restore AI sensitivity in 27 ER-positive BC patients progressing on AI for 3 months. BC patients progressed on AI received 5 mg of entinostat weekly in 28-day cycles. According to the results, one patient had confirmed PaR and another patient had SD for >6 months. A randomized Phase II placebo-controlled study conducted by Yardley *et al.* [129] investigated whether the entinostat and exemestane combination could overcome hormone therapy resistance in ER-positive BC patients (ENCORE 301 trial). Of the 130 patients enrolled, 64 patients (EE group) received 25 mg of exemestane daily with 5 mg of entinostat (oral-PO) one per week and 66 patients (EP group) received exemestane [25 mg PO daily (q.d)] and placebo. All the treatment cycles were 28 days and the study reported improved progression-free survival in the EE group compared with the EP group (4.3 months vs 2.3 months; hazard ratio 0.73; 95% confidence interval, 0.50–1.07). Improved overall survival was seen in the EE compared with the EP group (28.1 months vs 19.8 months; hazard ratio, 0.59; 95% confidence interval, 0.36–0.97). Moreover, hyperacetylation of lysine investigated in monocytes, T cells and B cells was associated with a 68% reduction in disease progression compared with the patients who did not show significant hyperacetylation profiles. Based on the clinical findings in the ENCORE 301 trial, trial E2112 (a double-blind Phase III study) is currently recruiting patients to evaluate effects of exemestane plus entinostat/placebo in the same population used for the ENCORE 301 trial (NCT02115282) [130] (Table 3). In a Phase I study, Tan *et al.* [131] investigated combined effects of panobinostat, a pan inhibitor, and letrozole in patients with metastatic BC.

Twelve metastatic BC patients were enrolled in the study and given two dose levels of panobinostat and letrozole [level 1–20 mg of panobinostat (PO 3-times weekly) plus 2.5 mg of letrozole (PO q.d) and level 2–30 mg of panobinostat (PO 3-times weekly) plus 2.5 mg of letrozole (PO q.d)] in 43 cycles. Of the 12 patients enrolled, two (17%) had PaR, whereas five (42%) showed SD at level 2.

HDACi with immunomodulatory agents

Immune checkpoint proteins, such as programmed cell death receptor 1 (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) are negative regulators of T cells. Binding of PD-1 to ligands PD-L1 or PD-L2 prevents T cell killing of cancer cells [132]. Inhibition of PD-1/PD-L1/2 interactions causes enhanced activation of T cells and permits T cell targeting of cancer cells [132]. So far, several immune checkpoint inhibitors such as ipilimumab (CTLA-4 target), pembrolizumab (PD-1 target), nivolumab (PD-1 target) and PDL-1 targets such as avelumab, durvalumab and atezolizumab have been approved by the FDA for clinical use and >500 immune checkpoint inhibitors are currently being investigated in clinical trials for a range of cancers [133]. Several HDACi have been proven to exert immunomodulatory effects, rationalizing their use as immunomodulatory agents. Combination treatment of HDACi with immune checkpoint inhibitors has demonstrated promising immunomodulatory effects *in vitro* and *in vivo* [134] and some ongoing clinical trials evaluate clinical efficacy of immunomodulatory agents in combination with HDACi in BC patients (Table 3). In addition to immunomodulatory agents, combination of several DNA hypomethylating agents with HDACi has also shown promising results in *in vitro* and *in vivo* experiments [133]. DNA hypomethylating agents decitabine and azacitidine have already been approved by the FDA for the treatment of chronic myelomonocytic leukemia (CMML), myelodysplatic syndromes and acute myeloid leukemia (AML) [133]. Clinical efficacy of a combined therapy comprising decitabine and VPA was evaluated in a Phase I/II clinical trial with 54 leukemia patients. Among the patients, 12 had objective responses and ten patients had complete remission [135]. In a Phase I trial, hypomethylating agent 5-azacitidine and phenylbutyrate were tested in patients with hematological malignancies and solid tumors. In this trial, subcutaneous administration of 5-azacitidine resulted in rapid absorption and it was found that elimination and exposure of 5-azacitidine could achieve pharmacodynamic effects in patients with solid tumors [136]. Li *et al*. [137] evaluated immunomodulatory effects of 5-azacitidine and entinostat in 40 BC patients. BC patients received 40 mg/m^2 of 5-azacitidine (subcutaneously for days 1–5 and 8–10) and 7 mg of entinostat (PO) on days 3 and 10 of 28 repeated cycles in the absence of cancer progression. Following 8 weeks of therapy, examination of biopsies revealed upregulation of 33 genes related to immunomodulatory pathways. A trial conducted by the ECOG-ACRIN cancer research group (E1104) investigated clinical efficacy of combination therapy containing monoclonal antibody trastuzumab and vorinostat in three-to-six (in Phase I) and ten (in Phase II) HER2-overexpressing metastatic BC patients. In Phase I, 200 mg of vorinostat (PO b.i.d) and 6 mg/kg of trastuzumab (intravenously; i.v.) of a 21-day cycle was found to be the recommended dose. However, Phase II of this trial was terminated owing to low efficacy of combination therapy [138]. Table 2 summarizes information of published clinical trials with HDACi in BC patients.

Concluding remarks and future perspectives

An imbalance in the opposing actions of HDACs and HATs has been associated with the development of a range of cancers including BC. HDACi can reestablish aberrant acetylation profiles of proteins associated with cancer pathways and reactivate several tumor-suppressor genes, causing cancer cells to undergo cell cycle arrest and apoptosis. Several selective and nonselective HDACi alone or in combination with other drugs have been investigated in preclinical and clinical studies for the treatment of BC. Although several clinical studies have been carried out to test the clinical efficacy of several HDACi in solid tumors including BC, positive therapeutic responses have always been reported in clinical trials conducted with hematological cancer patients (multiple myeloma, lymphomas and leukemia). Short-half-lives and the lack of a predictive biomarker have been identified as major challenges associated with HDACi-based therapies in BC. Resistance to HDACi has also been identified as a major drawback in HDACi therapy. Overexpression of drug efflux pumps in the ATP-binding cassette (ABC) transporter family, mutations in HDACs, genetic or epigenetic alterations (epigenetic silencing of genes owing to DNA methylation and histone acetylation), high levels of antioxidants (e.g., thioredoxin) and overexpression of antiapoptotic Bcl-2 or Bcl-XL and NF-κB in cancer cells have been identified as major mechanisms associated with HDACi resistance [133,139]. Histone acetylation profile is the commonly used pharmacodynamic marker in HDACi therapy. Apart from the histone acetylation profile, analysis of expression profiles of several oncogenic and tumor suppressor miRNA upon HDACi therapy would be helpful to improve the therapeutic efficacy of HDACi. Combination therapies have shown meaningful clinical outcomes compared with monotherapies, and combination therapies have been reported as the ideal approach to overcome HDACi-associated drug resistance [139,140]. So far, many HDACi under preclinical and clinical assessments are pan-HDACi. Because pan-HDACi exert broad HDAC inhibition, undesirable toxicities have been observed with broad-spectrum HDACi compared with selective HDACi [139,140]. Therefore, identification of new selective HDACi with low toxicity, predictive biomarkers for HDACi therapy and detailed mechanisms of action of HDACi will further support the use of HDACi in the treatment of BC. Moreover, discovery of novel synthetic derivatives of HDACi and preparation of compound libraries, will aid identification of new HDACi with potent anticancer and fewer toxic effects. For example, WW437 (novel synthetic derivative of SAHA) has been shown to exert potent antiproliferative effects in MDA-MB-231, 4T1 and BT549 BC cells with less cytotoxicity to normal mammary epithelial (MCF-10A) cells. Dose-dependent inhibition of HDAC2 and HDAC4 and increased acetylation of histone H3 and histone H4 were also evident in three BC cells exposed to WW437. WW437 also exerted *in vivo* antitumor effects in a BC tumor model. Furthermore, WW437 was found to inhibit the HDAC–EphA2 signaling axis in BC cells [141]. In parallel, iden-

tification of novel combination treatment strategies with broad therapeutic outcomes and fewer toxicities is essential. Additionally, the *in vivo* noninvasive real-time magnetic resonance spectroscopy technique described by Sankaranarayanapillai *et al*. [142] could be developed to monitor the histone acetylation profiles in cancer patients.

References

1 Torre, L.A. *et al*. (2017) Global cancer in women: burden and trends. *Cancer Epidemiol. Biomark. Prev*. 26, 444–457
2 Toss, A. *et al*. (2017) The impact of reproductive life on breast cancer risk in women with family history or BRCA mutation. *Oncotarget* 8, 9144–9154
3 Sørlie, T. *et al*. (2003) Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc. Natl. Acad. Sci. U. S. A*. 100, 8418–8423
4 Sørlie, T. *et al*. (2001) Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc. Natl. Acad. Sci. U. S. A*. 98, 10869–10874
5 Tang, Y. *et al*. (2016) Classification, treatment strategy, and associated drug resistance in breast cancer. *Clin. Breast Cancer* 16, 335–343
6 Li, Y. and Seto, E. (2016) HDACs and HDAC inhibitors in cancer development and therapy. *Cold Spring Harb. Perspect. Med*. 6 pii: a026831
7 Jones, P.A. and Baylin, S.B. (2002) The fundamental role of epigenetic events in cancer. *Nat. Rev. Genet*. 3, 415–428
8 Peterson, C.L. and Laniel, M.A. (2004) Histones and histone modifications. *Curr. Biol*. 14, R546–R551
9 Brown, C.E. *et al*. (2000) The many HATs of transcription coactivators. *Trends Biochem. Sci*. 25, 15–19
10 Baylin, S.B. and Herman, J.G. (2000) DNA hypermethylation in tumorigenesis: epigenetics joins genetics. *Trends Genet*. 16, 168–174
11 Berger, S.L. *et al*. (2009) An operational definition of epigenetics. *Genes Dev*. 23, 781–783
12 You, J.S. and Jones, P.A. (2012) Cancer genetics and epigenetics: two sides of the same coin? *Cancer Cell* 22, 9–20
13 Miremadi, A. *et al*. (2007) Cancer genetics of epigenetic genes. *Hum. Mol. Genet*. 16, R28–R49
14 Anzick, S.L. *et al*. (1997) AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. *Science* 277, 965–968
15 Spurdle, A.B. *et al*. (2006) The AIB1 polyglutamine repeat does not modify breast cancer risk in BRCA1 and BRCA2 mutation carriers. *Cancer Epidemiol. Biomark. Prev*. 15, 76–79
16 Hughes, D.J. *et al*. (2005) Breast cancer risk in BRCA1 and BRCA2 mutation carriers and polyglutamine repeat length in the AIB1 gene. *Int. J. Cancer* 117, 230–233
17 Burwinkel, B. *et al*. (2005) Association of NCOA3 polymorphisms with breast cancer risk. *Clin. Cancer Res*. 11, 2169–2174
18 Campbell, I.G. *et al*. (2004) No germline mutations in the histone acetyltransferase gene EP300 in BRCA1 and BRCA2 negative families with breast cancer and gastric, pancreatic, or colorectal cancer. *Breast Cancer Res*. 6, R366–R371
19 Cebrian, A. *et al*. (2006) Genetic variants in epigenetic genes and breast cancer risk. *Carcinogenesis* 27, 1661–1669
20 Gayther, S.A. *et al*. (2000) Mutations truncating the EP300 acetylase in human cancers. *Nat. Genet*. 24, 300–303
21 Chin, S.F. *et al*. (2007) Using array-comparative genomic hybridization to define molecular portraits of primary breast cancers. *Oncogene* 26, 1959–1970
22 Sjöblom, T. *et al*. (2006) The consensus coding sequences of human breast and colorectal cancers. *Science* 314, 268–274
23 Kuzmichev, A. *et al*. (2005) Composition and histone substrates of polycomb repressive group complexes change during cellular differentiation. *Proc. Natl. Acad. Sci. U. S. A*. 102, 1859–1864
24 Wang, Z. *et al*. (2009) Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. *Cell* 138, 1019–1031
25 Bertrand, P. (2010) Inside HDAC with HDAC inhibitors. *Eur. J. Med. Chem*. 45, 2095–2116
26 Suzuki, J. *et al*. (2009) Protein acetylation and histone deacetylase expression associated with malignant breast cancer progression. *Clin. Cancer Res*. 15, 3163–3171
27 Krusche, C.A. *et al*. (2005) Histone deacetylase-1 and-3 protein expression in human breast cancer: a tissue microarray analysis. *Breast Cancer Res. Treat*. 90, 15–23
28 Hu, X. *et al*. (2009) Genetic alterations and oncogenic pathways associated with breast cancer subtypes. *Mol. Cancer Res*. 7, 511–522
29 Müller, B.M. *et al*. (2013) Differential expression of histone deacetylases HDAC1, 2 and 3 in human breast cancer-overexpression of HDAC2 and HDAC3 is associated with clinicopathological indicators of disease progression. *BMC Cancer* 13, 215
30 Özdağ, H. *et al*. (2006) Differential expression of selected histone modifier genes in human solid cancers. *BMC Genom*. 7, 90
31 Saji, S. *et al*. (2005) Significance of HDAC6 regulation via estrogen signaling for cell motility and prognosis in estrogen receptor-positive breast cancer. *Oncogene* 24, 4531–4539
32 Zhang, Z. *et al*. (2004) HDAC6 expression is correlated with better survival in breast cancer. *Clin. Cancer Res*. 10, 6962–6968
33 Lee, Y.S. *et al*. (2008) The cytoplasmic deacetylase HDAC6 is required for efficient oncogenic tumorigenesis. *Cancer Res*. 68, 7561–7569
34 Rey, M. *et al*. (2011) HDAC6 is required for invadopodia activity and invasion by breast tumor cells. *Eur. J. Cell Biol*. 90, 128–135
35 Ververis, K. and Karagiannis, T.C. (2012) An atlas of histone deacetylase expression in breast cancer: fluorescence methodology for comparative semi-quantitative analysis. *Am. J. Transl. Res*. 4, 24–43
36 Eckschlager, T. *et al*. (2017) Histone deacetylase inhibitors as anticancer drugs. *Int. J. Mol. Sci*. 18, 1414
37 Damaskos, C. *et al*. (2017) Histone deacetylase inhibitors: an attractive therapeutic strategy against breast cancer. *Anticancer Res*. 37, 35–46
38 Dawson, M.A. and Kouzarides, T. (2012) Cancer epigenetics: from mechanism to therapy. *Cell* 150, 12–27
39 Iorio, M.V. and Croce, C.M. (2012) MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. *EMBO Mol. Med*. 4, 143–159
40 Calin, G.A. *et al*. (2006) MicroRNA signatures in human cancers. *Nat. Rev. Cancer* 6, 857–866
41 Lowery, A.J. *et al*. (2009) MicroRNA signatures predict oestrogen receptor, progesterone receptor and HER2/neu receptor status in breast cancer. *Breast Cancer Res*. 11, R27
42 Radojicic, J. *et al*. (2011) MicroRNA expression analysis in triple-negative (ER, PR and Her2/neu) breast cancer. *Cell Cycle* 10, 507–517
43 Zhang, L. *et al*. (2006) MicroRNAs exhibit high frequency genomic alterations in human cancer. *Proc. Natl. Acad. Sci. U. S. A*. 103, 9136–9141
44 Han, M. *et al*. (2012) Re-expression of miR-21 contributes to migration and invasion by inducing epithelial-mesenchymal transition consistent with cancer stem cell characteristics in MCF-7 cells. *Mol. Cell. Biochem*. 363, 427–436
45 Li, H. *et al*. (2011) miR-17-5p promotes human breast cancer cell migration and invasion through suppression of HBP1. *Breast Cancer Res. Treat*. 126, 565–575
46 Yu, F. *et al*. (2007) let-7 regulates self-renewal and tumorigenicity of breast cancer cells. *Cell* 131, 1109–1123
47 Singh, R. and Mo, Y.Y. (2013) Role of microRNAs in breast cancer. *Cancer Biol. Ther*. 14, 201–212
48 Ali, S.R. *et al*. (2015) Impact of histone deacetylase inhibitors on microRNA expression and cancer therapy: a review. *Drug Dev. Res*. 76, 296–317
49 Cho, J.H. *et al*. (2015) MicroRNA-31 is a transcriptional target of histone deacetylase inhibitors and a regulator of cellular senescence. *J. Biol. Chem*. 290, 10555–10567
50 Hsieh, T.H. *et al*. (2015) HDAC inhibitors target HDAC5, upregulate microRNA-125a-5p, and induce apoptosis in breast cancer cells. *Mol. Ther*. 23, 656–666
51 Eades, G. *et al*. (2011) miR-200a regulates Nrf2 activation by targeting Keap1 mRNA in breast cancer cells. *J. Biol. Chem*. 286, 40725–40733
52 Talbert, D.R. *et al*. (2013) The role of Myc and the miR-17∼92 cluster in histone deacetylase inhibitor induced apoptosis of solid tumors. *J. Cancer Ther*. 4, 907–918
53 Rhodes, L.V. *et al*. (2012) The histone deacetylase inhibitor trichostatin A alters microRNA expression profiles in apoptosis-resistant breast cancer cells. *Oncol. Rep*. 27, 10–16
54 Tu, C.Y. *et al*. (2014) Trichostatin A suppresses EGFR expression through induction of microRNA-7 in an HDAC-independent manner in lapatinib-treated cells. *Biomed. Res. Int*. 2014, 168949
55 Wang, S. *et al*. (2013) Functional cooperation of miR-125a, miR-125b, and miR-205 in entinostat-induced downregulation of erbB2/erbB3 and apoptosis in breast cancer cells. *Cell Death Dis*. 4, e556
56 Saito, Y. *et al*. (2009) Epigenetic therapy upregulates the tumor suppressor microRNA-126 and its host gene EGFL7 in human cancer cells. *Biochem. Biophys. Res. Commun*. 379, 726–731
57 Bian, X. *et al*. (2018) HDAC inhibitor suppresses proliferation and invasion of breast cancer cells through regulation of miR-200c targeting CRKL. *Biochem. Pharmacol*. 147, 30–37

58 Robertson, F.M. *et al.* (2010) Suberoylanilide hydroxamic acid blocks self-renewal and homotypic aggregation of inflammatory breast cancer spheroids. *Cancer* 116, 2760–2767
59 Lee, Y.J. *et al.* (2012) Molecular mechanism of SAHA on regulation of autophagic cell death in tamoxifen-resistant MCF-7 breast cancer cells. *Int. J. Med. Sci.* 9, 881–893
60 Palmieri, D. *et al.* (2009) Vorinostat inhibits brain metastatic colonization in a model of triple-negative breast cancer and induces DNA double-strand breaks. *Clin. Cancer Res.* 15, 6148–6157
61 Munster, P.N. *et al.* (2001) The histone deacetylase inhibitor suberoylanilide hydroxamic acid induces differentiation of human breast cancer cells. *Cancer Res.* 61, 8492–8497
62 Fiskus, W. *et al.* (2007) Hydroxamic acid analogue histone deacetylase inhibitors attenuate estrogen receptor-α levels and transcriptional activity: a result of hyperacetylation and inhibition of chaperone function of heat shock protein 90. *Clin. Cancer Res.* 13, 4882–4890
63 Kou, X. *et al.* (2017) Vorinostat and simvastatin have synergistic effects on triple-negative breast cancer cells via abrogating Rab7 prenylation. *Eur. J. Pharmacol.* 813, 161–171
64 Natarajan, U. *et al.* (2017) Treatment effects of vorinostat and letrozole combination on breast cancer cell survival and peripheral blood mononuclear cell differentiation. *FASEB J.* 31, lb161
65 Bali, P. *et al.* (2005) Activity of suberoylanilide hydroxamic acid against human breast cancer cells with amplification of her-2. *Clin. Cancer Res.* 11, 6382–6389
66 Chiu, H.W. *et al.* (2013) Suberoylanilide hydroxamic acid, an inhibitor of histone deacetylase, enhances radiosensitivity and suppresses lung metastasis in breast cancer in vitro and in vivo. *PLoS One* 8, e76340
67 Gameiro, S.R. *et al.* (2016) Inhibitors of histone deacetylase 1 reverse the immune evasion phenotype to enhance T-cell mediated lysis of prostate and breast carcinoma cells. *Oncotarget* 7, 7390–7402
68 Marchion, D.C. *et al.* (2004) Sequence-specific potentiation of topoisomerase II inhibitors by the histone deacetylase inhibitor suberoylanilide hydroxamic acid. *J. Cell. Biochem.* 92, 223–237
69 Ali, H.R. *et al.* (2015) PD-L1 protein expression in breast cancer is rare, enriched in basal-like tumours and associated with infiltrating lymphocytes. *Ann. Oncol.* 26, 1488–1493
70 Wimberly, H. *et al.* (2015) PD-L1 expression correlates with tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy in breast cancer. *Cancer Immunol. Res.* 3, 326–332
71 Silva, G.B. *et al.* (2013) Expression of the classical and nonclassical HLA molecules in breast cancer. *Int. J. Breast Cancer* 2013, 250435
72 Terranova-Barberio, M. *et al.* (2017) HDAC inhibition potentiates immunotherapy in triple negative breast cancer. *Oncotarget* 8, 114156–114172
73 Alao, J.P. *et al.* (2004) Histone deacetylase inhibitor trichostatin A represses estrogen receptor α-dependent transcription and promotes proteasomal degradation of cyclin D1 in human breast carcinoma cell lines. *Clin. Cancer Res.* 10, 8094–8104
74 Jang, E.R. *et al.* (2004) The histone deacetylase inhibitor trichostatin A sensitizes estrogen receptor α-negative breast cancer cells to tamoxifen. *Oncogene* 23, 1724–1736
75 Fan, J. *et al.* (2008) ERα negative breast cancer cells restore response to endocrine therapy by combination treatment with both HDAC inhibitor and DNMT inhibitor. *J. Cancer Res. Clin. Oncol.* 134, 883–890
76 Chen, L. *et al.* (2017) PI3K/mTOR dual inhibitor BEZ235 and histone deacetylase inhibitor trichostatin A synergistically exert anti-tumor activity in breast cancer. *Oncotarget* 8, 11937–11949
77 Chatterjee, N. *et al.* (2013) Histone deacetylase inhibitors modulate miRNA and mRNA expression, block metaphase, and induce apoptosis in inflammatory breast cancer cells. *Cancer Biol. Ther.* 14, 658–671
78 Tate, C.R. *et al.* (2012) Targeting triple-negative breast cancer cells with the histone deacetylase inhibitor panobinostat. *Breast Cancer Res.* 14, R79
79 Medon, M. *et al.* (2017) HDAC inhibitor panobinostat engages host innate immune defenses to promote the tumoricidal effects of trastuzumab in HER2+ tumors. *Cancer Res.* 77, 2594–2606
80 Matossian, M.D. *et al.* (2018) Panobinostat suppresses the mesenchymal phenotype in a novel claudin-low triple negative patient-derived breast cancer model. *Oncoscience* 5, 99–108
81 Zhou, Q. *et al.* (2007) Histone deacetylase inhibitor LBH589 reactivates silenced estrogen receptor alpha (ER) gene expression without loss of DNA hypermethylation. *Cancer Biol. Ther.* 6, 64–69
82 Chen, S. *et al.* (2010) The HDAC inhibitor LBH589 (panobinostat) is an inhibitory modulator of aromatase gene expression. *Proc. Natl. Acad. Sci. U. S. A.* 107, 11032–11037
83 Hsu, K.W. *et al.* (2018) The application of non-invasive apoptosis detection sensor (NIADS) on histone deacetylation inhibitor (HDACI)-induced breast cancer cell death. *Int. J. Mol. Sci.* 19, E452
84 Marijon, H. *et al.* (2018) Co-targeting poly(ADP-ribose) polymerase (PARP) and histone deacetylase (HDAC) in triple-negative breast cancer: higher synergism in BRCA mutated cells. *Biomed. Pharmacother.* 99, 543–551
85 Androutsopoulos, V.P. and Spandidos, D.A. (2017) Antiproliferative effects of TSA, PXD-101 and MS-275 in A2780 and MCF7 cells: acetylated histone H4 and acetylated tubulin as markers for HDACi potency and selectivity. *Oncol. Rep.* 38, 3412–3418
86 Tian, Y. *et al.* (2017) Valproic acid sensitizes breast cancer cells to hydroxyurea through inhibiting RPA2 hyperphosphorylation-mediated DNA repair pathway. *DNA Repair* 58, 1–12
87 Mawatari, T. *et al.* (2015) Valproic acid inhibits proliferation of HER2-expressing breast cancer cells by inducing cell cycle arrest and apoptosis through Hsp70 acetylation. *Int. J. Oncol.* 47, 2073–2081
88 Hao, Y. *et al.* (2017) Valproic acid induces decreased expression of H19 promoting cell apoptosis in A549 cells. *DNA Cell Biol.* 36, 428–435
89 Luo, Y. *et al.* (2016) Valproic acid causes radiosensitivity of breast cancer cells via disrupting the DNA repair pathway. *Toxicol. Res.* 5, 859–870
90 Aztopal, N. *et al.* (2018) Valproic acid, a histone deacetylase inhibitor, induces apoptosis in breast cancer stem cells. *Chem. Biol. Interact.* 280, 51–58
91 Mongan, N.P. and Gudas, L.J. (2005) Valproic acid, in combination with all-trans retinoic acid and 5-aza-2′-deoxycytidine, restores expression of silenced RARβ2 in breast cancer cells. *Mol. Cancer Ther.* 4, 477–486
92 Smith, J.G. *et al.* (1988) Butyric acid from the diet: actions at the level of gene expression. *Crit. Rev. Food Sci. Nutr.* 38, 259–297
93 Salimi, V. *et al.* (2017) Sodium butyrate promotes apoptosis in breast cancer cells through reactive oxygen species (ROS) formation and mitochondrial impairment. *Lipids Health Dis.* 16, 208
94 Louis, M. *et al.* (2004) The histone deacetylase inhibitor sodium butyrate induces breast cancer cell apoptosis through diverse cytotoxic actions including glutathione depletion and oxidative stress. *Int. J. Oncol.* 25, 1701–1711
95 Chopin, V. *et al.* (2004) Synergistic induction of apoptosis in breast cancer cells by cotreatment with butyrate and TNF-alpha, TRAIL, or anti-Fas agonist antibody involves enhancement of death receptors' signaling and requires P21waf1. *Exp. Cell Res.* 29, 560–573
96 Chopin, V. *et al.* (2004) P21 WAF1/CIP1 is dispensable for G1 arrest, but indispensable for apoptosis induced by sodium butyrate in MCF-7 breast cancer cells. *Oncogene* 23, 21–29
97 Chopin, V. *et al.* (2002) Sodium butyrate induces P53-independent, Fas-mediated apoptosis in MCF-7 human breast cancer cells. *Br. J. Pharmacol.* 135, 79–86
98 Knipstein, J. and Gore, L. (2011) Entinostat for treatment of solid tumors and hematologic malignancies. *Expert Opin. Investig. Drugs* 20, 1455–1467
99 Schech, A.J. *et al.* (2015) Histone deacetylase inhibitor entinostat in combination with a retinoid downregulates HER2 and reduces the tumor initiating cell population in aromatase inhibitor-resistant breast cancer. *Breast Cancer Res. Treat.* 152, 499–508
100 Schech, A. *et al.* (2015) Histone deacetylase inhibitor entinostat inhibits tumor-initiating cells in triple negative breast cancer cells. *Mol. Cancer Ther.* 14, 1848–1857
101 Lee, J. *et al.* (2014) A class I histone deacetylase inhibitor, entinostat, enhances lapatinib efficacy in HER2-overexpressing breast cancer cells through FOXO3-mediated Bim1 expression. *Breast Cancer Res. Treat.* 146, 259–272
102 Sabnis, G.J. *et al.* (2011) Functional activation of the estrogen receptor-α and aromatase by the HDAC inhibitor entinostat sensitizes ER-negative tumors to letrozole. *Cancer Res.* 71, 1893–1903
103 Shah, P. *et al.* (2014) Histone deacetylase inhibitor entinostat reverses epithelial to mesenchymal transition of breast cancer cells by reversing the repression of E-cadherin. *Breast Cancer Res. Treat.* 143, 99–111
104 Lee, K.W. *et al.* (2006) Antitumor activity of SK-7041, a novel histone deacetylase inhibitor, in human lung and breast cancer cells. *Anticancer Res.* 26, 3429–3438
105 Borbely, G. *et al.* (2015) Induction of USP17 by combining BET and HDAC inhibitors in breast cancer cells. *Oncotarget* 6, 33623–33635
106 Witt, A.E. *et al.* (2017) Identification of a cancer stem cell-specific function for the histone deacetylases, HDAC1 and HDAC7, in breast and ovarian cancer. *Oncogene* 36, 1707–1720
107 Robertson, F.M. *et al.* (2013) The class I HDAC inhibitor romidepsin targets inflammatory breast cancer tumor emboli and synergizes with paclitaxel to inhibit metastasis. *J. Exp. Ther. Oncol.* 10, 219–233
108 Choudhary, S. *et al.* (2010) FK228 and oncogenic H-Ras synergistically induce Mek1/2 and Nox-1 to generate reactive oxygen species for differential cell death. *Anti Cancer Drugs* 21, 831–840

109 Choi, D.S. and Chang, J.C. (2015) Chloroquine and romidepsin: combination therapy for treatment of breast cancer metastases. *AACR Cancer Res.* 75 Abstract 4129

110 Haigis, M.C. and Sinclair, D.A. (2010) Mammalian sirtuins: biological insights and disease relevance. *Annu. Rev. Pathol.* 5, 253–295

111 Hu, J. *et al.* (2014) Sirtuin inhibitors as anticancer agents. *Future Med. Chem.* 6, 945–966

112 Wang, J. *et al.* (2012) Sirtinol, a class III HDAC inhibitor, induces apoptotic and autophagic cell death in MCF-7 human breast cancer cells. *Int. J. Oncol.* 4, 1101–1109

113 Kuo, S.J. *et al.* (2013) SIRT1 suppresses breast cancer growth through downregulation of the Bcl-2 protein. *Oncol. Rep.* 30, 125–130

114 Ota, H. *et al.* (2006) Sirt1 inhibitor, sirtinol, induces senescence-like growth arrest with attenuated Ras–MAPK signaling in human cancer cells. *Oncogene* 25, 176–185

115 Borges, S. *et al.* (2014) A combination treatment with DNA methyltransferase inhibitors and suramin decreases invasiveness of breast cancer cells. *Breast Cancer Res. Treat.* 144, n79–n91

116 Rubin, E.H. *et al.* (2006) A study to determine the effects of food and multiple dosing on the pharmacokinetics of vorinostat given orally to patients with advanced cancer. *Clin. Cancer Res.* 12, 7039–7045

117 Vansteenkiste, J. *et al.* (2008) Early Phase II trial of oral vorinostat in relapsed or refractory breast, colorectal, or non-small cell lung cancer. *Invest. New Drugs* 26, 483–488

118 Luu, T.H. *et al.* (2008) A Phase II trial of vorinostat (suberoylanilide hydroxamic acid) in metastatic breast cancer: a California Cancer Consortium study. *Clin. Cancer Res.* 14, 7138–7142

119 O'Connor, O.A. *et al.* (2009) Clinical experience with intravenous and oral formulations of the novel histone deacetylase inhibitor suberoylanilide hydroxamic acid in patients with advanced hematologic malignancies. *J. Clin. Oncol.* 24, 166–173

120 Huang, L. and Pardee, A.B. (2000) Suberoylanilide hydroxamic acid as a potential therapeutic agent for human breast cancer treatment. *Mol. Med.* 6, 849–866

121 Munster, P. *et al.* (2007) Phase I trial of histone deacetylase inhibition by valproic acid followed by the topoisomerase II inhibitor epirubicin in advanced solid tumors: a clinical and translational study. *J. Clin. Oncol.* 25, 1979–1985

122 Munster, P. *et al.* (2009) Clinical and biological effects of valproic acid as a histone deacetylase inhibitor on tumor and surrogate tissues: Phase I/II trial of valproic acid and epirubicin/FEC. *Clin. Cancer Res.* 15, 2488–2496

123 Munster, P.N. *et al.* (2009) Phase I trial of vorinostat and doxorubicin in solid tumours: histone deacetylase 2 expression as a predictive marker. *Br. J. Cancer* 101, 1044–1050

124 Arce, C. *et al.* (2006) A proof-of-principle study of epigenetic therapy added to neoadjuvant doxorubicin cyclophosphamide for locally advanced breast cancer. *PLoS One* 1, e98

125 Ramaswamy, B. *et al.* (2012) Phase I–II study of vorinostat plus paclitaxel and bevacizumab in metastatic breast cancer: evidence for vorinostat-induced tubulin acetylation and Hsp90 inhibition in vivo. *Breast Cancer Res. Treat.* 132, 1063–1072

126 Connolly, R.M. *et al.* (2018) Tumor and serum DNA methylation in women receiving preoperative chemotherapy with or without vorinostat in TBCRC008. *Breast Cancer Res. Treat.* 16, 107–116

127 Munster, P.N. *et al.* (2011) A Phase II study of the histone deacetylase inhibitor vorinostat combined with tamoxifen for the treatment of patients with hormone therapy-resistant breast cancer. *Br. J. Cancer* 104, 1828–1835

128 Wardley, A.M. *et al.* (2010) Phase II data for entinostat, a class 1 selective histone deacetylase inhibitor, in patients whose breast cancer is progressing on aromatase inhibitor therapy. *J. Clin. Oncol.* 28, 1052

129 Yardley, D.A. *et al.* (2013) 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.* 31, 2128–2135

130 Exemestane with or without entinostat in treating patients with recurrent hormone receptor-positive breast cancer that is locally advanced or metastatic. Available at: https://clinicaltrials.gov/ct2/show/NCT02115282

131 Tan, W.W. *et al.* (2016) Phase I study of panobinostat (LBH589) and letrozole in postmenopausal metastatic breast cancer patients. *Clin. Breast Cancer* 16, 82–86

132 Pardoll, D.M. (2012) The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* 12, 252–264

133 Suraweera, A. *et al.* (2018) Combination therapy with histone deacetylase inhibitors (HDACi) for the treatment of cancer: achieving the full therapeutic potential of HDACi. *Front. Oncol.* 8, 92

134 Shen, L. *et al.* (2016) Histone deacetylase inhibitors as immunomodulators in cancer therapeutics. *Epigenomics* 8, 415–428

135 Garcia-Manero, G. *et al.* (2006) Phase 1/2 study of the combination of 5-aza-2′-deoxycytidine with valproic acid in patients with leukemia. *Blood* 108, 3271–3279

136 Rudek, M.A. *et al.* (2005) Pharmacokinetics of 5-azacitidine administered with phenylbutyrate in patients with refractory solid tumors or hematologic malignancies. *J. Clin. Oncol.* 23, 3906–3911

137 Li, H. *et al.* (2014) Immune regulation by low doses of the DNA methyltransferase inhibitor 5-azacitidine in common human epithelial cancers. *Oncotarget* 5, 587–598

138 Goldstein, L.J. *et al.* (2017) A Phase I/II study of suberoylanilide hydroxamic acid (SAHA) in combination with trastuzumab (Herceptin) in patients with advanced metastatic and/or local chest wall recurrent HER2-amplified breast cancer: a trial of the ECOG-ACRIN Cancer Research Group (E1104). *Breast Cancer Res. Treat.* 165, 375–382

139 Robey, R.W. *et al.* (2011) Histone deacetylase inhibitors: emerging mechanisms of resistance. *Mol. Pharm.* 8, 2021–2031

140 Fantin, V.R. and Richon, V.M. (2007) Mechanisms of resistance to histone deacetylase inhibitors and their therapeutic implications. *Clin. Cancer Res.* 13, 7237–7242

141 Zhang, T. *et al.* (2018) Inhibition of HDACs-EphA2 signaling axis with WW437 demonstrates promising preclinical antitumor activity in breast cancer. *EBioMedicine* 31, 276–286

142 Sankaranarayanapillai, M. *et al.* (2008) Monitoring histone deacetylase inhibition in vivo: noninvasive magnetic resonance spectroscopy method. *Mol. Imaging* 7, 7290

143 Cisplatin plus romidepsin & nivolumab in locally recurrent or metastatic triple negative breast cancer (TNBC). Available at: https://clinicaltrials.gov/ct2/show/NCT02393794

144 Randomized Phase 2 study of atezolizumab and entinostat in patients with aTN breast cancer with Phase 1b lead in. Available at: https://clinicaltrials.gov/ct2/show/NCT02708680

145 Entinostat, nivolumab, and ipilimumab in treating patients with solid tumors that are metastatic or cannot be removed by surgery or locally advanced or metastatic HER2-negative breast cancer. Available at: https://clinicaltrials.gov/ct2/show/NCT02453620

146 A pilot study of the combination of entinostat with capecitabine in high risk breast cancer after neo-adjuvant therapy (Breast 49). Available at: https://clinicaltrials.gov/ct2/show/NCT03473639

147 Reversing therapy resistance with epigenetic-immune modification. Available at: https://clinicaltrials.gov/ct2/show/NCT02395627