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Small molecule HDAC inhibitors: Promising agents for breast cancer treatment

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ABSTRACT

Breast cancer, a heterogeneous disease, is the most frequently diagnosed cancer and the second leading cause of cancer-related death among women worldwide. Recently, epigenetic abnormalities have emerged as an important hallmark of cancer development and progression. Given that histone deacetylases (HDACs) are crucial to chromatin remodeling and epigenetics, their inhibitors have become promising potential anticancer drugs for research. Here we reviewed the mechanism and classification of histone deacetylases (HDACs), association between HDACs and breast cancer, classification and structure–activity relationship (SAR) of HDACIs, pharmacokinetic and toxicological properties of the HDACIs, and registered clinical studies for breast cancer treatment. In conclusion, HDACIs have shown desirable effects on breast cancer, especially when they are used in combination with other anticancer agents. In the coming future, more multicenter and randomized Phase III studies are expected to be conducted pushing promising new therapies closer to the market. In addition, the design and synthesis of novel HDACIs are also needed.

1. Introduction

Breast cancer, a heterogeneous disease, is the most frequently diagnosed cancer and the second leading cause of cancer-related death among women worldwide [1]. Broadly, based on the gene expression profiling, breast cancer can be sub-classified into four intrinsic subtypes: luminal A, luminal B (Luminal B1 and Luminal B2), HER2 enriched, and basal-like (Table 1) [2]. When separating luminal A from luminal B1 subtypes, the cutoff point of Ki-67 was 14% previously. But recently, this cutoff was changed in 20% [3]. Enrichment of GATA3, PIK3CA and MAP3K1 mutations were commonly identified in the luminal A subtype [4]. GATA3 mutation was found to be significantly associated with improved overall survival [5]. In luminal B1 subtype, the frequency of p53 mutations was higher than luminal A, but PIK3CA mutation frequency was lower [6]. As reported, nearly half of HER2 positive breast cancer subtype was Luminal B2 subtype, which overexpressed GATA3, BCL2, and ESR1 genes [7]. In HER2 positive subtype, the overexpressed genes in Luminal subtype were down-regulated or deleted. But ERBB2 and GRB7 genes were overexpressed in 17q22.24 ERBB2 amplicon. Triple negative breast cancer (TNBC) represents 15–20% of all breast cancers and is characterized by higher rates of relapse, greater metastatic potential, and shorter overall survival [8]. Based on its heterogeneity, TNBC can be classified into six molecular subtypes: 2 basal like classes (BL1 and BL2), an immunomodulatory (IM), a mesenchymal (M), a mesenchymal stem cell (MSL) and the luminal androgen receptor (LAR) class (Fig. 1) [9]. AR, EGFR, and BRCA1 might be unique biomarkers for targeted therapy and prognosis in TNBC [10]. Nowadays, genomic advancements provide opportunities for precision medicine, based on the biomarkers for specific breast cancer subtype.

2. HDACs and their mechanism of action

Epigenetic abnormalities are considered one of the hallmarks of cancer development and progression, and have emerged as novel therapeutic targets [11]. As primary protein components of chromatin, histones (H1, H2A, H2B, H3 and H4) play important roles in establishing interactions between the nucleosomes [12]. There are at least eight types of histone post-translational modifications, namely acetylation, methylation, phosphorylation, ubiquitylation, sumoylation, ADP ribosylation, deamination and proline isomerization [13]. Lysine acetylation, a reversible post-translational modification of proteins, can

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Table 1
Molecular subtypes of breast cancer.

Molecular subtype	ER		PR		HER2	Ki67	Proportion in IBC	Gene profile
Luminal A	Positive	and/or	Positive (≥ 20%)		Negative	≤ 14%	30–40%	Overexpression of GATA3, XBP1, TFF3, HNF3, MAP3K1; 13% p53 mutation; 45% PIK3CA mutation
Luminal B1	Positive	and/or	Positive Negative (< 20%)	or	Negative	≥ 14%	20–30%	32% p53 mutation; 32% PIK3CA mutation
Luminal B2	Positive	and/or	Positive Negative	or	Positive	–	12–20%	Overexpression of GATA3, BCL 2, ESR1
HER2	Negative		Negative		Positive			ERBB2 and GRB7 overexpression; 71% p53 mutation; FGFR4, EGFR, HER-2 activation; 39% PIK3CA mutation
Basal-like	Negative		Negative		Negative		15–20%	AR, EGFR, and BRCA1

Notes: ER: estrogen receptor; HER2: human epidermal growth factor receptor 2; PR: progesterone receptor; IBC: invasive breast cancers.

be controlled by histone deacetylase (HDAC) and histone acetyltransferase (HAT). HDACs are enzymes that remove acetyl groups ($O{=}C{-}CH_3$) from ε-N-acetyl lysine amino acids on histones wrapped by DNA chains [14]. Histone acetylation by HAT was crucial to transcriptional activation, whereas deacetylation of histones promotes transcriptional repression and silencing of genes. Disruption of HAT and HDAC activities has been associated with the development of a wide variety of human cancers [15]. As reported, excessive level of histone deacetylation is linked to cancer pathologies by affecting several well characterized cellular oncogenes and tumor-suppressor genes, such as the p53, RUNX3, STAT3, β-catenin, estrogen receptor, Myc, EKLF, GATA family, HIF-1 α, MyoD, NF-κB or Foxp3 [16].

There exists 18 HDAC isoforms identified in humans and are classified into Class I (HDAC 1, 2, 3 and 8), Class II (HDACs 4, 5, 6, 7, 9, and 10), Class III (sirtuin family: sirt1-sirt7), and Class IV (HDAC 11) on the basis of homology to yeast HDACs. Class II HDACs can be further divided into two subgroups: Class IIA (HDAC 4, 5, 7 and 9), which has a large C-terminus, and Class IIB (HDAC 6 and 10), which has two deacetylase domains. Class I HDACs share sequence homology with yeast reduced potassium dependency-3 (Rpd3), and are mainly located in the nucleus of the cells. Class II family HDACs are homologous to the yeast histone deacetylases 1 (Hda1), and are primarily localized in the cytoplasm. Class II HDACs can also be shuttled between the cytoplasm and nucleus depending on the phosphorylation status. Class III HDACs are homologous with yeast silent information regulator-2 (Sir2) protein, and are located in nucleus, cytoplasm and mitochondrion. Class IV HDAC has a unique structure and is localized in the nucleus. HDAC 11, a class IV HDAC, displays an amino acid sequence frame of 347 residues, which is only slightly homologous to the other isoforms. Class IV HDAC shares some sequences of Class I and II enzymes [17]. Class I, II, and IV HDACs are zinc dependent and share a similar catalytic core for acetyl-lysine hydrolysis. Zn^{2+} ions are essential to stabilize their catalytic centers. The intrinsic activity of Class I, II, and IV HDACs can be suppressed by occupying the catalytic core of the zinc-binding site. Class III HDACs, are not zinc dependent and require a nicotinamide adenine dinucleotide for their enzyme activity. Zinc dependent deacetylase inhibitors cannot generally inhibit Class III HDACs (Fig. 1).

Nowadays, the crystal structure of HDAC2, HDAC4, HDAC7, HDAC8, HDLP (histone deacetylase-like protein) and HDAH (histone deacetylase-like amidohydrolase which is homologous to HDAC6) has been achieved (Fig. 2). Generally, the crystal structures of Class I HDACs possess commonly an 11 Å deep channel followed by a 14 Å internal cavity in proximity to the active site, where the catalytic Zn^{2+} ion is situated near the bottom of the pocket. Class IIa and IIb HDACs both consist of two functionally catalytic domains, a C-terminal catalytic domain and an *N*-terminal extension with 600 residues [18].

3. HDACs and breast cancer

Impairment in the balance between HATs and HDACs has been reported in the development of breast cancer. In 2004, Zhang et al. found a higher expression of HDAC6 mRNA in hormone positive breast cancer patients with small tumors and low histologic grade, indicating more responsive to endocrine treatment and better prognosis [19]. Similarly, HDAC6 expression maybe an independent prognostic indicator for ER-positive patients who received adjuvant treatment with TAM, indicating the biological significance of HDAC6 regulation via estrogen signaling [20]. In 2005, Krusche considered HDAC 1 as an independent prognostic marker for breast carcinoma. HDAC 1 expression analysis might be clinically useful to facilitate an individual, risk-directed, adjuvant systemic therapy in breast cancer patients [21]. In 2006, the overexpression of HDAC4 was found in breast cancer cells, in comparison with lung and colon cancer cells [22]. In 2008, Lee et al. approved the potential role of HDAC6 in anchorage independent growth of breast cancer cells [23]. In 2009, Suzuki et al. found marked reduction of HDAC1, HDAC2, and HDAC6 expression. Greater reductions

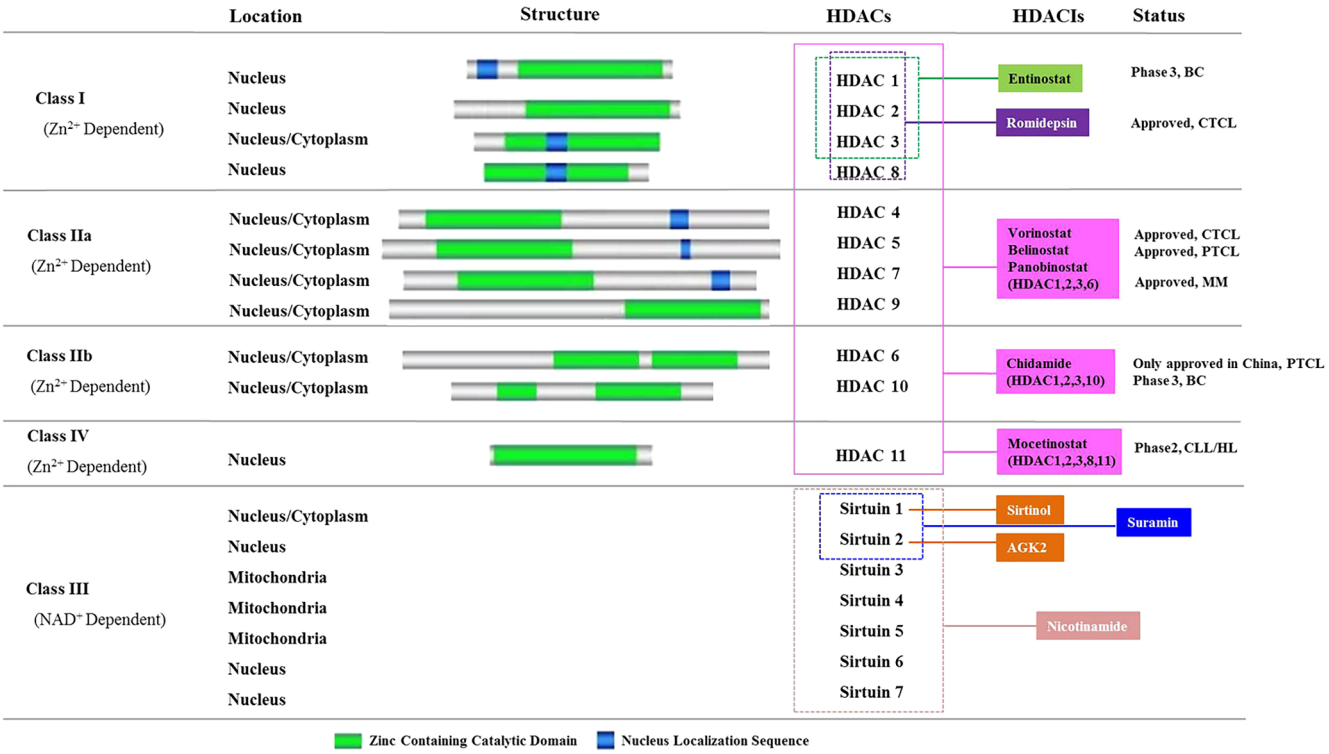

Fig. 1. The classification of HDACs and the represent selective inhibitors. Notes: CTCL: cutaneous T-cell lymphoma, BC: breast cancer, PTCL: peripheral T celll lymphoma, MM: multiple myeloma, CLL: chronic lymphocytic leukemia, HL: hodgkin lymphoma.

of H4 acetylation and HDAC1 levels were observed from synchronous normal epithelium to ductal carcinoma in situ (DCIS) in ER-negative compared with ER-positive, and in high-grade compared with non-high-grade tumors [24]. In ER- and PR-positive breast cancer cells, the deletion of HDAC2 gene was detected by Hu et al. [25]. In 2011, Rey et al. identified HDAC6 as a critical component of the invasive apparatus of MDA-MB-231 breast cancer cells, in both two- and three-dimensional matrices [26]. In 2012, Ververis et al. indicate higher expression of Class I histone deacetylases compared to Class II enzymes in breast cancer tissue [27]. In 2013, Müller et al. found different expression of

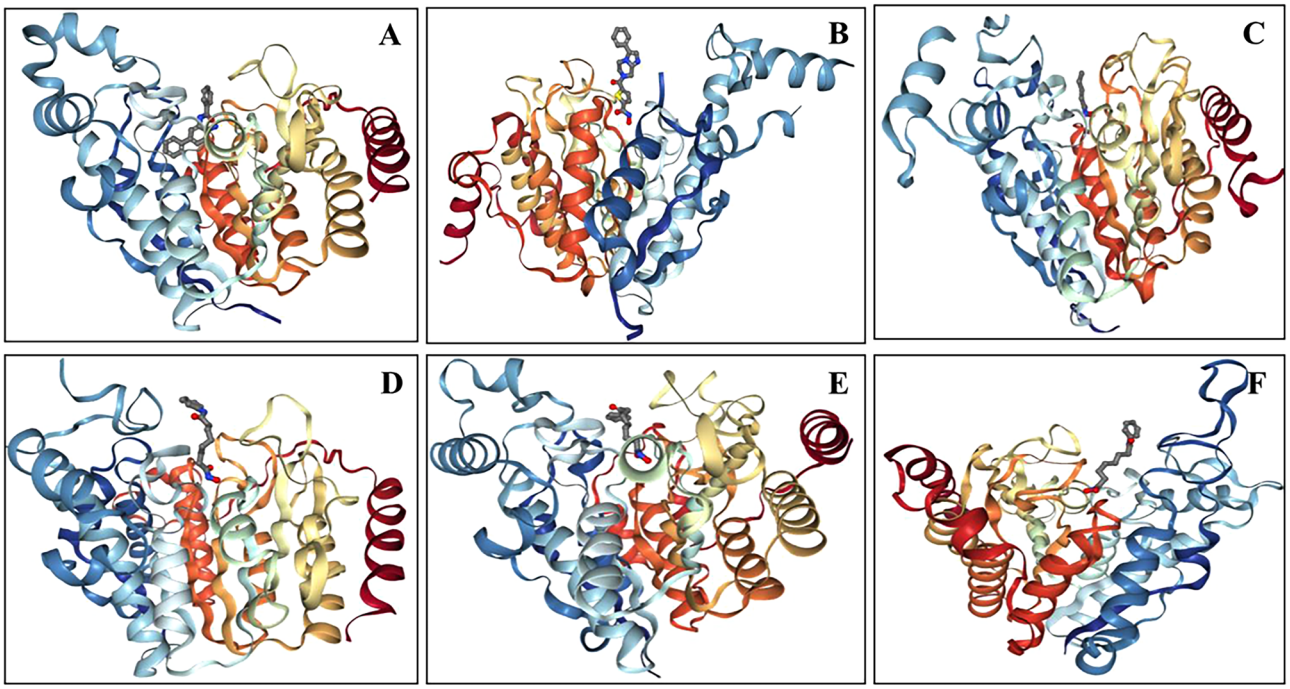

Fig. 2. The crystal structure of representative HDACs. (A) Crystal Structure of Human HDAC2 complexed with an N-(2-aminophenyl)benzamide; (B) Structure of HDAC4 catalytic domain bound to a hydroxamic acid inhibitor; (C) Crystal structure of catalytic domain of human histone deacetylase HDAC7 in complex with SAHA (Vorinostat); (D) Crystal Structure of human HDAC8 complexed with SAHA; (E) Crystal Structure of HDLP complexed with SAHA; (F) Crystal structure of HDAH with SAHA bound.

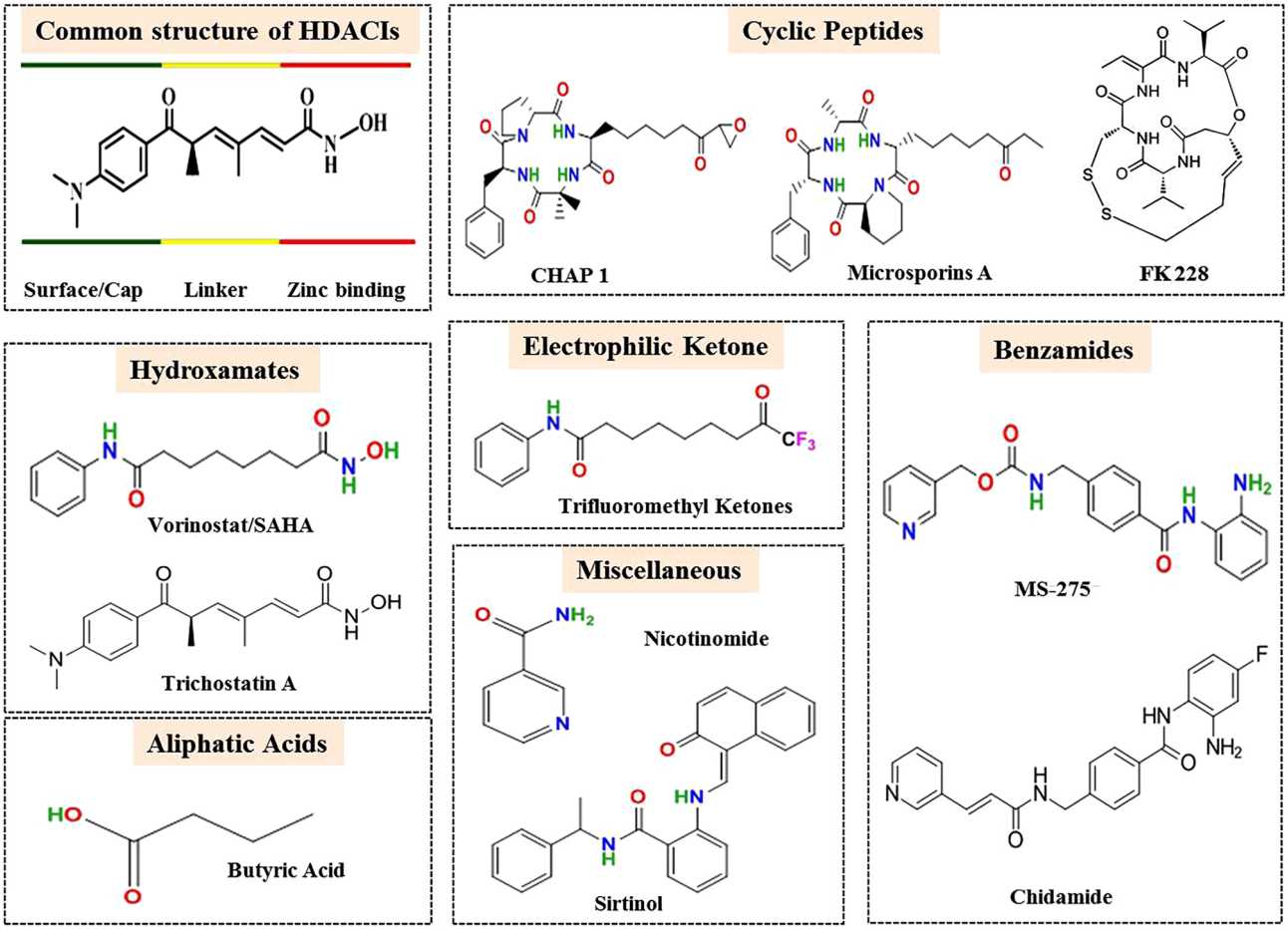

Fig. 3. Classification of HDACIs and the representative compounds.

class-1 HDAC isoenzymes 1, 2 and 3 in breast cancer. HDAC2 and HDAC3 were strongly expressed in subgroups of tumor with features of a more aggressive tumor type [28]. Hence, HDACs are crucial for breast cancer pathogenesis and progression, providing novel targets for breast cancer treatment.

4. Classification, structure–activity relationship (SAR) and quantitative structure–activity relationship (QSAR) of HDACIs

As HDACs-targeting inhibitors, HDACIs (histone deacetylase inhibitors) can enhance the acetylation of cellular proteins by blocking HDAC activity. Structurally, HDACIs are composed of a zinc binding group (ZBG), which chelates the zinc ion and engages in hydrogen bonds at the active site; a cap group, serving as a surface recognition motif; a hydrophobic cavity-binding linker region interacting with the HDAC substrate channel [29]. Based on the key function of the zinc catalytic domain, HDACIs are divided into several classes: hydroxamic acids [e.g. suberoylanilide hydroxamic acid (SAHA), Trichostatin A], cyclic peptides (e.g. CHAP 1, FK228), benzamides (e.g. MS-275, Chidamide), electrophilic ketones (e.g. trifluoromethyl ketones), miscellaneous and aliphatic acid compounds (e.g. butyric acid) (Fig. 3). In addition, HDACIs can also be classified according to their specificity for HDACs subtypes or classes: Class I HDACIs, Class IIa HDACIs, Class IIb HDACIs, Class III HDACIs, and Class IV HDACIs. For instance, SAHA is a pan-HDAC inhibitor and MS-275 is a class I HDACs inhibitor. Taking the crystal structure of HDLP in complex with SAHA as an example, the interaction between HDACs and HDACs inhibitors could be explained (Fig. 4). Visually, the active pocket seems like a funnel, containing a large cap region, a deep channel and a cavity (Fig. 4A). The capping group interacts with protein residues Asn-20, Tyr-91, Glu 92 and Pro-22 of the opposite molecule, forming an extensive hydrophobic sandwich. The linker interacts with residues Gly-140, Phe-141, Phe-198, and Tyr-297. In zinc binding region, residues Asp-168, His-131, and His-132 (Fig. 4B–D).

Several existing models of structure–activity relationship (SAR) and quantitative structure–activity relationship (QSAR) of the HDACIs—as well as their interaction with the HDACs from the data obtained by docking and molecular dynamics—have been reported to identify and optimize HDACIs. In 2004, Xie et al. reported a comprehensive quantitative structure–activity relationship (QSAR) study of HDACIs in the hope of identifying the structural determinants for anticancer activity. Finally, a highly predictive QSAR model with R2 of 0.76 and leave-one-out cross-validated R2 of 0.73 was obtained. The overall rate of cross-validated correct prediction of the classification model is around 92% [30]. In 2006, Ragno et al. showed the first 3D QSAR application on a broad molecular diversity training set of HDACIs. The results of 3D QSAR and docking studies validated each other and provided insight into the structural requirements for anti-HDAC activity [31]. In 2009, Tang et al. illustrates the power of the combined QSAR-VS (virtual screening) method as a general approach for the effective identification of structurally novel HDACIs [32]. In 2016, Choubey et al. produced a statistically significant QSAR model with a high predictive power with r^2 (o) value of 0.88 and r^2 (m) value of 0.58 to carry out further in silico studies [33]. In 2017, Abdizadeh demonstrated that both CoMFA (q2, 0.663; rncv 2, 0.909) and CoMSIA models (q2, 0.628; rncv 2, 0.877) showed a good predictive ability in both internal and external validation, which could be used for designing new biaryl benzamides as potent HDAC1 inhibitors in cancer treatment [34]. Pham-The et al.

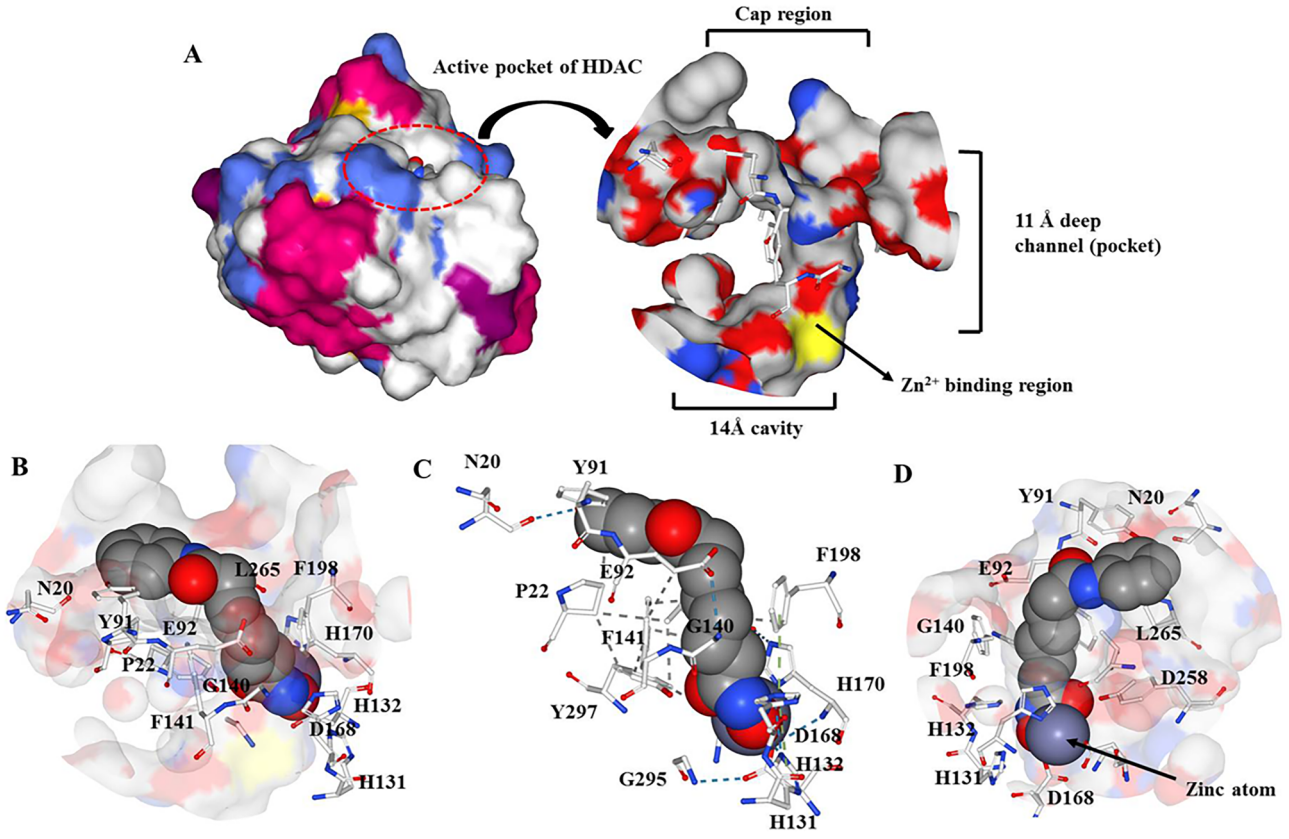

Fig. 4. Interaction between HDACs and HDACs inhibitors (taking HDLP in complex with SAHA as an example). (A) The active pocket of HDLP. The pocket seems like a funnel, containing a large cap region, a deep channel and a cavity. (B-D) Interaction between HDLP and SAHA. The capping group interacts with protein residues Asn-20, Tyr-91, Glu 92 and Pro-22 of the opposite molecule, forming an extensive hydrophobic sandwich. The linker interacts with residues Gly-140, Phe-141, Phe-198, and Tyr-297. In zinc binding region, residues Asp-168, His-131, and His-132.

developed a ML-based QSAR model with global accuracy in the external set ranging from 0.83 to 0.90 and screened potent, isoform-selective HDACIs [35]. In 2018, Shi et al. predict several HDAC1 inhibitors using computational QSAR model combined molecular descriptors and fingerprints. The best predicting model was CDK fingerprint with support vector machine, which exhibited an accuracy of 0.89 [36]. Adhikari proved that selective benzamide-based HDAC3 inhibitors might be potential validated weapon to cancers, through comparative SAR/QSAR/QAAR approaches [37]. Above all, the QSAR and classification models were significant to provide direct guidance for HDACIs development.

5. Pharmacokinetic and toxicological properties of the HDACIs

As anti-breast cancer agents, it is important to disclose the pharmacokinetic and toxicological properties of the HDACIs. In general, HDACs inhibition by HDACIs leads to inhibition of tumor growth, and apoptosis of cancer cells, whereas normal tissue is not particularly affected [38]. The biology of HDACIs transport is relevant to physiological and pharmacological benefits [39]. In 2013, Wilson et al. evaluated the effectiveness of HDACIs vorinostat and panobinostat in a dose- and exposure-dependent manner in order to better understand the dynamics of drug action and antitumor efficacy. Results indicated that although HDACIs exert both potent growth inhibition and cytotoxic effects, cancer cells could demonstrate an inherent ability to survive HDACI concentrations and exposure times that exceed those clinically achievable [40]. In 2015, Pilon et al. improved pharmacokinetic parameters of class I HDACIs using IV bolus delivery at 5 mg/kg [41]. As the first oral subtype-selective HDACI approved in China, twice-weekly chidamide monotherapy has been recommended based on its tolerable toxicity. But its clinical efficacy could be further increased by combination with multidrug chemotherapy or chemo-free regimens [42]. Cai et al. found that interactions between HDACI MGCD0103 and other drugs would increase the risk of either diminished efficacy or adverse effects [43]. Recently, various zinc binding groups have been designed and tested to improve the activity and selectivity of HDACIs, and to overcome the pharmacokinetic limitations of current HDACIs [44]. Wide application of novel zinc binding groups in the HDACIs design will contribute to the emergence of new HDACIs. In addition, building a pharmacokinetic/pharmacodynamic (PK/PD) model was also important for early understanding of toxicities and pharmacokinetic determination [45].

6. HDACs inhibitors for breast cancer treatment

Clinical trials using HDACIs have been performed and their results indicate that HDACIs have antitumor activity and may be clinically beneficial. Several HDACIs have been approved by the US FDA (Food and Drug Administration) for the treatment of several cancers, such as cutaneous *T*-cell lymphoma (CTCL), peripheral T cell lymphoma (PTCL) and multiple myeloma (MM). However, no HDACI has been approved by the US FDA for breast cancer treatment. So far, 62 clinical studies have been registered in ClinicalTrials.gov for breast cancer treatment (Table 2). Among them, three phase III studies were designed to explore the efficacy of HDACIs (entinostat and chidamide) when combined with endocrine therapy for advanced breast cancer. Sixteen phase II studies were conducted to observe the effects of HDACIs monotherapy or combination chemotherapy on breast cancer. The remaining studies are under phase I stage. Entinostat (MS-275), vorinostat (SAHA), and panobinostat (LBH-589) are inhibitors that commonly used in clinical

Table 2
The registered clinical trials for breast cancer treatment using HDACIs.

NO	NCT Number	Title	Interventions	Phases	Enrollment	Sponsor/Collaborators	Status	Outcome Measures
1	NCT00993642	ERB-B4 After Treatment With HDAC Inhibitor in ER + Tamoxifen Refractory Breast Cancer	Panobinostat (LBH589)	Early Phase 1	0	Tulane University Health Sciences Center; Novartis; Board of Regents, State of Louisiana; Tulane University	Withdrawn	Expression of ERBB4 pre- and post- treatment with LBH589
2	NCT00719875	HDAC Inhibitor Vorinostat (SAHA) With Capecitabine (Xeloda) Using a New Weekly Dose Regimen for Advanced Breast Cancer	Vorinostat	Phase 1	24	Yale University; Merck Sharp & Dohme Corp.	Completed	Safety, dose-limiting toxicities and maximum tolerated dose of oral capecitabine in combination with oral vorinostat; the clinical benefit, time to progression, and duration of response
3	NCT02833155	Entinostat in Chinese Postmenopausal Women Patients With Locally Recurrent or Metastatic Breast Cancer	Entinostat; Exemestane	Phase 1	18	EddingPharm Oncology Co., LTD.	Recruiting	Adverse events, 12-lead ECG, blood pressure/ pulse, temperature, laboratory parameters and physical examination
4	NCT00574587	Trial for Locally Advanced Her2 Positive Breast Cancer Using Paclitaxel, Trastuzumab, Doxorubicin and Cyclophasmide on a Weekly Basis	Vorinostat; Paclitaxel; Trastuzumab; Doxorubicin; Cyclophosphamide	Phase 1; Phase 2	54	Albert Einstein College of Medicine; Merck Sharp & Dohme Corp.	Unknown status	Recommended phase II dose of vorinostat in combination with weekly paclitaxel/ trastuzumab; pCR rate
5	NCT02393794	Cisplatin Plus Romidepsin & Nivolumab in Locally Recurrent or Metastatic Triple Negative Breast Cancer (TNBC)	Romidepsin; Cisplatin; Nivolumab	Phase 1; Phase 2	54	Priyanka Sharma; Celgene Corporation; Bristol-Myers Squibb; University of Kansas Medical Center	Recruiting	Phase I: Recommended Phase II Dose of romidepsin in combination with cisplatin; Phase II: Objective response rate, Clinical Benefit Rate, Pharmacokinetics, Median Progression-Free Survival and Overall Survival
6	NCT00395655	Hydralazine and Valproate Added to Chemotherapy for Breast Cancer	Hydralazine; magnesium valproate	Phase 2	43	National Institute of Cancer; National Council of Science and Technology, Mexico; Psicofarma S.A. de C.V.	Terminated	Global DNA methylation; Histone Deacetylase Activity; Global gene expression; Pathological response; Hydralazine plasma levels; Valproic acid plasma levels
7	NCT03742245	Olaparib in Combination With Vorinostat in Patients With Relapsed/Refractory and/or Metastatic Breast Cancer	Olaparib; Vorinostat	Phase 1	28	Jenny C. Chang, MD; AstraZeneca; The Methodist Hospital System	Not yet recruiting	MTD; Dose-limiting toxicities (DLTs) and other adverse events; Recommended Phase 2 dose (RP2D); Antitumor activity
8	NCT02569489	Dose Escalation Study of HBI-8000 in Combination With Paclitaxel and Trastuzumab in Women With Advanced or Metastatic HER2 + Breast Cancer	HBI-8000; Trastuzumab; Paclitaxel	Phase 1	0	HUYA Bioscience International	Withdrawn	MTD
9	NCT01194908	Re-expression of ER in Triple Negative Breast Cancers	Decitabine; LBH589; Tamoxifen	Phase 1; Phase 2	5	Emory University; Novartis; Eisai Inc	Terminated	Maximum Tolerated Dose; the Safety of Tamoxifen in Combination With Decitabine and LBH589
10	NCT02820961	Drug-Drug Interaction Study of Entinostat and Exemestane in Postmenopausal Women With ER + Breast Cancer	Entinostat; exemestane	Phase 1	40	Syndax Pharmaceuticals	Active, not recruiting	Cmax, maximum plasma concentration; Tmax, time at which maximum plasma concentration; adverse events
11	NCT01349959	Azacitidine and Entinostat in Treating Patients With Advanced Breast Cancer	Azacitidine; Entinostat	Phase 2	40	National Cancer Institute (NCI)	Completed	Confirmed Response Rate; Clinical Benefit Rate; Progression-free Survival
12	NCT01007695	Molecular Signature of Valproic Acid in Breast Cancer With Functional Imaging Assessment - a Pilot	Valproic Acid	Phase 1	31	University of Utah	Terminated	Sensitivity and specificity of the genomic sensitivity signature of VPA (GDSS-VPA); the change in immunohistochemical markers of proliferation, apoptosis, and tumor grade following treatment with valproic acid in newly diagnosed breast cancer; dose-limiting toxicities for valproic acid
13	NCT02115594	Phase 2 Study of Fulvestrant With and Without Entinostat in Postmenopausal Women With ER + Advanced Breast Cancer	Fulvestrant; Entinostat; Placebo	Phase 2	0	Syndax Pharmaceuticals	Withdrawn	Progression Free Survival; Objective Response Rate (CR or PR); Clinical Benefit Rate; Overall Survival; safety parameters
14	NCT00567879	A Trial of Panobinostat and Trastuzumab for Adult Female Patients With HER2 Positive Metastatic Breast Cancer (MBC) Whose Disease Has Progressed on or After Trastuzumab	Panobinostat; Trastuzumab	Phase 1; Phase 2	56	Novartis Pharmaceuticals; Novartis	Terminated	Dose Limiting Toxicities (DLTs); Overall Response
15	NCT01434303	Entinostat, Lapatinib Ditosylate and Trastuzumab in Treating Patients With Locally Recurrent or	Entinostat; Lapatinib	Phase 1	37	National Cancer Institute (NCI)	Active, not recruiting	Incidence of grade III or IV toxicities

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Table 2 (*continued*)

NO	NCT Number	Title	Interventions	Phases	Enrollment	Sponsor/Collaborators	Status	Outcome Measures
16	NCT02708680	Distant Relapsed Metastatic Breast Cancer Previously Treated With Trastuzumab Only Randomized Phase 2 Study of Atezolizumab and Entinostat in Patients With aTN Breast Cancer With Phase 1b Lead In	Entinostat; atezolizumab; Placebo	Phase 1; Phase 2	88	Syndax Pharmaceuticals; Roche Pharma AG	Active, not recruiting	DLT and the MTD and/or RP2D; Progression-free survival; Overall response rate (ORR); Clinical benefit rate (CBR); Overall survival (OS); Duration of response (DOR); Time to response (TTR)
17	NCT00788931	A Trial l of Panobinostat Given in Combination With Trastuzumab and Paclitaxel in Adult Female Patients With HER2 Positive Metastatic Breast Cancer	LBH589; trastuzumab; paclitaxel	Phase 1	15	Novartis Pharmaceuticals; Novartis	Completed	The maximum tolerated dose; Safety and tolerability
18	NCT02453620	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	Entinostat	Phase 1	45	National Cancer Institute (NCI)	Recruiting	Adverse events; Changes in ratio of effector T cell (Teff) to regulatory T cell (Treg); Objective response rate; Progression-free survival (PFS); Duration of overall response, stable disease
19	NCT02115282	Exemestane With or Without Entinostat in Treating Patients With Recurrent Hormone Receptor-Positive Breast Cancer That is Locally Advanced or Metastatic	Entinostat; Exemestane; Goserelin Acetate	Phase 3	600	National Cancer Institute (NCI)	Active, not recruiting	Progression-free survival (PFS); Overall survival (OS); Objective response; Incidence of toxicity; Time to treatment deterioration (TTD)
20	NCT00843167	Broccoli Sprout Extract in Treating Women Who Have Had a Mammogram and Breast Biopsy	Broccoli sprout extract; placebo	Phase 2	54	OHSU Knight Cancer Institute; National Cancer Institute (NCI)	Completed	Change in Isothiocyanate, Ki-67, Histone Deacetylase (HDAC) Activity; Treatment Compliance
21	NCT01695057	Vorinostat Before Surgery in Treating Patients With Triple-Negative Breast Cancer	Vorinostat	Not Applicable	0	University of Southern California; National Cancer Institute (NCI)	Withdrawn	Combined PR/ER response; Grade 3 or 4 toxicities
22	NCT00754312	A Phase I, Multicenter, Open Label Study on the Effects of SNDX-275 on Expression of Biomarkers in Subjects With Newly Diagnosed Breast Cancer	SNDX-275	Phase 1	30	Syndax Pharmaceuticals	Withdrawn	Targeted biomarkers; safety and tolerability of SNDX-275
23	NCT00416130	Clinical Trial of SAHA in Patients With Breast Cancer	Vorinostat	Phase 1; Phase 2	49	National University Hospital, Singapore; Merck Sharp & Dohme Corp.	Unknown status	Clinical laboratory tests; Vital signs; Electrocardiograms; Vorinostat concentration in serum samples; Level of histone H3 acetylation; Known functional single nucleotide polymorphisms; Baseline plasma protein profiles and changes in response to chemotherapy
24	NCT00777049	Study of Panobinostat Monotherapy in Women With HER2-negative Locally Recurrent or Metastatic Breast Cancer	Panobinostat	Phase 2	54	Translational Research in Oncology	Completed	Objective Response Rate
25	NCT00777335	Study of Panobinostat Monotherapy in Women With v-ERB-B2 Avian Erythroblastic Leukemia Viral Oncogene Homolog 2 (HER2) Positive Locally Recurrent or Metastatic Breast Cancer	Panobinostat - LBH589	Phase 2	4	Translational Research in Oncology	Terminated	Overall Response (OR) Rate
26	NCT00132002	Suberoylanilide Hydroxamic Acid in Treating Patients With Progressive Stage IV Breast Cancer	Vorinostat	Phase 2	14	National Cancer Institute (NCI)	Terminated	Objective Tumor Response Rate; Overall Survival; Progression-free Survival
27	NCT00262834	Vorinostat in Treating Women Who Are Undergoing Surgery For Newly Diagnosed Stage I -III Breast Cancer	Vorinostat	Not Applicable	54	National Cancer Institute (NCI)	Completed	Adverse Events; Change in Tissue Proliferation, apoptosis, tissue Histone Acetylation
28	NCT03361800	Window of Opportunity Trial of Entinostat in Patients With Newly Diagnosed Stage I-IIIC, TNBC	Entinostat	Early Phase 1	20	UNC Lineberger Comprehensive Cancer Center; Syndax Pharmaceuticals; National Cancer Institute (NCI)	Recruiting	Decrease in Ki-67 mRNA, proliferation signature; adverse events
29	NCT00788112	Vorinostat in Treating Women With Ductal Carcinoma in Situ of the Breast	Vorinostat	Phase 1	17	University of California, San Francisco; National Cancer Institute (NCI)	Completed	Reduction in Ki-67; Changes in HDAC1 and HDAC6 expression and histone H4
30	NCT02897778	Cardiac Safety Study of Entinostat in Men and Women With Advanced Solid Tumors	Entinostat; Placebo	Phase 1	37	Syndax Pharmaceuticals	Completed	

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Table 2 (*continued*)

NO	NCT Number	Title	Interventions	Phases	Enrollment	Sponsor/Collaborators	Status	Outcome Measures
								Relationship between entinostat plasma concentrations and placebo controlled change from baseline QTc; Cmax
31	NCT01118975	GCC 0845:Vorinostat and Lapatinib in Advanced Solid Tumors and Advanced Breast Cancer to Evaluate Response and Biomarkers	Vorinostat; Lapatinib	Phase 1; Phase 2	12	University of Maryland; University of Maryland Greenebaum Cancer Center	Terminated	Dose Limiting Toxicities; Clinical Benefit Rate
32	NCT02632071	ACY-1215 + Nab-paclitaxel in Metastatic Breast Cancer	ACY-1215; Nab-paclitaxel	Phase 1	24	Kevin Kalinsky; Acetylon Pharmaceuticals Incorporated; National Cancer Institute (NCI); Columbia University	Recruiting	Maximum tolerated dose (MTD) of ACY-1215 (Ricolinostat); adverse events
33	NCT00258349	Vorinostat and Trastuzumab in Treating Patients With Metastatic or Locally Recurrent Breast Cancer	Vorinostat; Trastuzumab	Phase 1; Phase 2	16	National Cancer Institute (NCI)	Completed	Response Rate; Time to Progression|Overall Survival
34	NCT03473639	A Pilot Study of the Combination of Entinostat With Capecitabine in High Risk Breast Cancer After Neoadjuvant Therapy	Entinostat; Capecitabine	Phase 1	55	University of Virginia; Syndax Pharmaceuticals	Recruiting	Maximum tolerated dose; adverse events
35	NCT00511576	Study to Evaluate Combination Treatment of MGCD0103 and Docetaxel (Taxotere®) for Subjects With Advanced Cancer Tumors	MGCD0103; Docetaxel	Phase 1	54	Mirati Therapeutics Inc.	Terminated	Maximum tolerated dose (MTD), dose limiting toxicities (DLTs), and safety profile; plasma pharmacokinetics (PK) of MGCD0103; overall tumor response
36	NCT00020579	MS-275 in Treating Patients With Advanced Solid Tumors or Lymphoma	Entinostat	Phase 1	75	National Institutes of Health Clinical Center (CC); National Cancer Institute (NCI)	Completed	Dose-limiting toxicities and maximum tolerated dose; Pharmacology and pharmacokinetics; Acetylation of histones in peripheral blood; Tumor response
37	NCT00828854	A Phase 2, Multicenter Study of the Effect of the Addition of SNDX-275 to Continued Aromatase Inhibitor (AI) Therapy in Postmenopausal Women With ER + Breast Cancer Whose Disease is Progressing	Entinostat (SNDX-275)	Phase 2	25	Syndax Pharmaceuticals	Completed	Clinical benefit rate (CBR); Progression free survival (PFS); Objective response rate (ORR)
38	NCT00365599	Phase II Trial of SAHA & Tamoxifen for Patients With Breast Cancer	SAHA; tamoxifen	Phase 2	43	H. Lee Moffitt Cancer Center and Research Institute; Merck Sharp & Dohme Corp.	Completed	Objective Response (OR); Time to Progression (TTP); Adverse Events
39	NCT01105312	Panobinostat and Letrozole in Treating Patients With Metastatic Breast Cancer	Letrozole; panobinostat	Phase 1; Phase 2	28	Alliance for Clinical Trials in Oncology; National Cancer Institute (NCI)	Completed	Maximum-tolerated Dose (Phase I); Response Rate; Survival Time (Phase II); Time-to-disease Progression (Phase II); Progression-free Survival (Phase II); Duration of Response (Phase II); Clinical Benefit Rate; Time to Treatment Failure; Confirmed Response Rate (Phase I)
40	NCT03538171	Entinostat Versus Placebo Combined With Endocrine Therapy in Chinese Patients With Advanced Breast Cancer in Chinese Patients With Hormone Receptor-Positive Advanced Breast Cancer	Entinostat; Placebo; Exemestane	Phase 3	512	EddingPharm Oncology Co., LTD.	Recruiting	Cmax; Tmax; PFS; OS; Preliminary efficacy (PFS); TTD; CBR
41	NCT00616967	Carboplatin and Nab-Paclitaxel With or Without Vorinostat in Treating Women With Newly Diagnosed Operable Breast Cancer	Carboplatin; paclitaxel; vorinostat; placebo	Phase 2	68	Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins; National Cancer Institute (NCI)	Completed	Pathological Complete Response (pCR) Rate; Safety; Clinical Complete Response (cCR) Rate
42	NCT03291886	Phase 2 Study of KHK2375 in Subjects With Advanced or Recurrent Breast Cancer	Entinostat; Entinostat; Exemestane	Phase 2	124	Kyowa Hakko Kirin Co., Ltd	Active, not recruiting	Progression Free Survival(PFS); Overall survival (OS)|Antitumor effect
43	NCT01084057	Ixabepilone and Vorinostat in Treating Patients With Metastatic Breast Cancer	Vorinostat; ixabepilone	Phase 1	56	City of Hope Medical Center	Active, not recruiting	Dose limiting toxicity; Objective response rate and/or clinical benefit rate; Toxicity profile
44	NCT00676663	Study to Evaluate Exemestane With and Without SNDX-275 in Treatment of Postmenopausal Women With Advanced Breast Cancer	Entinostat; exemestane	Phase 2	130	Syndax Pharmaceuticals	Completed	Progression free survival (PFS); compare objective response rate (ORR) and clinical benefit rate (CBR); safety and tolerability of entinostat
45	NCT00632489			Phase 1	20		Completed	

(*continued on next page*)

Table 2 (*continued*)

NO	NCT Number	Title	Interventions	Phases	Enrollment	Sponsor/Collaborators	Status	Outcome Measures
		LBH589 in Combination With Capecitabine Plus/Minus (⊠) Lapatinib in Breast Cancer Patients	LBH589; Capecitabine; Lapatinib			SCRI Development Innovations, LLC; Novartis		Maximum Tolerated Doses (MTD) and Dose-limiting Toxicities (DLT) of LBH589; Antitumor Activity of LBH589
46	NCT01194427	A Study of Vorinostat and Tamoxifen in Newly Diagnosed Breast Cancer	Vorinostat; Tamoxifen	Phase 2	2	Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins; Merck Sharp & Dohme Corp.	Terminated	Changes in Markers of Proliferation
47	NCT00368875	Phase I-II Study of Vorinostat, Paclitaxel, and Bevacizumab in Metastatic Breast Cancer	Vorinostat; paclitaxel	Phase 1; Phase 2	54	National Cancer Institute (NCI)	Completed	Recommended Phase II Dose; Objective Response Rate (CR + PR); Progression-free Survival (PFS); Time to Treatment Failure (TTF); Overall Survival(OS)
48	NCT01153672	Vorinostat in Treating Patients With Stage IV Breast Cancer Receiving Aromatase Inhibitor Therapy	Vorinostat; anastrozole; letrozole; exemestane	Not Applicable	8	University of Washington; National Cancer Institute (NCI)	Completed	Rate of Clinical Benefit; Duration of Response; Progression-free Survival; Overall Survival; Adverse Events
49	NCT01720602	Vorinostat in Treating Patients With Stage IV Breast Cancer Receiving Hormone Therapy	Vorinostat; anastrozole; letrozole; exemestane	Not Applicable	15	University of Washington; National Cancer Institute (NCI)	Active, not recruiting	Clinical Benefit; Response Rate; Progression-free Survival (PFS); Overall Survival
50	NCT01594398	Study to Assess Food Effect on Pharmacokinetics of Entinostat in Subjects With Breast Cancer or Non-Small Cell Lung Cancer	Entinostat; Erlotinib; Exemestane	Phase 1	14	Syndax Pharmaceuticals	Completed	Difference in pharmacokinetics of entinostat; Change in laboratory values; Change in ECG; Difference in pharmacodynamics from baseline; Adverse events
51	NCT00126451	A Clinical Trial of Oral Suberoylanilide Hydroxamic Acid (SAHA) in Patients With Relapsed or Refractory Breast, Colorectal and Non-Small Cell Lung Cancer (0683–011)(TERMINATED)	MK0683; vorinostat; SAHA	Phase 2	16	Merck Sharp & Dohme Corp.	Terminated	Response rate; Positron emission tomography (PET); safety and tolerability of SAHA
52	NCT02395627	Reversing Therapy Resistance With Epigenetic-Immune Modification	Tamoxifen; Vorinostat; pembrolizumab	Phase 2	38	Pamela Munster; University of California, San Francisco	Active, not recruiting	Overall Response Rate; Adverse Events; Progression Free Survival; Median Progression Free Survival; Overall Survival; Tumor Responses; Response of PD-L1 expression
53	NCT01234532	Entinostat and Anastrozole in Treating Postmenopausal Women With TNBC That Can Be Removed by Surgery	Entinostat; anastrozole	Phase 2	5	University of Maryland; Syndax Pharmaceuticals	Terminated	Recommended phase II dose of entinostat; Safety and tolerability; Change in Ki67, ER, PR, HER2, EGFR, CK5/6, aromatase, tissue histone H3 and H4 acetylation; Response to entinostat and anastrozole
54	NCT02890069	A Study of PDR001 in Combination With LCL161, Everolimus or Panobinostat	PDR001; LCL161; Everolimus; Panobinostat; QBM076; HDM201	Phase 1	350	Novartis Pharmaceuticals	Recruiting	Dose limiting toxicities (DLTs); adverse events (AEs); Dose intensities; Best overall response (BOR)
55	NCT03432741	Direct Tumor Microinjection and FDG-PET in Testing Drug Sensitivity in Patients With Relapsed or Refractory Non-Hodgkin Lymphoma, Hodgkin Lymphoma, or Stage IV Breast Cancer	Belinostat; Carfilzomib; F-18; Gemcitabine Hydrochloride	Phase 1	26	Mayo Clinic; National Cancer Institute (NCI)	Recruiting	Incidence of drug sensitivity; Cutaneous response rate; adverse events; Nodal disease response rate
56	NCT02307240	Open Label, Multi-center Study to Assess the Safety, Tolerability and Pharmacokinetics of CUDC-907 in Subjects With Advanced/Relapsed Solid Tumors	CUDC-907	Phase 1	60	Curis, Inc.	Active, not recruiting	Safety and tolerability of oral CUDC-907; pharmacokinetics (PK) of CUDC-907; biomarkers of CUDC-907 activity; anti-cancer activity of CUDC-907
57	NCT01249443	Paclitaxel and Carboplatin in Treating Patients With Metastatic or Recurrent Solid Tumors and HIV Infection	Vorinostat; carboplatin; paclitaxel	Phase 1	17	AIDS Malignancy Consortium; National Cancer Institute (NCI); The EMMES Corporation|University of Arkansas	Terminated	Adverse events; Response rates; Effects of therapy
58	NCT00838929	Study of the Combination of Vorinostat and Radiation Therapy for the Treatment of Patients With Brain Metastases	Vorinostat	Phase 1	17	Sidney Kimmel Cancer Center at Thomas Jefferson University; Merck Sharp & Dohme Corp.; Thomas Jefferson University	Completed	Maximum Tolerated Dose (MTD) and Recommended Phase II Dose (RP2D) of Vorinostat
59	NCT00045006	Suberoylanilide Hydroxamic Acid in Treating Patients With Advanced Cancer	Vorinostat	Phase 1	null	Memorial Sloan Kettering Cancer Center; National Cancer Institute (NCI)	Completed	
60	NCT00413075	Study of Oral PXD101 in Patients With Advanced Solid Tumors or Lymphoma	Oral belinostat	Phase 1	121	Onxeo; Spectrum Pharmaceuticals, Inc	Completed	Safety, tolerability and maximum tolerated dose of orally administered PXD101;

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Table 2 (*continued*)

NO	NCT Number	Title	Interventions	Phases	Enrollment	Sponsor/Collaborators	Status	Outcome Measures
								pharmacokinetics; anti-tumor activity; safety, tolerability, and anti-tumor activity
61	NCT00413322	Study of PXD101 Alone and in Combination With 5-Fluorouracil (5-FU) in Patients With Advanced Solid Tumors	Belinostat; 5-FU	Phase 1	35	Onxeo	Completed	Maximum tolerated dose of PXD101; down-regulation of thymidylate synthase
62	NCT02482753	A Phase III Trial of Chidamide in Combination With Exemestane in Patients With Hormone Receptor-Positive Advanced Breast Cancer	Chidamide; Exemestane; placebo	Phase 3	365	Chipscreen Biosciences, Ltd	Active, not recruiting	progression-free survival (PFS); pharmacokinetic profiles of chidamide and exemestane; acetylation level of histone H3; OS; duration of response (DOR); objective response rate (ORR); clinical benefit rate (CBR)

research. Considering the distribution of the registered studies, most of them are located in the USA (Fig. 5). Specifically, in China, chidamide is a representative HDACI applied for breast cancer treatment and has shown promising efficacy. Here we reviewed the progress of these representative inhibitors in breast cancer research: entinostat, vorinostat, panobinostat and chidamide.

6.1. Entinostat for breast cancer treatment

Entinostat (MS-275), a synthetic benzamide derivative HDACI with oral bioavailability, potently and selectively inhibits class I and IV HDAC enzymes. The pharmacokinetics of entinostat were linear over dosages ranging from 2 to 12 mg/m^2. Meanwhile, entinostat has a long half-life ranging from an average of 33–150 h [46]. Preclinical experiments in vitro and in vivo have demonstrated promising antitumor activity of entinostat in lung cancer, prostate cancer, breast cancer, pancreatic cancer, among other [47]. For breast cancer, entinostat was shown to increase ER expression and induce the re-expression of ER-α, androgen receptor and the aromatase enzyme, providing a strong rationale for the combination of HDAC and aromatase inhibitors to treat ER-negative and endocrine-resistant breast cancers [48]. In 2013, the combination of entinostat and letrozole was indicated to restore responsiveness of letrozole-resistant MCF-7Ca xenografts to aromatase inhibitors through modulation of Her-2, compared with treatment with either agent alone [49]. Moreover, entinostat was also found to enhance anti-tumor efficacy in combination with HER2-targeted agents, which prompted the authors to conduct a clinical trial of entinostat in combination with lapatinib, and trastuzumab in patients with HER2-over-expressing breast cancer resistant to trastuzumab-based treatment [50]. Hence, addition of epigenetic therapy may be an effective approach to targeting resistance pathways in breast cancer.

However, entinostat has not been approved for clinical use by the US FDA, in spite of the promising studies that have been conducted [51]. A number of Phase I and II combination trials using one or more drugs which operate through modifying epigenetic strategies are being investigated (Table 1). For example, in 2013, a randomized phase II, double-blind, placebo-controlled study evaluating entinostat combined with the aromatase inhibitor exemestane versus exemestane alone (ENCORE301 trial, NCT00676663) was conducted. Results showed that entinostat added to exemestane was generally well tolerated in patients with ER + advanced breast cancer and acetylation changes may provide an opportunity to maximize clinical benefit with entinostat [52]. Based on the outcome, in 2018, Yeruva SLH et al. reported the protocol of a phase III registration trial (E2112 trial, NCT02115282), investigating the efficacy of entinostat/placebo in combination with exemestane. This study will validate the preclinical and clinical findings supporting the role of HDAC inhibitors in overcoming resistance to endocrine therapy in breast cancer [53]. In 2018, a randomized phase III trial was also registered to evaluate the clinical benefit of the addition of entinostat to exemestane among Chinese patients with HR-positive, Her-2-negative locally advanced or metastatic breast cancer who have previously progressed on a non-steroidal aromatase inhibitor (AI). Identification and treatment of the right patients who may benefit from these agents may not be in the too distant future.

6.2. Vorinostat for breast cancer treatment

SAHA (vorinostat) is one of the most widely studied small-molecule pan-HDAC inhibitors, approved by the US Food and Drug Administration in October 2006 for the treatment of cutaneous T-cell lymphoma [54]. SAHA inhibits the activity of 11 known human class I and class II HDACs, reducing histone acetylation and modifying downstream effects on apoptotic pathways. Preclinical data on various breast cancer cell lines have shown that SAHA induces cell-cycle phase arrest and apoptosis at low micromolar or sub-micromolar concentrations. For triple-negative breast cancer, a heterogeneous disease

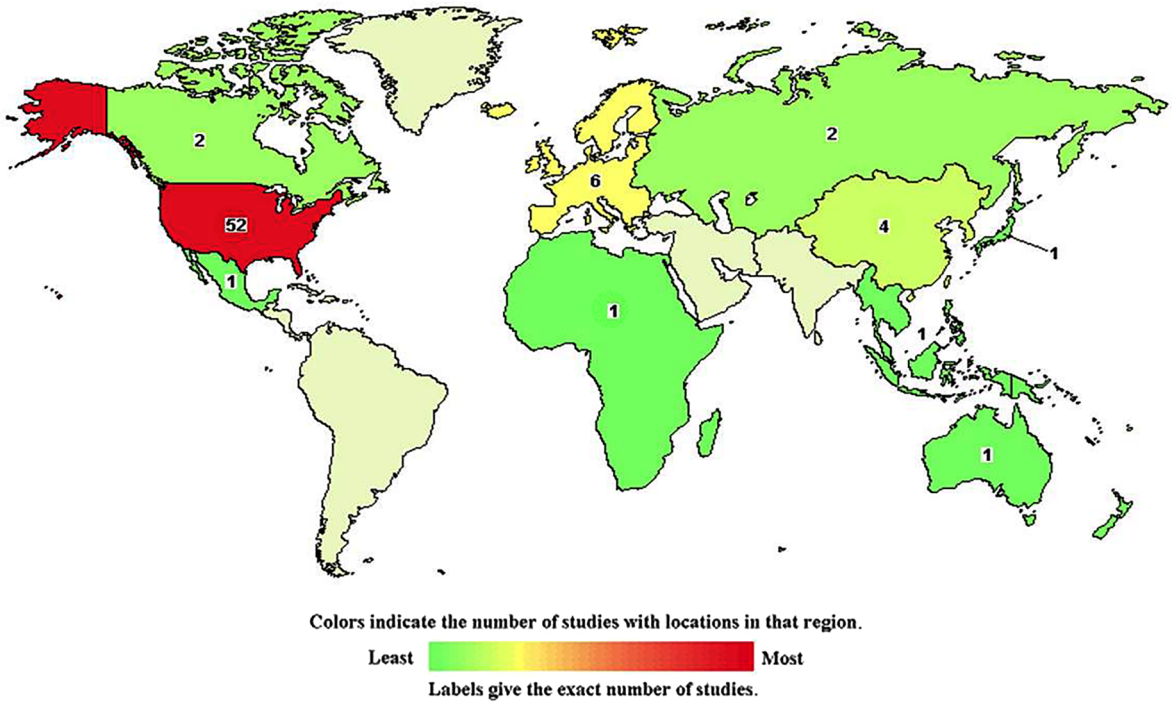

Fig. 5. Distribution of the clinical trials studying the effects of HDACIs on breast cancer around the world.

characterized by poor outcomes, SAHA was has been shown to exert an anti-proliferative effect and control tumor growth in mouse models [55,56]. Preclinical studies showed that SAHA could significantly promote the activation of epithelial mesenchymal transition (EMT), induce down-regulation of FOXA1 transcription and upregulation of N-cadherin and vimentin [57]. In combination studies with PARP inhibitor olarparib, compared with olaparib or SAHA treatment alone, the combinational treatment regimen with olaparib and SAHA suppressed proliferation, induced apoptotic and autophagic cell death, and promoted inhibition of cell proliferation in TNBC breast cancer cell lines [58]. Besides, Carlisi et al. found the combination of parthenolide (PN, a sesquiterpene lactone isolated from feverfew) and SAHA inhibited the cytoprotective responses induced by the single compounds, but did not alter the mechanisms leading to the cytotoxic effects. Combined treatment maintained both hyperacetylation of histones H3 and H4 induced by SAHA and down-regulation of DNMT1 expression induced by PN. Inhibition of the DNA-binding activity of NF-kB, which is determined by PN, was also observed after combined treatment [59]. Combining ferrocifen (FcTAM) and SAHA structural motifs to form the unprecedented FcTAM–SAHA hybrid molecule led to an increased cytotoxicity in triple-negative MDA-MB-231 breast cancer cells. Estrogen receptor alpha (ERα) and HDAC are not the main targets of the hybrid compounds. But, p21waf1/cip1 gene expression was induced in accordance with their anti-proliferative activity [60]. Moreover, SAHA could serve as a radiosensitizer against TNBC. In 2013, Chiu et al. found that SAHA could enhance radio-sensitivity and suppresses lung metastasis in TNBC in vitro and in vivo [61]. Besides, SAHA not only enhances radiosensitivity but also suppresses lung metastasis in breast cancer, suggesting that SAHA alone or combined with ionizing radiation could serve as a potential therapeutic strategy for breast cancer [61].

In spite of the promising results with SAHA in the treatment of cutaneous T-cell lymphoma, its application in breast cancer has been challenging. As shown in Table 1, there are 22 clinical trials registered to study the effects of SAHA alone or in combination for the treatment of breast cancer. However, SAHA treatment for breast cancer appeared to achieve only modest clinical benefits, especially monotherapy regimens. In 2008, Luu et al. evaluated the response rate SAHA as a single-agent in patients with metastatic breast cancer. The results failed to meet the Response Evaluation Criteria In Solid Tumors (RECIST) response criteria [62]. Recently, SAHA was considered for combination therapy, due to its favorable toxicity and ease of administration. In 2011, Munster et al. found that the combination of SAHA and tamoxifen was well tolerated in reversing hormone resistance and HDAC2 expression was a predictive marker for the combinational efficacy [63]. In 2012, Ramaswamy observed 55% objective responses in metastatic breast cancer and the adverse event profile was consistent with paclitaxel-bevacizumab [64]. In 2014, Tu et al. designed a phase I/II study and found that the combination of SAHA with weekly paclitaxel plus trastuzumab (Herceptin) followed by doxorubicin-cyclophosphamide is associated with a high pCR (pathological complete response) rate in locally advanced Her2/neu positive breast cancer [65]. In 2015, Connolly et al. demonstrated that preoperative CP (carboplatin and nanoparticle albumin-bound paclitaxel) with SAHA or placebo is associated with similar pCR rates [66]. In 2017, a Phase I/II study (E1104) of SAHA in combination with trastuzumab in patients with advanced metastatic and/or local chest wall recurrent Her-2-amplified breast cancer was registered by Goldstein et al. Results showed no dose-limiting toxicity (DLTs) with SAHA 200 mg twice daily combined with trastuzumab, but there was insufficient statistical evidence that adding SAHA reversed trastuzumab resistance in these patients [66]. Chiaradonna et al. found it could be relevant to evaluate the expression of antioxidant genes that may favor tumor resistance as a factor to consider for potential clinical application and treatment with epigenetic drugs (HDACis) in breast cancer [55]. Because of the potentiation effects of SAHA on regular anticancer drugs, its classes are still worthy of further exploration as part of a combination therapy in the treatment of breast cancer.

6.3. Panobinostat (LBH589) for breast cancer treatment

Panobinostat (LBH589) is a potent inhibitor with activity against pan-Class I, II, and IV HDAC enzymes. Panobinostat can block multiple cancer related pathways and reverse epigenetic events implicated in cancer progression [57]. In preclinical studies, panobinostat has shown potent inhibitory activity at low nanomolar concentrations to

hematologic malignancies and non-responsive solid tumors. In 2010, Fortunati demonstrated that the pan-HDAC inhibitor panobinostat is a multi-functional agent in breast cancer cells: anti-tumor drug and inducer of sodium-iodide symporter (NIS) [67]. In 2012, Tate et al. revealed that pano binostat is overtly toxic to TNBC cells in vitro and decreases tumorigenesis in vivo, via up-regulating anti-proliferative, tumor suppressor, and epithelial marker genes and initiating partial reversal of the epithelial-to-mesenchymal transition [68]. In 2014, Fortunati et al. demonstrated that the use of panobinostat could alter the invasive breast cancer cell phenotype, suggesting the use of panobinostat in aggressive breast cancer refractory to hormonal therapy [69]. In 2015, Kai et al. found combination of panobinostat and salinomycin has a synergistic inhibitory effect on TNBC breast cancer stem cells by inducing apoptosis, arresting the cell cycle, and regulating epithelial-mesenchymal transition, with no apparent associated severe toxicity [70]. In 2017, a potential therapeutic role for mevastatin plus panobinostat in targeting aggressive TNBC was explored, presenting a novel therapeutic strategy for further clinical study [71]. In 2019, Qin et al. indicated that panobinostat inhibited tumor growth and metastasis via upregulating APCL expression in breast cancer cells, which is a novel mechanism of panobinostat [72]. To date, panobinostat has demonstrated favorable therapeutic benefits against breast tumors in the clinic. However, clinical research with panobinostat for breast cancer treatment is limited. As shown in Table 1, most reports were phase I clinical studies. In 2016, Tan et al. designed a phase I study of panobinostat and letrozole in postmenopausal metastatic breast cancer patients and recommended their phase II starting dose [56]. Looking forward, promising results from phase II-III studies can be anticipated.

6.4. Chidamide for breast cancer treatment

Chidamide (CS055/Tucidinostat/Epidaza®) is the first oral subtype-selective histone deacetylase inhibitor (HDACI) approved in China as well as the first HDACI of the benzamide class approved for the treatment of relapsed and refractory peripheral T-cell lymphoma (PTCL) [42]. Chidamide can selectively inhibit HDAC1, 2, 3 and 10 [73]. For breast cancer treatment, the combination of chidamide with exemestane was generally well tolerated with promising preliminary efficacy in HR + advanced breast cancer patients [68]. The overall results from this study encourage further pivotal trials in this patient population. A phase III trial of chidamide in combination with exemestane in patients with hormone receptor-positive advanced breast cancer (NCT02482753) was designed by professor Jiang. In 2018, Jiang reported the safety and efficacy data of the trial at a ESMO (European Society for Medical Oncology) meeting. In 2019, the detail outcomes of the study was published in《The Lancet Oncology》. Results showed 6.5 months improvement of progression-free survival (7.4 vs 3.8) after 13.9 months follow-up. The most common grade 3 or 4 adverse events in either group were neutropenia and thrombocytopenia. Serious adverse events of any cause occurred in 51 (21%) of 244 patients in the tucidinostat group and seven (6%) of 121 patients in the placebo group. No treatment-related deaths were reported. The study provided evidence of the safety and efficacy of chidamide for the treatment of breast cancer in a Chinese population [74].

7. Limitations and future direction of HDACIs

As shown, HDACIs seem to be a promising group of anti-cancer drugs, particularly in combination with other anti-cancer drugs and/or radiotherapy. However, the samples of the registered studies were relatively small. More promising evidence need to be obtained from large scale, multi-center clinical trials. Meanwhile, their use in the combination with other drugs and the schedule of such drug combinations need to be further investigated in both preclinical and clinical studies. Furthermore, it is necessary to disclose all their functions and cellular interactions, which might result in development of more efficient therapy with HDACIs. Finally, predictive factors for evaluation of HDACIs should be discovered.

8. Conclusion

HDAC inhibitors (HDACIs), the first successful epigenetic therapy against cancer, have been increasingly reported in breast cancer research. However, initial studies of HDACIs commonly failed to meet the desired response criteria. HDACIs have proven to be more useful in combination therapy, due to their favorable toxicity and ease of administration. In combination treatment, multiple oncogenic signaling pathways can be simultaneously targeted increasing the likelihood of overcoming resistance in difficult-to-treat advanced breast cancer. However, despite clear responses with HDACIs in the treatment of breast cancer being limited, there is enough positive data to justify further research into the best combinations. More multicenter and randomized Phase III studies should be conducted to realize the full potential and specificity of HDACIs therapy in various subtypes of breast cancer. Further clinical studies should include the most promising novel HDACIs and isozyme-specific inhibitors.

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Declaration of Competing Interest

The authors declare that they do not have any conflict of interest.

Appendix A. Supplementary material

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