

The emergence of specific HDAC inhibitors and their clinical efficacy in the treatment of hematologic malignancies and breast cancer

Abstract

The dynamic balance between histone acetylation and deacetylation is an important epigenetic mechanism controlling gene expression, and is associated with the etiology and progression of many human diseases. Early success of histone deacetylase (HDAC) inhibition in the treatment of hematological cancers paved the way for the development and testing of many different pan/global histone deacetylase inhibitors (HDIs). Dose limiting toxicities in these early trials, coupled with increased understanding of the differential expression patterns of HDACs in different tissues and disease states, led to the emergence of more specific HDIs that target specific HDAC classes and isotypes. Whereas a large number of clinical trials have been undertaken using pan-HDIs, which have demonstrated varying success in the treatment of hematological and solid malignancies, both as single agents and in combination with other drugs, there have been far fewer undertaken with the emerging repertoire of specific HDIs, which could potentially overcome the limitations seen in the pan inhibitors. In this review we describe the classification and development of HDIs as well the roles of HDACs in cancers, and the rationale behind moving toward more selective inhibition. We then examine the clinical efficacy of both pan- and specific HDI treatment by reviewing a number of clinical trials, focusing on hematological malignancies, where numerous trials have demonstrated single agent and combinatory efficacy, as well as breast cancers, where fewer trials have been undertaken showing limited efficacy, but also where promising pre-clinical findings necessitate further clinical investigation.

Keywords: histone deacetylases, histone deacetylase inhibitors, targeted therapy, hematological malignancies, breast cancers

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Abbreviations: HDACs, histone deacetylase; HDIs, histone deacetylase inhibitors

Introduction

Epigenetic modification of gene expression through inhibition of histone deacetylases (HDAC) represents a promising potential clinical strategy for a variety of conditions, including cancer, autoimmunity and transplantation, polyglutamine disorders, neurological diseases, and heart disease.¹⁻⁴ Numerous histone deacetylase inhibitors (HDI) have been developed, showing varying degrees of specificity across classes or isotypes of HDACs. Early experience demonstrated the efficacy of less specific, “pan/global” HDIs (e.g. vorinostat, panbinostat, and abexinostat) in the treatment of specific hematological malignancies, but was tempered by significant toxicity exhibited by the inhibitor class. As the preclinical and clinical evaluation of this strategy has progressed, the field has shifted toward a focus on the development and investigation of more specific HDIs,⁵ hoping to limit toxicity and unintended non-target effects of HDI. Herein, we seek to review the trend in clinical research toward the development of class and isoform-specific HDIs, highlighting their clinical efficacies and limitations in the treatment of hematologic and breast cancers in order to underscore their potential to allow for the improvement of clinical outcomes with less toxicity and unintended non-target effects.

HDACS and the development of HDIS

Histone proteins are heavily modified on their N-Terminal tails

which play essential role in chromatin architecture and the regulation of gene accessibility and expression. Although many different types of modification take place at these sites, the acetylation is a key regulatory mark that has been most thoroughly investigated.⁶ This acetylation/deacetylation of histone proteins two enzyme families with antagonistic effects: histone acetyl-transferases (HATs) that add acetyl groups to lysine residues, and HDACs that remove these groups. Hyperacetylated histones usually result in transcriptionally active genes, and hypoacetylation generally results in repressed transcription.⁷ Histone proteins are not the only substrates of these enzymes, other proteins have also been revealed to be substrates for reversible acetylation including α -tubulin (HDAC 6 target).^{6,8,9} There are currently 18 human HDACs genes that have been identified and are classified into four distinct families based on their homology to yeast proteins. Class I (homologous to yeast Rpd3), including HDACs 1, 2, 3, and 8, are ubiquitously expressed and are active in complexes with other corepressor proteins such as NCOR and SMRT.¹⁰ These enzymes are primarily located within the nucleus, with the exception of HDAC3 that has been detected in both the nucleus and cytoplasm.¹¹ Class II (homologous to Hda1 in yeast) HDACs also can be found in the nucleus as well as the cytoplasm and are divided into two separate subclasses: IIa containing HDACs 4, 5, 7, 9, and IIb containing HDACs 6, and 10.¹² Class III (homologous to yeast Sir2) HDACs, termed sirtuins 1-7, are NAD⁺ dependent and have been found to localize to the nucleus, mitochondria, and cytoplasm.^{13,14} Finally, class IV (“hybrid” sharing similarities to class I, II) consists solely of HDAC 11.¹⁵

The development of HDIs has resulted in an array of small molecules that target a many key cancer phenotypes including proliferation, apoptosis, differentiation, autophagy, and anti-angiogenic effects.¹⁶ Relatively weak HDAC inhibition was initially observed with sodium butyrate in a clinical setting. This compound served as the prototype of the aliphatic acid group that was later found to include sodium valproate and phenylbutyrate.¹⁷ Following the identification of these compounds a range of much more potent and structurally diverse HDIs have been produced. These compounds are generally classified as hydroxamates, benzamides, tetrapeptides/desmipeptides, and sirtuin inhibitors.¹⁸

HDACs in cancer

Cancer results not only from the mutation of genes, but also from changes in epigenetic modifications, such as DNA methylation and histone modification. Unlike genetic mutations, these epigenetic modifications are often reversible.⁶ Because of the ability to potentially change these aberrant epigenetic states, particularly acetylation, that are associated with cancer, HDACs have become viable and promising therapeutic targets.⁶ Atypical expression of HDACs and changes in acetylation levels in human tumors have been demonstrated in numerous correlative studies and overexpression of HDACs 1, 5, and 7 can serve as biomarkers to differentiate tumor from normal tissue.^{19,20} Clearly demonstrated in many studies, abnormal expression and activity of HDACs is correlated to key oncogenic outcomes such as the direct deacetylation of p53 leading to decreased transcriptional activity and HDAC-mediated activation of transcription factors SP1 and C/EBP, which leads to upregulation of oncogenes such as BCL-2.^{19,21,22} While inappropriate binding of HDACs to promoters has been shown to play a role in tumorigenesis, there is also evidence that fusion proteins created by chromosomal translocations can physically interact with HDACs and lead to deregulated functions that are tumorigenic.⁶

Rationale for selective inhibition

Despite their relative success in clinical applications, with vorinostat (SAHA, Zolima), and belinostat (PXD101, Beleodaq) that are approved for the treatment of hematologic tumors, HDIs that inhibit a broad spectrum of HDACs have all exhibited several significant dose-limiting toxicities (DLT), including fatigue, nausea, vomiting, thrombocytopenia, and neutropenia.^{5,23} In addition, treatment by some HDIs, notably vorinostat,²⁴ and panbinostat,²⁵ have been associated with serious cardio-toxicity in the treatment of solid cancers.^{18,26} As class II HDACs have been demonstrated to be

abundant in heart tissue, designing inhibitors inactive against class II HDACs could potentially alleviate some of these cardiotoxicities. Due the prevalence of these significant toxicities, increased effort has been directed toward developing HDIs that selectively inhibit certain classes or single isoforms.

In conjunction with the goal of reducing some of the dose limiting toxicities observed with broad-spectrum HDIs, the transition to class- and isotype-specific inhibition could also yield greater therapeutic value. HDACs are frequently over-expressed in many human diseases, predominantly cancers. In particular, class I HDACs are often overexpressed in human tumors, and selective knockdown of HDAC 1 or 2 has been shown to be sufficient to decrease tumor growth.²⁷ Accordingly, development of new inhibitors has focused on the inhibition of class I HDACs. While numerous class I specific HDIs have been developed, with many showing preclinical promise, only a few, including the FDA approved romidepsin (FK-228, Istodax),^{28–30} CHR-3966,³¹ entinostat,³² mocetinostat,³³ chidamide (CS055/HBI-8000),³⁴ have entered the clinic.¹⁹ In addition to these class I specific HDIs, class I and II specific HDIs, including AR-42³⁵ and the hydroxamide quisinostat (JNJ26481585),³⁶ have been developed and put to clinical trial.¹⁹

Treatment of hematologic malignancies with HDI

Unlike many types of solid cancers, hematologic malignancies have proved particularly sensitive to HDI treatment (Table 1). The first disease to have an FDA designated HDI as a treatment option, following failed attempts of other systemic treatments, was cutaneous T-cell lymphoma (CTCL). CTCL consists of extranodal non-Hodgkin's lymphomas (NHLs) made up of malignant, clonal T-cells presenting in the skin. Numerous phase I and II clinical trials have been undertaken for CTCL using the panHDIs vorinostat,^{37,38} panbinostat,³⁹ and belinostat,^{40,41} both as single agents and in combination with other chemotherapeutic compounds. Notably, panbinostat has been investigated in patients after treatment with the retinoid bexarotene,⁴² and belinostat has been investigated in combination with many agents including bortezomib, 5-fluorouracil, 5-azacytidine, carboplatin, paclitaxel, and isotretinoin.⁴³ These drugs were generally well tolerated in CTCL patients as well as in peripheral T-cell lymphoma (PTCL),^{44,45} Hodgkin's lymphoma,^{46–48} multiple myeloma,^{49,50} diffuse B-cell lymphoma,⁵¹ myelodysplastic syndrome,⁵² and acute myeloid leukemia,⁵³ often achieving complete remission (CR), partial remission (PR), and sustained disease (SD), while exhibiting manageable dose limiting toxicities such as fatigue, nausea, anemia, and vomiting.⁴³

Table 1 Trials of Pan/Global HDIs in Hematological Malignancies

Agent	Combinations	Disease	Phase	Number	Response	Citation
Vorinostat	Single agent	CTCL	II	33	PR 8	37
Vorinostat	Single agent	CTCL	IIb	74	CR 1, PR 21	38
Vorinostat	Single agent	Hodgkin's Lymphoma	II	25	PR 1, SD 7	47
Vorinostat	Single agent	Advanced Leukemias, and Myelodysplastic syndrome	I	29	CR 3	52
Vorinostat	Carfilzomib, lenalidomide, dexamthasone	MM	I	17	PR 9, 5 SD, PD 2	50
Panbinostat	Single agent	Hodgkin's Lymphoma	II	129	PR 30, CR 5	48

Table Continued

Agent	Combinations	Disease	Phase	Number	Response	Citation
Panbinostat	Bexarotene	CTCL	II	118	CR/PR 24	42
Belinostat	Single agent	PTCL	II	31	CR 13, PR 18	45
Belinostat	Single agent	CTCL/PTCL	II	CR, PR, SD, PD	CR, PR, SD, PD	41
Abexinostat	Single agent	Relapsed lymphoma	I/II	21	CR 2, PR 14	85

Abbreviations: SD, sustained disease; CR, complete remission; PR, partial remission; DR, durable response; WT, well-tolerated; NR, no response; CTCL, cutaneous T-cell lymphoma; AML, acute myeloid leukemia; PTCL, peripheral T-cell lymphoma; MM, multiple myeloma.

Whereas hundreds of clinical trials have been undertaken with panHDIs in the treatment of hematologic malignancies, fewer have been undertaken with class and isoform specific HDIs (Table 2). In

order to investigate the efficacy of these specific HDIs in treating hematologic malignancies, we review the results of a selection of clinical trials.

Table 2 Trials of Specific HDIs in Hematological Malignancies

Agent	Combinations	Disease	Phase	Number	Response	Citation
Romidepsin	Single agent	CTCL	II	71	CR 1, PR 20	57
Romidepsin	Single agent	CLL, AML	I	20	NR	53
Romidepsin	Single agent	CTCL	II	96	CR 5, PR 21	56
Romidepsin	Single agent	PTCL	II	45	CR 8, PR 9	55
Entinostat	Single agent	Lymphoid malignancies and refractory solid tumors	I	22	WT	32
Chidamide	Single agent	Advanced solid tumors, lymphomas	II	31	PR 5	34
AR-42	Single agent	Relapsed MM, lymphoma	I	17	WT	35
Mocetinostat	Single agent	lymphocytic leukemia	II	21	NR	33

Abbreviations: SD, sustained disease; CR, complete remission; PR, partial remission; DR, durable response; WT, well-tolerated; NR, no response; CTCL, cutaneous T-cell lymphoma; AML, acute myeloid leukemia; PTCL, peripheral T-cell lymphoma; MM, multiple myeloma.

Romidepsin is a bicyclic peptide that, although originally developed for its ability to reverse the ras-transformed phenotype to normal, was found to potently inhibit class I HDACs in 1998.²⁹ Phase I and II clinical trials initiated by the National Cancer Institute achieved therapeutic responses in patients with CTCL and PTCL.^{44,53,54} Phase II studies into the efficacy of romidepsin were conducted using a dosage of 14 mg/m² infused over four hours on days 1, 8, and 15 of a 8-day cycle, and resulted in a number of PRs.^{29,44} Further phase II studies confirmed this efficacy achieving both CR and PR in CTCL and PTCL patients.^{55,56} These successes were tarnished by the sudden cardiac death of two patients in a trial conducted by Piekarczyk et al.,⁴⁴ which indicated cardio-toxicity as observed in panHDIs.^{29,57} This finding, however, led to a systematic study into the potential cardiac effects of romidepsin treatment, which confirmed the drug's safety.³⁰

Entinostat (MS-275), selectively inhibits class I HDACs and exhibited promising pre-clinical results showing not only potent antiproliferative functions via the induction of (CIP1/WAF1), but also increased expression of differentiation markers (CD11b) and induction of apoptosis via increases in reactive oxygen species, mitochondrial damage, and caspase activation.^{19,32,58} A phase I clinical trial also established that unlike other HDI treatments, treatment with Entinostat resulted in no detectable cardiac events.³² Investigation into the combination treatment of myeloid malignancies with Entinostat and 5-Azacytidine in which, participants were treated for ten consecutive days with various doses of 5-Azacytidine and received Entinostat orally on intermittent days on a 28 or 29 day cycle.^{6,59} Within the group of 30 patients who received at least 4 therapy cycles, three had a complete remission, four had a partial remission, and

seven showed hematologic improvements.⁵⁹ Alone or in combination with standard therapies HDIs are showing promise.

Chidamide (CS055/HBI-8000) represents a benzamide class HDI that is designed to inhibit the catalytic site of class I HDACs, while exhibiting improved metabolic stability as compared to other hydroxamic acid and benzamide type inhibitors.^{34,60,61} Studies into enzymatic inhibition by chidamide have shown that it effectively inhibits class I HDACs and class II HDAC 10.^{34,61} In addition, pre-clinical studies have shown broad-spectrum in vitro and in vivo anti-tumor activity as well as oral bioavailability with chidamide treatment.^{34,61} A phase I clinical trial was performed on a cohort of 31 patients with advanced solid tumors or lymphomas where doses between 5 to 50 mg chidamide were administered several times per week for four consecutive weeks every six weeks. Overall, the treatments were well tolerated within the trial period. Chidamide was also shown to display favorable pharmacokinetic and pharmacodynamic qualities that had a relatively long half-life while demonstrating significant preliminary anti-tumor activity.³⁴ Finally, consistent with previous clinical studies that demonstrated HDI's significant single-agent anti-tumor activity in lymphomas, chidamide treatment produced four partial responses out of five patients enrolled with T-cell lymphoma, while also producing partial responses in a patient with adenoid cystic carcinoma of the submandibular gland.^{34,38,56}

Mocetinostat is an isotype-specific aminophenylbenzamide that inhibits HDAC classes I and IV with very little class II inhibition that has been involved in different phase I and phase II studies for hematologic malignancies.⁶² The results of a phase II trial in patients with advanced chronic lymphocytic leukemia (CLL), where patients received

mocetinostat with an escalating dose or the addition of rituximab was permitted after two or more cycles without response, demonstrated limited anti-cancer activity as no responses were observed.³³ The limitations of mocetinostat use in CLL were additionally apparent with the observation of several toxicities including infections, thrombocytopenia, anemia, diarrhea, and fatigue.^{6,33} These findings demonstrate the need for further investigations into combinatory treatments with mocetinostat.⁶ However, a later phase II trial in the treatment of classical Hodgkin's lymphoma showed mocetinostat as a promising single-agent with controllable toxicity in patients with relapsed classical Hodgkin's lymphoma.⁶³

HDIS in breast cancer

No longer viewed as a single disease, breast cancer consists of a diverse group of tumors and is the most common cancer diagnosed in women worldwide.⁶⁴ The heterogeneity of breast tumors has long been observed in histological and clinical outcomes, however, recent advances in molecular biology have allowed for more refined classifications that link molecular characteristics with disease mechanisms to clinical outcomes.^{65,66} Comprehensive gene expression profiling has allowed for the classification of breast tumors into at several major subtypes each with their own risk factors, treatment responses, risk of disease progression, and organ sites of metastases.⁶⁵ Luminal tumors are positive for estrogen (ER) and progesterone receptors (PR) and respond well to hormonal interventions. Human epidermal growth receptor 2+(HER2+) tumors are characterized by overexpression of the ERBB2 oncogene, and have been effectively

controlled with a diverse array of anti-HER2 therapies. Finally, basal like tumors in general lack hormone receptors and HER2 and are therefore referred to as triple-negative breast cancer (TNBC).^{67,68} This group, which is one of the highest priority areas of research in breast cancer, responding approximately 20% of the time to standard chemotherapy, can further be divided into additional subclasses, each with its own molecular features and sensitivity to treatment.⁶⁵

Corresponding with other solid cancers, but contrasting hematologic malignancies, far fewer clinical trials have been undertaken using HDIs in the treatment of breast cancer. Phase I and II clinical trials thus far have focused on the panHDIs vorinostat,⁶⁹⁻⁷¹ and panbinostat⁷² in metastatic breast cancer (Table 3). Further contrasting the use of HDIs in hematologic malignancies, nearly all HDI regimens in breast cancer treatments are administered in combination with other chemotherapeutic drugs. Vorinostat has been combined with paclitaxel and bevacizumab,⁷⁰ and tamoxifen⁷³ in phase I and II trials, where treatment regimens have been well tolerated with response rates of approximately 40 percent. Panbinostat has been combined with letrozole in a phase I trial in postmenopausal metastatic breast cancer, with a moderate response showing 2 and 5 of 12 participants experiencing PR and SD respectively.⁷² Vorinostat has also been investigated as a single-agent treatment in breast cancer, however response rates were poor.⁶⁹ These trials demonstrated that, although HDI has not been seen to be particularly effective in the treatment of breast cancer as single-agents, the promise that combinatory treatments exhibit necessitates further investigation.

Table 3 Trials of Pan/Global HDIs in Breast Cancer

Agent	Combinations	Disease	Phase	Number	Response	Citation
Vorinostat	Single agent	Metastatic breast cancer	II	14	SD 1	69
Vorinostat	Paclitaxel, bevacizumab	Metastatic breast cancer	I/II	44	CR 2, PR 24, SD 16	70
Vorinostat	Tamoxifen	Hormone-therapy resistant breast cancer	II	43	DR 8, SD 9	73
Panbinostat	Letrozole	Post-menopausal metastatic breast cancer	I	12	PR 2, SD 5	72

Abbreviations: SD, sustained disease; CR, complete remission; PR, partial remission; DR, durable response.

Although the efficacy of HDI in breast cancer clinical trials has not been demonstrated quite as extensively as in hematological malignancies, numerous preclinical studies have implicated HDACs as potential therapeutic targets. Treatment of TNBC cell lines MDA-MB-157, MDA-MB-231, MDA-MB-468, and BT-549 with panbinostat resulted in decreased cell proliferation and survival, apoptosis induction (excluding MDA-MB-468), and induced changes in cell morphology consistent with reversal from the mesenchymal phenotype.⁷⁴ Additionally, panbinostat treatment, in combination with salinomycin, of TNBC cells and an in vivo xenograft mouse model synergistically inhibited cell proliferation through the induction of apoptosis, cell cycle arrest, and epithelial mesenchymal transition (EMT) regulation.⁷⁵ These findings implicate both single-agent and combinatory efficacy for HDI treatment in TNBC, although these results have been tempered by limited results in a phase II trial of entinostat.⁷⁶ Further evidencing the promise of HDI treatment in combination with other drugs, the inhibitor LAQ824 was demonstrated to down-regulate HER2 and sensitize breast cancer cells to trastuzumab, taxotere, gemcitabine, and epothilone B.⁷⁷

Due to observed overexpression of class I HDACs 1, 2, and 3 in

varying breast tumor types much of the research on HDI in breast cancer has focused on the class I HDACs and their inhibitors.⁷⁸ HDACs 2 and 3 expression was demonstrated to be higher in less differentiated tumors and was correlated with the negative hormone receptor expression phenotype associated with more aggressive tumor types.⁷⁸ Concordantly, HDAC 2 overexpression was significantly correlated with HER2 expression and nodal metastasis. In contrast, HDAC 1 was highly expressed in hormone receptor positive tumors, which generally exhibit less aggressive features.⁷⁸ This finding, however, conflicts with earlier experimental evidence showing negative regulation of the estrogen receptor alpha (ER) by HDAC 1.⁷⁹ In addition to HDACs 1, 2, and 3 the class I HDAC 8 has been implicated in the progression of breast cancer. Upregulated HDAC 8 expression was demonstrated in paired breast cancer tissue from both a TCGA data set and a cohort of Taiwanese patients, and was associated with poor prognosis in early-stage breast cancer, late-stage disease, and tumor progression.⁸⁰ Knockdown by si-HDAC8 and inhibition with the novel HDAC 8 inhibitor PCI-34051 markedly reduced (by 89.0%) cell migration, as evidenced in both Transwell and wound-healing assays, in the highly invasive breast cancer cell

line MDA-MB-231.

In addition to the class I HDACs, class II HDACs 5 and 9 have been implicated as potential therapeutic targets in breast cancer.^{81,82} HDAC 5 overexpression has been demonstrated in human breast cancer cell lines, where it functionally interacts with lysine-specific demethylase 1 (LSD1) to promote cancer progression. Depletion of HDAC 5 in MDA-MB-231 cells resulted in decreased cellular LSD1 levels, hindered cellular proliferation, induced G1 cell-cycle arrest, and attenuated cell migration and colony formation.⁸¹ Similarly, HDAC 9 overexpression was shown in highly aggressive basal breast cancer cell lines, as well as basal tumors, and was associated with higher rates of gene transcription and epigenetic marking of the HDAC 9 promoter.⁸² Ectopic expression of HDAC 9 in luminal breast cancer cells resulted in increased cell proliferation and decreased apoptosis. These effects, associated with deregulated expression of HDI controlled genes such as CDKN1A, BAX, and TNFRSF10A, were reversed in basal breast cancer cells after HDAC 9 knock-down.⁸² These findings implicate the involvement of HDACs 5 and 9 in the regulation of mammary carcinogenesis and HDI response, and could potentially serve as targets in personalized HDI treatment.

Conclusion

Histone deacetylase inhibition represents a promising epigenetic strategy for the treatment of a number of diseases, in particular in hematologic malignancies and other cancers. Following the early successes of panHDI in the treatment of hematologic malignancies, and the subsequent discovery of dose-limiting toxicities and unintended non-target effects of the same, there has been a trend toward the development of class- and isoform-specific HDIs that can be individualized toward specific diseases, improving patient outcomes while limiting the drugs' toxic effects.⁵ While these novel HDIs have been demonstrated to have higher potencies in preclinical investigation, and have produced positive results in early clinical trials, further investigation is needed in order to determine if selective HDAC inhibition will remain effective at treating disease with minimal adverse effects.⁴³ The clinical success of single agent and combinatory HDI treatments of hematologic malignancies stands in contrast to the limitations of HDI treatment in solid cancers, particularly breast cancer. However, strong preclinical evidence has been elucidated implicating the potential for selective HDI success in breast cancer.^{74–78,80–84} The contrast between the success of panHDI in the treatment of hematologic malignancies, where selective HDI treatments have only showed modestly improved outcomes, with the newly emerging implications for success in selective HDI treatment in breast cancer, demonstrates the need for further clinical investigation into class/isoform specific HDAC inhibition.

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None.

Conflict of interests

The authors have declared that no conflict of interest exists.

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