Epigenetic modification has recently been recognized as an important factor in the development of therapy resistance in cancer. Hence, histone deacetylase inhibitors as potential epigenetic modifiers are an emerging class of novel cancer therapeutics and have been extensively studied. Here, we review the role of histone deacetylase inhibitors in the treatment of breast cancer as single agents as well as in combination with chemotherapeutic, hormonal, and targeted agents.

Keywords: breast cancer • entinostat • epigenetics • HDAC • histone deacetylase inhibitors • histone deacetylases • valproic acid • vorinostat

Despite the recent introduction of several promising novel therapies, breast cancer is the most common cancer among women and remains a leading cause of cancer death for them, second only to lung cancer. In 2012, an estimated 229,060 women in the USA were diagnosed with breast cancer and 39,920 succumbed to the disease [1]. Drug development in breast cancer continues to explore strategies utilizing chemotherapy with novel mechanisms as well as targeted biologics directed towards signaling pathways that contribute to the disease, such as the estrogen receptor (ER) or HER2. A recent emerging field of interest involves modalities that target the epigenome. The most extensively studied representative compounds are the histone deacetylase (HDAC) inhibitors and demethylation agents. Both classes of drugs have been routinely integrated into the clinical management of hematological malignancies, yet their optimal use in solid tumors including breast cancer has yet to be defined.

Mechanism of action
HDACs and histone acetyltransferases (HATs) play an important role in the development and progression of cancer through epigenetic modification of gene expression [2,3]. By regulating acetylation of lysine residues on histones they affect chromatin function and maintenance as well as activity of transcription factors. Dysregulation of HDACs’ and HATs’ function has been implicated in the development of cancers such as acute promyelocytic leukemia and non-Hodgkin’s lymphoma [4,5]. In addition to their role in transcription through acetylation of histones, they also regulate acetylation of many nonhistone targets and may thereby perform key functions in post-translational regulation of gene expression. This central regulatory role of HDACs and HATs provided the mechanistic rationale for development of HDAC inhibitors as anticancer agents.

Different classes & structures of HDAC inhibitors
Currently, there are more than 20 different HDAC enzymes, divided into four classes by their homology (Table 1). HDAC1, 2, 3, and 8 belong to class 1, HDAC4–7, 9, and 10 to class 2. The sirtuins are grouped as class 3 HDAC enzymes and HDAC11 is the sole member of class 4. While embryonic and conditional depletion of select
HDAC enzymes have pointed to their specific molecular and biologic roles, clinically their functions have not been as clearly distinguished.

HDAC inhibitors are differentiated by their structure and further characterized into different subgroups. There are short chain fatty acids (valproic acid [VPA]), benzamide (entinostat), cyclic tetrapeptides (romidepsin, Istodax®) and hydroxamic type (Trichostatin A, vorinostat [Zolinza®], suberoylanilide hydroxamic acid), LBH589 (panobinostat), PXD101 (belinostat), PCI24721 (abexinostat), CHR-3996, and JNJ 26481585 [6–13]. HDAC inhibitors have been evaluated as a single-agent therapy in several Phase I and II trials as well as in combination with other targeted agents or chemotherapy. Vorinostat received approval in October 2006 for the treatment of refractory cutaneous T-cell lymphoma. A second HDAC inhibitor, romidepsin, received approval for the same indication in 2009.

Several clinical studies are currently studying whether there are differences in efficacy achieved when using a pan-HDAC inhibitor versus a selective HDAC inhibitor. Furthermore, the clinical toxicities observed with HDAC inhibitors have not been linked to the inhibition of specific HDAC enzymes. Many of the common toxicities, such as thrombocytopenia, fatigue, diarrhea and anorexia are seen with select or nonselect HDAC inhibitors and are seen across the different structural classes. The somnolence seen with VPA but not with other HDAC inhibitors may reflect its high CNS penetrance.

Key findings in preclinical studies of HDAC inhibitors in breast cancer cell lines & xenograft models

In in vitro models, HDAC inhibitors affect a broad range of biologic functions. HDAC inhibition is associated with G1 growth arrest and anti-proliferative effects. In several cell-line models, these agents cause a reversal of dedifferentiation and induction of autophagy. HDAC inhibition is further associated with apoptosis and mitotic exit block. However, in breast cancer and other solid tumor models, the induction of cell death by apoptosis or autophagy may require concentrations that are higher than those achieved in clinical testing [14–17].

HDAC inhibitors have been extensively studied in combination with DNA-damaging agents. Preclinical studies in breast cancer cell lines found that HDAC inhibitor-induced chromatin decondensation facilitates access of these agents to their DNA substrates, with increased DNA interaction with the DNA-damaging agents and, hence, enhanced apoptosis [18]. The abrogation of DNA repair gene response seen with many cytotoxic agents further suggests a benefit to adding HDAC inhibitors to DNA-damaging agents [12,19–23].

<table>
<thead>
<tr>
<th>Class</th>
<th>Members</th>
<th>Cellular location</th>
<th>Main Substrates</th>
<th>Commonly used inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>HDAC1</td>
<td>Nuclear</td>
<td>Histones</td>
<td>Belinostat</td>
</tr>
<tr>
<td></td>
<td>HDAC2</td>
<td></td>
<td></td>
<td>Vorinostat</td>
</tr>
<tr>
<td></td>
<td>HDAC3</td>
<td></td>
<td></td>
<td>Panobinostat</td>
</tr>
<tr>
<td></td>
<td>HDAC8</td>
<td></td>
<td></td>
<td>Entinostat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Valproic acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Romidepsin</td>
</tr>
<tr>
<td>IIa</td>
<td>HDAC4</td>
<td>Nuclear/cytoplasmic</td>
<td>Histones</td>
<td>Belinostat</td>
</tr>
<tr>
<td></td>
<td>HDAC5</td>
<td></td>
<td></td>
<td>Vorinostat</td>
</tr>
<tr>
<td></td>
<td>HDAC7</td>
<td></td>
<td></td>
<td>Panobinostat</td>
</tr>
<tr>
<td></td>
<td>HDAC9</td>
<td></td>
<td></td>
<td>Entinostat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Valproic acid</td>
</tr>
<tr>
<td>IIb</td>
<td>HDAC6</td>
<td>Nuclear/cytoplasmic</td>
<td>Histones; α-tubulin;</td>
<td>Belinostat</td>
</tr>
<tr>
<td></td>
<td>HDAC10</td>
<td></td>
<td>Hsp90</td>
<td>Vorinostat</td>
</tr>
<tr>
<td>III</td>
<td>Sir2</td>
<td>Nuclear/cytoplasmic/ mitochondrial</td>
<td>Histones; Tubulin; p53; TAF</td>
<td>Nicotinamides</td>
</tr>
<tr>
<td></td>
<td>SIR homologs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>HDAC11</td>
<td>Nuclear</td>
<td>Related to Rpd3 protein</td>
<td>Belinostat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Vorinostat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Panobinostat</td>
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<td></td>
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<td>Entinostat</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Romidepsin</td>
</tr>
</tbody>
</table>
Histone deacetylase inhibitors for the treatment of breast cancer

Review: Clinical Trial Outcomes

Severa lstudies suggest that the interaction between HDAC inhibitors and DNA-damaging agents is sequence-specific and requires the HDAC inhibitor to be administered prior to exposure to these cytotoxic agents [21,24]. HDAC inhibitors cause conformational changes in chromatin by expression changes in chromatin remodeling genes, which render tumor DNA more vulnerable to DNA-damaging agents. These effects were found to require pre-exposure to HDAC inhibitors for at least 24–48 h. Pre-exposure to HDAC inhibitors enhanced cytotoxic effects of several chemotherapeutic agents including VP-16,

Table 2. Published clinical trials of histone deacetylase inhibitors in breast cancer patients.

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients (breast/total)</th>
<th>Trial Design</th>
<th>Regimen</th>
<th>Response</th>
<th>Correlatives</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arce et al.</td>
<td>16/16</td>
<td>Phase II</td>
<td>Hydralazine 182 or 83 mg PO q.d. and VPA 30 mg/kg t.i.d. from day 7 through cycle 4 Doxorubicin 60 mg/m² and cyclophosphamide start 600 mg/m² on day 1 for 21 days</td>
<td>Clinical: Five CR/ Eight PaR/ three SD Pathologic: one CR, ten with residual disease &lt;3 cm</td>
<td>DNA methylation and HDAC activity assays Microarray gene expression analysis</td>
<td>[65]</td>
</tr>
<tr>
<td>Luu et al.</td>
<td>14/14</td>
<td>Phase II</td>
<td>Vorinostat 200 mg PO b.i.d., 2 weeks on, 1 week off</td>
<td>Four SD</td>
<td>Genomic profiling</td>
<td>[60]</td>
</tr>
<tr>
<td>Vansteenkiste et al.</td>
<td>3/16</td>
<td>Early Phase II</td>
<td>Vorinostat at 200/300/400 mg b.i.d., 2 weeks on, 1 week off where 200 mg b.i.d. was the MTD</td>
<td>Eight SD overall One SD in breast</td>
<td>NA</td>
<td>[59]</td>
</tr>
<tr>
<td>Munster et al.</td>
<td>Phase I: 10/44 Phase II: 15/15</td>
<td>Phase I/II</td>
<td>Phase I: VPA (15–160 mg/kg/day); Epirubicin/FEC (75–100 mg/m²) for 21 days Phase II:expansion: VPA 120 mg/kg/day; FEC 100 for 21 days</td>
<td>Phase I: nine PaR/ 13 SD Phase II: one CR/ eight PaR/three SD in breast</td>
<td>PBMC and tumor histone acetylation HDAC gene expression</td>
<td>[32]</td>
</tr>
<tr>
<td>Munster et al.</td>
<td>5/32</td>
<td>Phase I</td>
<td>Vorinostat 400/600/800/1000 mg on days 1–3 Doxorubicin 20 mg/m² on day 3 for 21 days</td>
<td>Two PaR/five SD overall One PaR/one SD in breast</td>
<td>PBMC and tumor histone acetylation HDAC gene expression</td>
<td>[62]</td>
</tr>
<tr>
<td>Munster et al.</td>
<td>43/43</td>
<td>Phase II</td>
<td>Vorinostat 400 mg q.d. for 3 weeks on, 1 week off Tamoxifen 20 mg PO q.d.</td>
<td>Eight OR Nine SD</td>
<td>PBMC histone acetylation and HDAC gene expression</td>
<td>[72]</td>
</tr>
<tr>
<td>Ramaswamy et al.</td>
<td>54/54</td>
<td>Phase I/II</td>
<td>Phase I: Vorinostat 200/300 mg b.i.d. on days 1–3, 8–10, 15–17 for 28 days; paclitaxel 90 mg/m² on days 2, 9 and 16 for 28 days; bevacizumab 10 mg/kg on days 2 and 16 for 28 days Phase II: vorinostat 300 mg b.i.d.; paclitaxel 90 mg/m² on days 2, 9 and 16 of 28 days; bevacizumab 10 mg/kg on days 2 and 16 of 28 days</td>
<td>Phase I: two OR Phase II: 24 OR Phase I/II: 16 SD &gt;24 weeks</td>
<td>PBMC and tumor Hsp 90, histone, and tubulin acetylation</td>
<td>[69]</td>
</tr>
<tr>
<td>Yardley et al.</td>
<td>130 (64 exemestane, 66 placebo)</td>
<td>Phase II, randomized, placebo-controlled</td>
<td>Entinostat 5 mg PO once weekly versus placebo Exemestane 25 mg PO q.d.</td>
<td>PFS: 4.3 vs 2.3 months OS: 28.1 vs 19.8 months</td>
<td>PBMC lysine acetylation</td>
<td>[74]</td>
</tr>
</tbody>
</table>

b.i.d.: Twice per day; CR: Complete response; FEC: Fluouracil, epirubicin and cyclophosphamide; HDAC: Histone deacetylase; MTD: Maximum tolerated dose; OR: Overall response; PaR: Partial response; PBMC: Peripheral blood mononuclear cells; PFS: Progression free survival; PO: By mouth; q.d.: daily; SD: Stable disease; t.i.d.: Three times per day; VPA: Valproic acid.
doxorubicin, and cisplatin in \textit{in vitro} and \textit{in vivo} breast cancer models, whereas a reverse sequence showed no benefit or was even antagonistic [19]. Synergistic or additive interaction with HDAC inhibitors has also been reported for other cytotoxic agents including microtubule inhibitors, antifolates, or nucleoside analogs [25–27]. The underlying mechanism of HDAC inhibitor potentiation with these classes of agents is not as well understood and does not appear to depend on schedule.

In addition to studying HDAC inhibitors in combination with chemotherapy, HDAC inhibitors have raised interest for the treatment of breast cancer due to their transcriptional and post-transcriptional regulation of ER and HER kinase family members, including HER2 [28–30]. The HER kinase family is down-regulated by HDAC inhibitors, most prominently by the hydroxamic acid type HDAC inhibitors, which act synergistically when combined with a monoclonal antibody in preclinical models [28,31]. Multiple studies further suggest that HDACs also play an important role in hormone receptor signaling; and HDAC expression has been linked to prognosis.

### Table 3. Ongoing clinical trials of histone deacetylase inhibitors in breast cancer patients.

<table>
<thead>
<tr>
<th>Study</th>
<th>Institute (location)</th>
<th>Trial title</th>
<th>Regimen</th>
<th>Correlates</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chumsri et al.</td>
<td>University of Chicago Comprehensive Cancer Center (IL, USA)</td>
<td>A pilot and Phase II study of entinostat and anastrozole in postmenopausal women with operable triple negative breast cancer to evaluate biomarkers and surrogates for response</td>
<td>Entinostat PO q.d. on days 1, 8, 15, 22, and 29 Anastrozole PO q.d. on days 4–29</td>
<td>Tumor IHC Histone acetylation Tumor and CTC gene expression Genomic profiling</td>
<td>[104]</td>
</tr>
<tr>
<td>Stearns et al.</td>
<td>Mayo Clinic (MN, USA)</td>
<td>Phase II study of azacitidine and entinostat (SNDX-275) in patients with advanced breast cancer</td>
<td>Azacitidine sc. on days 1–5 and 8–10 every 28 days Entinostat PO on days 3 and 10 every 28 days</td>
<td>Cytidine deaminase pharmacogenetics and activity Tumor gene expression DNA methylation assay</td>
<td>[105]</td>
</tr>
<tr>
<td>Ueno et al.</td>
<td>MD Anderson Cancer Center (TX, USA)</td>
<td>Phase I/II study of entinostat and lapatinib in patients with HER2-positive metastatic breast cancer in whom trastuzumab has failed</td>
<td>Entinostat PO on days 1 and 15 Lapatinib tosylate PO on days 1–28</td>
<td>Tumor and CTC candidate gene expression</td>
<td>[106]</td>
</tr>
<tr>
<td>Esserman et al.</td>
<td>UCSF Helen Diller Family Comprehensive Cancer Center (CA, USA)</td>
<td>A window trial of vorinostat in patients with ductal carcinoma \textit{in situ} of the breast</td>
<td>Vorinostat PO b.i.d. for 3 days, followed by lumpectomy/mastectomy 2 h after last dose</td>
<td>PBMC and tumor histone acetylation Tumor IHC HDAC gene expression</td>
<td>[107]</td>
</tr>
<tr>
<td>O’Regan et al.</td>
<td>Emory University Winship Cancer Institute (GA, USA)</td>
<td>Phase I/II trial of tamoxifen following epigenetic regeneration of estrogen receptor using decitabine and LBH 589 in patients with triple negative metastatic breast cancer</td>
<td>Decitabine 5 mg/m² iv. on days 1–5 LBHS89 10 mg/m² iv. on days 1 and 8 Tamoxifen</td>
<td>NA</td>
<td>[108]</td>
</tr>
<tr>
<td>Werner et al.</td>
<td>Huntsman Cancer Institute (UT, USA)</td>
<td>Molecular signature of valproic acid in breast cancer with functional imaging assessment – a pilot</td>
<td>VPA 30–max 50 mg/kg/day PO b.i.d.</td>
<td>DCE MRI PBMC histone acetylation Genomic profiling (sensitivity signature) Tumor IHC</td>
<td>[109]</td>
</tr>
</tbody>
</table>

b.i.d.: Twice daily; CTC: Circulating tumor cells; DCE: Dynamic contrast enhanced; EMT: Epithelial mesenchymal transition; FDG-PET: [18F]Fluorodeoxyglucose positron emission tomography; FES-PET: [18F]Fluoroestradiol positron emission tomography; HDAC: Histone deacetylase; IHC: Immunohistochemistry; iv.: Intravenously; PBMC: Peripheral blood mononuclear cells; PO: By mouth; q.d.: Daily; sc.: Subcutaneously; VPA: Valproic acid.
Histone deacetylase inhibitors for the treatment of breast cancer

Review: Clinical Trial Outcomes

in hormone-sensitive breast cancer and response to therapy [32–34].

Hormonal therapy has been one of the most successful strategies to treat breast cancer; however, its efficacy is dependent on both expression and function of the estrogen and progesterone receptor (PR) [35–39]. Much emphasis has been placed on gaining a better understanding of resistance to hormonal therapy, where the epigenome is believed to be central to the development of hormone therapy resistance. Hence, the role of HDAC enzymes and their inhibitors in hormone therapy resistance has been more extensively studied.

Several investigators have since suggested a correlation between HDAC1 and HDAC3 expression and specific breast cancer characteristics, where higher expression of HDAC1 mRNA levels as well as HDAC1 and HDAC3 protein expression were associated with smaller, ER and PR positive, node negative tumors in addition to better clinical outcomes [40,41]. Furthermore, HDAC6 may also convey a favorable response to tamoxifen (TAM) [42]. However, larger prospective studies to determine the prognostic and predictive roles of select HDAC expression are needed.

Many of the HDAC family members regulate the acetylation of core histones and nonhistone targets and may be involved in the transcriptional regulation of ER signaling [43,44]. Acetylation regulates both transcription and turnover of ER, and the regulation of ER is complex [30,32,33,45]. ER may be silenced by promoter methylation, which is reversible by HDAC and DNA methyl transferase inhibition resulting in ER re-expression upon drug exposure [43,44,46–51]. Further studies suggested that the re-expression of the ER was associated with re-sensitization of ER-negative breast cancer cell

<table>
<thead>
<tr>
<th>Study</th>
<th>Institute (location)</th>
<th>Trial title</th>
<th>Regimen</th>
<th>Correlatives</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sparano et al.</td>
<td>Montefiore Medical Center (NY, USA)</td>
<td>Phase I–II trial of vorinostat plus weekly paclitaxel and trastuzumab followed by doxorubicin-cyclophosphamide in patients with locally advanced breast cancer</td>
<td>Vorinostat 200 or 300 mg PO b.i.d. on days 1–3 of each weekly paclitaxel dose Paclitaxel 80 mg/m² per week for 12 weeks Trastuzumab 4 mg/kg (loading dose), then 2 mg/kg per week for 12 weeks including loading dose Doxorubicin 60 mg/m² every 2 weeks for 8 weeks Cyclophosphamide 600 mg/m² every two weeks for 8 weeks, then surgery followed postoperatively by trastuzumab 8 mg/kg (loading dose), then 6 mg/kg every 3 weeks for 14 doses</td>
<td>NA</td>
<td>[110]</td>
</tr>
<tr>
<td>Chumsri et al.</td>
<td>University of Maryland Greenebaum Cancer Center (MA, USA)</td>
<td>Pilot and Phase II – vorinostat and lapatinib in patients with advanced solid tumor malignancies and women with recurrent local-regional or metastatic breast cancer to evaluate response and biomarkers of EMT and breast cancer stem cells</td>
<td>Vorinostat 300–400 mg PO, 4 days on 3 days off Lapatinib 1250 mg PO q.d.</td>
<td>Breast cancer stem cells and biomarkers of EMT</td>
<td>[111]</td>
</tr>
<tr>
<td>Linden et al.</td>
<td>Fred Hutchinson Cancer Research Center (WA, USA)</td>
<td>A pilot study of vorinostat to restore sensitivity to aromatase inhibitor therapy part B</td>
<td>Vorinostat PO 5 days/week for 3 weeks Anastrozole PO q.d.; or Letrozole PO q.d.; or Exemestane PO q.d.</td>
<td>FES-PET; FDG-PET; Change in hormone levels Tumor gene expression</td>
<td>[112]</td>
</tr>
</tbody>
</table>

b.i.d.: Twice daily; CTC: Circulating tumor cells; DCE: Dynamic contrast enhanced; EMT: Epithelial mesenchymal transition; FDG-PET: [18F]Fluorodeoxyglucose positron emission tomography; FES-PET: [18F]Fluoroestradiol positron emission tomography; HDAC: Histone deacetylase; IHC: Immunohistochemistry; iv.: Intravenously; PBMC: Peripheral blood mononuclear cells; PO: By mouth; q.d.: Daily; sc.: Subcutaneously; VPA: Valproic acid.
lines to TAM or aromatase inhibitors (AIs) [45,49,51,52]. However, the degree of estrogen re-expression by HDAC inhibitors is modest and requires co-exposure to a demethylation agent [43,44,46–51]. Whether the effects on ER re-expression can be translated into clinical care is currently being determined in several clinical studies.

Opposite to the findings in ER-negative breast cancer cells, HDAC inhibition in ER-positive cells results in direct transcriptional down-regulation of ERα and its response genes and subsequent loss of ER protein with sensitization to hormone therapy in hormone-sensitive and hormone-resistant settings [32]. In addition to transcriptional down-regulation of ER, the receptor expression may be further reduced by proteasomal degradation involving HSP90 chaperone-mediated effects [53]. There is considerable variability in the degree of ER suppression and re-expression seen with various HDAC inhibitors, classes and concentrations [30,52,54]. These effects further vary by cell line and tissue type.

Whether the effects of HDAC inhibitors on ERα transcription are direct, indirect, or both, may depend on the agent and the setting. Several clinical studies are now underway to determine which of these phenomena can be most successfully explored in patients. Taken together, these findings point to the considerable challenges that underlie efforts to define the optimal clinical setting for further testing of these agents and for the selection of the best HDAC inhibitor. The effects on ER down-regulation have also led to the conduct of several studies demonstrating additive and synergistic interactions between HDAC inhibitors and anti-estrogens and AIs in in vitro and in vivo models. The observed positive interaction between ER signaling, hormonal therapy and HDAC inhibition in preclinical models provided the rationale for further testing of such combinations in clinical studies.

Clinical studies of HDAC inhibitors in breast cancer

Single-agent activity for HDAC inhibitors has led to approval in cutaneous T-cell lymphoma and has shown promising results in hematologic malignancies. However, the efficacy of HDAC inhibitors as single agents in solid tumors overall has been disappointing. Several Phase II trials studied the hydroxamic acid type HDAC inhibitor, vorinostat. In a study of recurrent or refractory ovarian cancer and peritoneal carcinoma, nine out of 27 female patients had stable disease and one out of 27 had a partial response. This study closed early because it did not meet its own continuation criteria of four patients with progression-free survival >6 months [55]. Similar Phase II studies with single-agent vorinostat in advanced head and neck and thyroid carcinoma also closed early because no confirmed responses were observed [56,57].

Early clinical patient data for HDAC inhibitors in breast cancer was available mainly with vorinostat, where single-agent data was first gathered in the Phase I setting. Rubin et al. found durable benefit of 15 months in one of four breast cancer patients with metastatic disease in a Phase I study evaluating the pharmacokinetics of vorinostat with food ingestion and multiple dosing [58]. Vansteenkiste et al. conducted an early open-label Phase II trial of single agent vorinostat in a number of patients with solid tumors, including refractory breast cancer (Table 2) [59]. Patients received 200, 300, or 400 mg twice daily for 14 of 21 days. One breast cancer patient had a durable response despite multiple prior treatments.

Luu et al. conducted the first breast cancer-specific single-arm Phase II trial evaluating single-agent vorinostat in metastatic breast cancer patients (Table 2) [60]. Patients with confirmed stage IV metastatic breast cancer, measurable disease, and up to two prior chemotherapy regimens for metastatic breast cancer received 200 mg of vorinostat twice daily for 14 of the 21 days. The trial was halted after 14 patients were enrolled, when the predefined Response Evaluation Criteria In Solid Tumors (RECIST) criteria response threshold for continuation was not met. While no objective RECIST responses were recorded, four of 14 patients (29%) had a clinical benefit with stable disease with median time-to-progression of 8.5 months (range: 4–14 months). Side effects were reported to be manageable with grade 3/4 fatigue, diarrhea, cytopenia, and mucositis. Pre-treatment tumor gene expression data was collected; however, given that no formal RECIST responses were observed, no candidate gene expression profile was identified to correlate with therapeutic response.

■ HDAC inhibitors in combination with chemotherapy in breast cancer

The limited single-agent activity with vorinostat seen initially in breast cancer is not completely surprising given the observed plasma levels of vorinostat in clinical studies of 1–2 µM [13]. At these concentrations, significant apoptosis is not seen in most breast cancer models [15–17]. However, pre-clinical studies also suggested that there was a rationale for combination with chemotherapeutic agents as concentrations needed to achieve enhanced synergistic efficacy were considerably lower than those required for single-agent activity [18,21,24]. Activity of combining an HDAC inhibitor with chemotherapy combinations was also observed to be sequence-specific and required for the HDAC inhibitor to be given prior to exposure of the cytotoxic agent [21,24].

Our group conducted a proof-of-principle Phase I trial of VPA and the topoisomerase inhibitor, epirubicin,
where 44 patients with advanced solid tumors were enrolled in a dose escalation study with an expansion of 15 patients with breast cancer (Table 2) [61,62]. Prior anthracycline exposure was allowed. Epirubicin was administered at 100 mg/m² and given together with VPA at dose levels between 15 to 160 mg/kg/day. Dose-limiting toxicities (DLT) associated with high doses of VPA included confusion and somnolence. The median number of prior treatment regimens was three (range: 0–10 prior regimens) and nine out of 41 of the treated patients (22%) showed a partial response. Stable disease was seen in 16 of the 41 patients (39%). A total of 13 (32%) patients discontinued study therapy after reaching maximum epirubicin life-time dose rather than progression of disease.

As VPA has a long history of use in neurology, where it is used as an antiseizure medication and its bioavailability is followed via serum plasma levels, we used total and free VPA plasma concentrations as a correlative marker. As such, we identified that VPA levels increased linearly with dose and also correlated linearly with histone acetylation in peripheral blood mononuclear cells (PBMCs). A benefit was observed in several patients who had previously progressed on anthracyclines and in diseases not typically considered anthracycline-sensitive such as melanoma. While these findings support preclinical studies suggesting a potentiation of anthracyclines, VPA did not enhance epirubicin-induced toxicities in nontumor tissues [62]. In the breast cancer dose-expansion cohort, patients received a median of six cycles of therapy (range: 1–7 cycles) with nine out of 14 evaluable patients obtaining an objective response (64%) with one complete response, eight partial responses, and three patients with stable disease. Importantly, the addition of an HDAC inhibitor did not appear to impact tolerability of the cytotoxic regimen and the recommended Phase II dose was determined to be 120 mg/kg/day loading dose followed by 60 mg/kg every 12 h for five doses. While no further DLTs were observed in the expansion cohort in the DLT period at this dose, 20% somnolence was seen in the post-DLT period at 120 mg/kg/day dosing requiring dose modifications in several patients. Another commonly observed toxicity was myelosuppression, which was attributed to epirubicin.

In this study, histone acetylation was measured by immunofluorescence in PBMCs and tumor samples obtained on days 1 and 3 of cycle 1, where histone acetylation was expressed as a change from baseline value and normalized to the control gene lamin. Results showed that histone acetylation in PBMCs were comparable to tumor samples, suggesting that PBMCs would be a good surrogate marker for tumor histone acetylation. In this particular study, our group did also find a correlation between histone acetylation and VPA dose in the dose escalation cohort, and a strong correlation between HDAC2 expression and histone acetylation. However, no correlation was found between HDAC6 expression and histone acetylation. These results support preclinical findings suggesting that VPA’s effects on chromatin act through its inhibition of HDAC2 rather than other HDAC enzymes [63]. As such, HDAC2 may function as a potential biomarker that could be followed to indicate degree of therapeutic response. The combination of an HDAC inhibitor and topoisomerase inhibitor was further evaluated with the more potent HDAC inhibitor, panobinostat; and the study is currently under way with a focus on sarcoma [64].

Concurrently, Arce et al. used similar agents to conduct a proof-of-principle single-arm Phase II trial to confirm the rationale for combining cytotoxic agents with HDAC inhibitors in advanced breast cancer with a different correlative approach (Table 2) [65]. In addition to combining an HDAC inhibitor and a topoisomerase inhibitor, by administering VPA and doxorubicin, they added a demethylating agent with hydralazine. These three drugs were given together with cyclophosphamide to simulate a neoadjuvant breast cancer regimen with an acetyl backbone, where the addition of a demethylating agent and an HDAC inhibitor was intended to evaluate specific gene expression changes, notably enhancing favorable profiles such as reactivating tumor suppressor genes or those that potentiate therapy.

Patients were given a single oral 500-mg dose of sulfamethazine to determine their acetylator phenotype and control for different metabolizing polymorphisms that would affect hydralazine concentrations. Depending on the outcome of this screening step, patients were then assigned to either hydralazine at 182 mg dose for rapid-acetylators and 83 mg for slow-acetylators. Patients would receive either dose in combination with VPA, doxorubicin 60 mg/m³, and cyclophosphamide 600 mg/m³. Similar to prior combinations with chemotherapy, the addition of hydralazine did not significantly impact toxicity and the regimen remained well-tolerated. In total, 16 patients were treated and evaluable. Following the trial’s neoadjuvant regimen, clinical evaluation found five (30%) patients had a complete response, eight (50%) patients had a partial response and three (20%) had stable disease. A total of 15 out of the 16 enrolled patients underwent surgery, at which time one of 15 patients had a pathologic complete response and 70% had residual disease of <3 cm.

This study found that hydralazine and magnesium valproate up-regulated at least threefold 1091 and 89 genes, respectively, and both drugs’ plasma levels were
significantly different between slow and fast acetylators. While the study sample was small, global gene expression profiling was conducted and a total of 3,117 genes were found to be up- or down-regulated in the clinical samples as compared to normal breast tissue. Specifically, the authors identified that NDUFV13 and DAPPER gene expression was up-regulated following study therapy, where these genes and others in their family have been implicated in enhancing apoptosis and found down-regulated in doxorubicin-resistant cells [66,67].

Several groups evaluated vorinostat in combination with cytotoxic agents. Our group conducted a Phase I trial evaluating the combination of vorinostat with doxorubicin in solid tumors (Table 2) [68]. The study enrolled 32 patients, of whom five had breast cancer. Patients had received a median of two (range 0–6) prior systemic therapies prior to enrollment. Vorinostat was dosed at 400, 600, 800, or 1000 mg on days 1–3 followed by doxorubicin on day 3 of 4 weeks. The maximally tolerated dose for vorinostat given for three days per week was 800 mg. One of five patients with breast cancer had a partial response. Histone hyperacetylation in PBMCs versus tumor cells was comparable in determining target effect. Similar to our findings with the combination of VPA and 5-fluorouracil, epirubicin and cyclophosphamide, we found here that histone hyperacetylation correlated with baseline HDAC2 expression, further supporting HDAC2 as a marker predictive of HDAC inhibition and drug efficacy. Discontinuation from study was more common with this combination and its efficacy appears to be less than that of the combination of VPA and epirubicin.

Ramaswamy et al. evaluated vorinostat in combination with paclitaxel and bevacizumab in 54 patients with metastatic breast cancer who had not received prior chemotherapy in a Phase I/II study (Table 2) [69]. The patients received vorinostat (200 or 300 mg orally, twice daily) on days 1–3, 8–10, and 15–17. Paclitaxel was administered on days 2, 9, and 16, and bevacizumab 10 mg/kg was given on days 2 and 16 every 28 days. At the recommended Phase II dose of 300 mg vorinostat twice daily, 24 of 44 (55%) chemotherapy-naive patients had an objective response. Similar to other chemotherapy combinations with HDAC inhibitors, the regimen was well-tolerated and adverse events reflected mostly the toxicity of the paclitaxel–bevacizumab combination, with the exception of increased diarrhea attributed to vorinostat.

This trial added further new correlatives to evaluate the drug–target effect by evaluating both histone and nonhistone targets of HDAC inhibitors. In addition to H3 and H4 acetylation, the authors evaluated Hsp90 and α-tubulin acetylation following vorinostat administration. HDAC6 mediates α-tubulin acetylation which may contribute to the potentiation of paclitaxel by HDAC inhibitors. Acetylation of Hsp90 in turn disrupts Hsp90's association with other members of the survival pathway that are known to be active in breast cancer such as AKT, c-RAF, and Her2. These signaling pathways are also thought to promote recovery following cytotoxic therapy. The authors reported an induction of p27 and p21, two cyclin-dependent kinase inhibitors, and inhibition of CKD4. Acetylation of both core histones and α-tubulin was seen in seven patients, where tumor biopsies were available, suggesting a clinically relevant inhibition of HDAC6. The class 2B HDAC enzyme, HDAC6, is expected to be inhibited by the hydroxamic acid type HDAC inhibitors such as vorinostat.

- HDAC inhibitors in combination with HER2-targeting agents

Based on several preclinical studies suggesting a direct modification of the HER kinase family, a Phase I/II trial tested the combination of vorinostat and the HER2 targeting monoclonal antibody trastuzumab in patients with HER2 positive breast cancer [28,70]. This National Cancer Institute-sponsored clinical trial was halted due to insufficient activity [101].

More recently, a trial combining vorinostat with the small-molecule HER-kinase inhibitor, lapatinib, in patients with locally advanced or metastatic HER2 positive breast cancer, is currently ongoing. The study plans to enroll 47 patients to determine the response rate of the combination (Table 3) [102].

A similar ongoing trial tests the effects of entinostat and lapatinib on HER2 signaling in an estimated 70 patients with HER2 positive metastatic or inflammatory breast cancer patients who had previously received trastuzumab (Table 3) [103].

- HDAC inhibitors to potentiate hormonal therapy in breast cancer

Concurrently with the development of HDAC inhibitors to augment chemotherapeutic agents’ activity in breast cancer, preclinical work also suggested a strong rationale to explore the role of this drug class in enhancing the efficacy of hormonal therapy. HDAC inhibitors’ effects on ER down-regulation in ER-positive breast cancer cells as well as their effect on ER re-expression in hormone-negative tumors has gone on to lead to their study in several clinical studies.

Based on preclinical findings suggesting a potentiation of TAM in hormone-sensitive breast cancer cell lines and reversal of hormone therapy resistance irrespective of serum estradiol levels, our group enrolled 43 breast cancer patients in a single-arm Phase II study to receive the combination of the HDAC inhibitor vorinostat with TAM (Table 2) [14,32,71,72]. Pre- and post-menopausal women with ER- or PR-positive metastatic breast cancer who had
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Wardley et al. performed a Phase II study (presented at the ASCO 2010 meeting), where the authors added the HDAC inhibitor, entinostat, to postmenopausal ER-positive breast cancer patients’ hormonal treatment regimen who had progressed following 3 months of therapy with an AI [73]. These patients continued to take the AI they had received and, upon study enrollment, received entinostat at 5 mg weekly in 28-day cycles upon study enrollment. Patients had to have measurable disease by RECIST and ≤1 prior chemotherapy for metastatic disease. Of 27 enrolled patients, 11 (41%) received study treatment for greater than 4 months and three (11%) for >6 months. One patient had a confirmed partial response, and one patient had stable disease for >6 months. The addition of the HDAC inhibitor resulted in expected toxicities, notably nausea, diarrhea, and mostly low-grade fatigue. Grade 3 or higher toxicities included fatigue, dyspnea, diarrhea, and lethargy. Biomarker analysis showed an increased protein lysine acetylation in CD8, CD14, and CD19/20 cells as well as increased apoptosis.

In the ENCORE 301 trial, Yardley et al. went on to further investigate the role of HDAC inhibitors in enhancing the efficacy of hormonal therapy through a randomized, placebo-controlled Phase II study of exemestane with or without entinostat in ER positive postmenopausal women with metastatic breast cancer [74]. The international trial randomized 130 patients from 38 sites in North America, Central Europe, and Russia to two arms, one in which patients received exemestane and entinostat (EE; n = 64), and another in which patients received exemestane and placebo (n = 66). Patients who progressed or relapsed following therapy with a nonsteroidal AI and had received fewer than two prior lines of chemotherapy were eligible. Measurable disease was not required. Following an intention-to-treat analysis, the authors reported prolonged progression-free survival (4.3 vs 2.3 months; hazard ratio: 0.73; 95% CI: 0.5–1.07) for the EE arm and also found that addition of entinostat extended median overall survival compared to exemestane alone (28.1 vs 19.8 months; hazard ratio: 0.59; 95% CI: 0.36–0.97). Overall response and clinical benefit rate were similar for both groups. Analysis of baseline characteristics, subsequent treatment, and subsets of prognostic factors did not identify any contributing factors that account for the extended survival benefit in the EE treatment group. The EE arm did have a higher incidence of grade 3 and 4 adverse events, notably neutropenia (14%), thrombocytopenia (14%), and fatigue (6%).

This study also evaluated an early marker of response in PBMCs by measuring protein lysine acetylation, in B cells, T cells, and monocytes pre- and post-treatment, on day 1, 8, and 15. Results showed that lysine acetylation correlated with clinical benefit, where patients with hyperacetylation had a 68% reduced risk of disease progression (8.5 vs 2.7 months progression-free survival) compared to patients who did not have sustained elevated levels of acetylation.

Future perspective

Per the clinical trials database of the NIH (www.clinicaltrials.gov), there are ongoing clinical trials investigating the role of HDAC inhibitors in combination with targeted agents, including hormonal and HER2-targeted therapy, in breast cancer (Table 3), notably in addition to combinations of HDAC inhibitors in hormone-sensitive tumors, there are now trials
evaluating HDAC inhibitors in triple-negative breast cancer. Chumsri et al. have been running an open trial since 2010 that is recruiting for the combination of entinostat and anastrozole as neoadjuvant therapy in patients with triple negative breast cancer (Table 3). There are also several new HDAC inhibitors being investigated.

A major challenge in the development of HDAC inhibitors is the absence of a robust biomarker. Acetylation has been generally accepted as a pharmacodynamic marker. However, many of the HDAC inhibitors have a relatively short half-life. Vorinostat’s half-life, for example, was reported to range from 21 to 58 min [75]. Also, the dosing and schedule of HDAC inhibitors differs by the agents themselves, as determined by their respective pharmacology, toxicity profile, and with which oncologic agents (chemotherapy vs targeted agents) they are paired.

That said, several studies have shown that hyperacetylation often exceeds the plasma half-life of the agent [6,13,61,75]. Munster et al. and Yardley et al. show that sustained histone acetylation beyond the pharmacological half-life is predictive of response [68,74]. Furthermore, acetylation is linked to baseline expression of HDAC2 [72]. An alternative would be the clinical adaptation of novel functional imaging such as magnetic resonance spectroscopy to determine the degree of histone acetylation in a noninvasive real-time method as proposed by Ronen et al. in xenograft models [76].

The stratification of patients with the ability to maintain sustained acetylation may enrich patients more likely to respond to HDAC inhibitors. Ongoing evaluation of such correlative markers will be important in moving this class of agents forward.

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Executive summary

Background
- Histone deacetylase (HDAC) inhibitors target the epigenome of cancers and are being researched as novel targeted agents in breast cancer.

Mechanism of action
- HDACs and histone acetyltransferases regulate acetylation of lysine residues on histone and nonhistone targets, thereby affecting chromatin function and activity of transcription factors as well as post-translational regulation of gene expression.

Different classes & structures of HDAC inhibitors
- HDAC inhibitors are differentiated by their structure and further characterized into different subgroups.
- Many of the common toxicities, such as thrombocytopenia, fatigue, diarrhea and anorexia are seen with select or nonselect HDAC inhibitors and across different structural classes.

Key findings in preclinical studies of HDAC inhibitors in breast cancer cell lines & xenograft models
- In in vitro models, HDAC inhibitors affect a broad range of biologic functions, including growth arrest, reversal of dedifferentiation, apoptosis, and induction of autophagy.
- HDAC inhibitor-induced chromatin changes facilitate access of cytotoxic agents to their DNA substrate and render tumor DNA more vulnerable.
- HDAC inhibitors are also of interest for the treatment of breast cancer due to their transcriptional and post-transcriptional regulation of estrogen receptors and HER kinase family members.

Clinical studies of HDAC inhibitors in breast cancer
- Early clinical data for HDAC inhibitors for single-agent use in breast cancer was disappointing.

HDAC inhibitors in combination with chemotherapy in breast cancer
- The activity of the combination of HDAC inhibitors and chemotherapy is sequence-specific and requires the HDAC inhibitor to be given prior to the cytotoxic agent.
- Combination of HDAC inhibitors and chemotherapy shows promising efficacy and correlates with biomarkers such as HDAC2 expression or protein acetylation.

HDAC inhibitors to potentiate hormonal therapy in breast cancer
- Clinical trials studying the combination of hormone therapy and HDAC inhibitors show promising efficacy in hormone-positive metastatic breast cancer that also correlates with surrogate tumor tissue protein acetylation.

Future directions in the drug development of HDAC inhibitors in breast cancer therapy
- The role of incorporating HDAC inhibitors in therapy for triple-negative breast cancer is now being studied.
- Further research into more robust biomarkers will be important in moving HDAC inhibitors forward as a class of cancer drugs.
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References

Papers of special note have been highlighted as:

- of interest
- of considerable interest

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Truong & Munster


This is a Phase II trial with vorinostat that was the first to evaluate single-agent histone deacetylase (HDAC) inhibitor use in a dedicated breast cancer population and showed disappointing efficacy.
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This is the first randomized Phase II study evaluating the role of an HDAC inhibitor in the setting of hormone therapy resistance in a large multicenter and multinational trial. It found improved overall survival in the HDAC inhibitor arm as compared to placebo.

Websites

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