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The clinical development of histone deacetylase inhibitors as targeted anticancer drugs

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Abstract

Importance of the field—Histone deacetylase (HDAC) inhibitors are being developed as a new, targeted class of anticancer drugs.

Area covered in this review—This review focuses on the mechanisms of action of the HDAC inhibitors, which selectively induce cancer cell death.

What the reader will gain—There are 11 zinc-dependent HDACs in humans and the biological roles of these lysine deacetylases are not completely understood. It is clear that these different HDACs are not redundant in their activity. This review focuses on the mechanisms by which HDAC inhibitors can induce transformed cell growth arrest and cell death, inhibit cell mobility and have antiangiogenesis activity. There are more than a dozen HDAC inhibitors, including hydroxamates, cyclic peptides, benzamides and fatty acids, in various stages of clinical trials and many more compounds in preclinical development. The chemically different HDAC inhibitors may target different HDACs.

Take home message—There are extensive preclinical studies with transformed cells in culture and tumor-bearing animal models, as well as limited clinical studies reported to date, which indicate that HDAC inhibitors will be most useful when used in combination with cytotoxic or other targeted anticancer agents.

Keywords

apoptosis; HDAC inhibitor; histone deacetylases; mechanism of action; reactive oxygen species; ROS; suberoylanilide hydroxamic acid

1. Histone deacetylases (HDACs)

Epigenetics refers to the regulation of gene expression though post-translational modification of protein complexes associated with DNA without changes in DNA sequence

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Declaration of interest
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[1,2]. DNA is packaged in chromatin, which is structurally complex and dynamic, consisting of DNA, histones, and non-histone proteins. These proteins are subject to post-translational modification by acetylation of lysines, methylation of lysines and arginines, phosphorylation of serines, ubiquitination of lysines, sumoylation, proline, isomerization, and ADP-ribosylation. Acetylation and deacetylation, perhaps the best studied of these post-translational protein modifications, are regulated by the activity of two sets of enzymes, histone deacetylases (HDACs) and histone acetyltransferases (HATs) [3,4]. HATs catalyze the acetylation of lysines, which neutralizes the positive charge on histone and allow the negatively charged DNA to assume a transcription-competent conformation. HDACs remove acetyl groups from lysines, allowing interaction between negatively charged DNA and positively charged histone proteins, which can result in heterochromatin and transcriptional silencing of genes. Patterns of acetylation and methylation of lysines, as well as other post-translational modifications, appear to be a code for specific recruitment of protein complexes regulating gene expression and DNA replication and stability [5]. HDACs and HATs have many non-histone protein substrates including proteins of transcription complexes that have a role in regulating gene expression, and proteins in pathways that regulate cell proliferation, cell migration, cell death and angiogenesis [6,7].

Eighteen HDACs have been identified in humans that are classified based on their homology to yeast HDACs (Table 1). Eleven of these HDACs are zinc-dependent enzymes: class I, HDAC1, 2, 3, and 8 have homology to yeast RPD3; class IIa, HDACs 4, 5, 7, and 9 have homology to yeast HDA1; class IIb, HDACs 6 and 10 have two catalytic sites, and class IV, HDAC11, has conserved residues shared with both class I and class II deacetylases [6,8]. Class III HDACs include sirtuins 1 – 7, which have homology to yeast Sir2 and have an absolute requirement for NAD^+.

Analysis of lysine acetylation targets in transformed cells found 3600 acetylated lysines in 1750 proteins [7]. Inhibition of HDACs with vorinostat (suberoylanilide hydroxamic acid; SAHA, see Section 3) altered only about 10% of these acetylation sites. These sites were identified on proteins that regulate gene expression, RNA signaling, DNA damage repair, cell cycle progression, nuclear transport, cytoskeleton function, protein chaperones, and ribosome formation and function [7]. HDACs have many non-histone protein targets; phylogenic studies indicate that HDACs preceded histones in evolution of organisms [9].

Class I HDACs are primarily localized in the nucleus (Table 1). HDAC3 can shuttle between the nucleus and cytoplasm. Class II HDACs are primarily localized in the cytoplasm and shuttle between the nucleus and the cytoplasm. Class I HDACs are expressed in all tissue and have histone as substrates. Class I HDACs are found in protein complexes with transcription factors and co-repressors. HDACs do not bind directly to DNA and are recruited to target genes via association with transcriptional activators and repressors, incorporated into large multiprotein transcription complexes.

Class I HDACs have a simpler structure than class IIa or IIb HDACs, with relatively short amino acid and carboxy terminal extensions from the catalytic site. Class I HDACs have a primary role in cell survival and proliferation, based on, knockout studies and inhibition with class I-selective inhibitors [10,11]. Deletion of both HDAC1 and 2, but not either

alone, causes cancer cell death and neural precursor maturation defects. HDAC1 and 2 redundantly regulate cardiac morphogenesis, growth and contractility [12]. HDAC2 is a regulator of chromatin compaction status and its downregulation or inhibition causes chromatin decondensation and sensitization to DNA-targeted anticancer drugs [13]. HDAC3 deletion causes early embryonic lethality. Inactivation of HDAC3 was associated with cessation in cell cycle progression, DNA damage, and impaired repair and apoptosis [14].

Class II HDACs have more tissue-specific regulatory function than class I HDACs [6,8,11]. Class IIa HDACs have conserved binding sites for transcription factors and the chaperone proteins 14-3-3, which are involved in regulation of the shuttling of these enzymes between the nucleus and cytoplasm. HDAC4 regulates neural survival in normal and diseased retinas [15]. The expression of HDAC4 is repressed by miRNA 206, facilitating reinnervation [16]. Mice lacking HDAC4 expression have premature ossification of developing bones, while overexpression of HDAC4 inhibits chondrocyte and osteocyte differentiation. HDAC5 knock-down mice have large hearts [8]. HDAC5 and HDAC9 are involved in the development of myocardium and skeletal muscle. HDAC7 is involved in the regulation of vascular endothelial development and vascular integrity [17].

HDAC6 is unique among the 11 zinc-dependent HDACs in having two catalytic sites and an ubiquitin binding site (see Section 4.7). Specific substrates of HDAC6 include α-tubulin, cortactin, transmembrane proteins such as IFN-αR, HSP90 and other chaperone proteins, and peroxiredoxins [18–21]. Paradoxically, HDAC6 inhibition can increase resistance of cancer cells to oxidative stress by causing the accumulation of acetylated peroxiredoxins. Acetylated peroxiredoxins have increased activity as H_2O_2 reductases [19]. The ubiquitin binding site toward the c-terminal end of HDAC6 plays a critical role in aggresome formation in the pathway of proteolysis of misfolded proteins [22,23].

Little is known about the function of HDAC10. HDAC11 negatively regulates expression of the gene encoding IL-10 in antigen-presenting cells which induce T-cell activation as well as T-cell tolerance [24]. These findings suggest that HDAC11 has molecular targets that influence immune activation.

Although HDACs are widely distributed in chromatin, inhibition of these enzymes alters the transcription of a relatively small proportion (2 – 10%) of expressed genes in transformed cells [25–28].

The regulation of HDAC activity can occur at multiple levels including protein–protein interaction, post-translational modification (sumoylation, phosphorylation, proteolysis, subcellular localization) and by metabolic co-factors [29,30]. For example, HDAC1 promoter is inducible by IL-2. HDAC4 promoter is regulated in part by P1/SP3 transcription factor. Phosphorylation and subsequent association with 14-3-3 regulates subcellular localization of HDAC4, HDAC5, HDAC7, and HDAC9.

The crystalline structure of the catalytic site of a histone deacetylase-like protein was solved with binding of inhibitors, trichostatin A (TSA) and SAHA [31]. More recently, the crystal structure of human HDAC4, 7 and 8 have been solved [32–35].

2. Aberrant HDACs and cancers

Structural mutations in HDACs associated with cancers appear to be rare. A mutation of HDAC2 has been reported in colon cancer and endometrial cancer cell lines [36]. HDAC4 mutations have been identified in breast cancer in a large scale sequencing study of breast and rectal colon cancers [37].

Altered expression and aberrant recruitment of HDACs have been reported in many human cancers [6,8,38,39]. Systematic analysis of cultured cancer cell lines (primarily cultures of human cancer cells and various human tumor biopsy samples) found that many had higher levels of expression of the zinc-dependent HDACs than in corresponding normal tissues. Overexpression of class I HDACs has been reported in esophageal, prostate, non-small cell lung, gastrointestinal, and oral cancers [38–42]. Analysis of the expression of histone deacetylases in lymphomas found that the most frequently altered HDAC was HDAC6, which was weakly expressed or undetected in 9 out of 14 lymphoid cell lines and in 83 of 89 primary lymphoma tissue specimens [43]. HDAC5 and HDAC10 have been reported as decreased in expression in lung cancer associated with poor prognosis. HDAC6 is overexpressed in many cutaneous T-cell lymphoma (CTCL) and peripheral T-cell lymphoma (PTCL) cells. HDAC8 expression correlates with poor outcome in neuroblastoma and inhibition of HDAC8 induces differentiation of transformed cells [44].

Fusion proteins involving chromosomal translocation have been identified in acute myelocytic leukemia (AML) and acute lymphoblastic leukemia (ALL) [45,46]. The chromosomal translocation results in a fusion protein (MLL-CBP) consisting of the CBP and mixed linage protein, MLL. Aberrant recruitment of HDACs to the transcription factors that involve oncogenetic DNA binding fusion proteins resulting from chromosomal translocations or overexpression of repressive transcription factors also include oncogenetic PMA–RARα or PLZF–RARα and AML1–ETO fusion proteins in acute pro-myelocytic and acute myelogenous leukemia.

3. Histone deacetylase inhibitors (HDACi)

HDACi have been discovered that have different chemical structures including hydroxamic acids, cyclic peptides, electrophilic ketones, short-chain fatty acids, benzamides, boronic acid-based compounds, benzofuranone- and sulfonamide-containing molecules, and α/β peptide structures (Table 2) [47–53]. Various chemical modifications have incorporated solubilizing functional groups, which may improve pharmacokinetic properties. Considerable efforts are being made to develop isoform-selective deacetylase inhibitors such as tubacin, which selectively inhibits HDAC6, and PC-34051, a selective HDAC8 inhibitor [18,50,53].

The hydroxamic class of HDACi, of which vorinostat (SAHA) is the first HDACi developed and approved by the US FDA for clinical use [47], generally have common structural characteristics: zinc-binding moiety (ZBM) in the catalytic pocket, opposite capping group, and straight-chain alkyl, vinyl, or aryl linker connecting the two. These functional groups interact with three relatively conserved regions of the catalytic pocket of HDACs. The zinc facilitates hydrolysis of the acetyl–lysine bond and is at the bottom of the narrow catalytic

pocket. A hydrophobic chain with six carbons is generally the optimal link of the ZBM and the opposite capping group. The capping group about the catalytic site opening is generally a hydrophobic structure that interacts with the rim amino acids. The amino acid sequence of the rim surrounding the catalytic site of the different HDACs has greater sequence diversity compared with the other domains, and thus may have the most potential to be manipulated to develop selective HDACi.

A new concept in HDACi structures is combining inhibition of protein kinases and HDACs in one molecule [54]. That structurally diverse compounds are effective inhibitors of HDACs suggests that the mechanism of action of these compounds may involve not only blocking the catalytic site but also binding of the rim of the enzyme with other proteins independent of the deacetylase activity.

4. Mechanism of action of histone deacetylase inhibitors

HDACi can induce transformed cell death by one or more pathways (Figure 1). HDACi can cause transformed cell growth arrest, cell death and inhibition of angiogenesis. The pathway leading to cell death of transformed cells induced by HDACi depends, in part, on the HDACi, concentration and time of exposure to the agent and the molecular characteristics of a particular transformed cell. Normal cells are relatively resistant to HDACi-induced cell death [55,56].

4.1 DNA damage and repair

There is no evidence that HDACi are directly mutagenic. Histone acetylation, induced by HDACi, does cause structural alterations in chromatin, which can expose DNA to damaging agents, such as UV, radiation, cytotoxic drugs, and reactive oxygen, resulting in DNA double-strand breaks (DSB) [6,8,57].

HDACi can induce the accumulation of reactive oxygen species (ROS), resulting in DNA damage [55,56,58]. HDACi induce the accumulation of the phosphorylated form of H2AX, a marker of DSB [59]. HDACi can downregulate the expression of genes for DNA repair proteins involved in homologous recombination, including, RAD51, BRACA1, and BRAC2, and in non-homologous DSB repair including Ku70, Ku86 and DNA-PKcs [60–65]. The accumulation of DSB causes altered gene expression and leads to apoptotic cell death. Transformed cells may have many defects in pathways of DSB repair and, unlike normal cells, lack the capacity to repair DNA damage.

The synergy of HDACi and DNA-damaging agents, such as cytotoxic drugs or radiation, may result from the combined effects of HDACi in inhibiting DNA repair processes as well as activating intrinsic and extrinsic cell apoptotic death pathways (see Section 4.4).

4.2 Altered gene expression

HDACi altered gene transcription by inducing acetylation of histones, transcription factors and other proteins regulating gene expression [6–8]. Early experiments with lymphoid cell lines cultured with TSA showed that only about 2% of expressed genes were altered, either increased or decreased, compared with untreated cells [25]. More recent studies, using

cDNA arrays, found as many as 10 – 20% of expressed genes altered in their transcription in cell lines of leukemia, multiple myeloma, and colon, kidney, prostate, and breast cancer cells cultured with the HDACi [26–28]. The number of genes with altered expression increased with time of culture and concentration of HDACi. Some changes in gene expression are probably direct effects of the HDACi, while many may be downstream effects. The pattern of alterations of gene expression are similar for different HDAC inhibitors, but there are differences induced by different agents in various transformed cells [28].

The cyclin-dependent kinase (CDK) inhibitor, p21 (WAF1/CIP1), is one of the most commonly induced genes by HDACi [66]. HDACi-induced expression of p21 is independent of p53. In ARP-1 cells, vorinostat caused specific modifications in the pattern of acetylation and methylation of lysines in histones H3 and H4 associated with the proximal promoter region of the p21 gene [67]. Histone acetylation or methylation in the promoter region of the expressed p27 (KIP1) or the silent epsilon globin gene in HDACi-cultured ARP-1 cells were not altered, nor was the expression of these genes. Vorinostat caused a marked decrease in HDAC1 and Myc and recruitment of RNA polymerase II in the protein complex associated with the proximal promoter region of the p21 gene, with little detectable change in HDAC2, Brg1, GCN5, P300 pr Sp1 proteins in the complex. These findings suggest that the selective alteration of transcription of a gene by HDACi may be determined by the composition of proteins in the transcription factor complex including the HDACs.

HDACi can inhibit gene expression mediated by STAT5 [68]. HDACi can repress the transcription of the androgen receptor (AR) gene [69] and block AR-mediated transcriptional activation of genes. HDACi, such as SAHA, can alter the miRNA expression profile in transformed cells [70]. These miRNAs have target genes related to angio-genesis, apoptosis, chromatin modification cell proliferation and differentiation. HDACi can activate a Sp1/Sp3- mediated induction of multiple immediate early (fos, Juh, egr1, egr3, atj3, arc, mr4a1) and stress response genes (mdrg4, Mt1B, MtiE, Mt1f, ME1H) associated with apoptosis of colon cancer cells [71].

4.3 Cell growth arrest

HDACi can induce cell growth arrest in both normal and transformed cells in culture. Vorinostat causes predominantly G1 arrest at low concentration and both G1 and G2/M arrests at higher concentration [66].

In cells cultured with HDACi, increased levels of the CDK inhibitors and decreased levels of cyclins may account for reduced CDK activity, causing dephosphorylation of Rb, blocking E2F activities in the transcription of genes for G1 progression and G1/S transition [72,73]. HDACi can kill both proliferation and non-proliferating transformed cells [56,74]. This is in contrast to the action of many chemotherapy drugs, which are effective only on proliferating transformed cells.

4.4 Induced apoptosis

HDACi can induce death of transformed cells by activating the extrinsic and/or intrinsic apoptotic pathways [6,8,11,75–80]. A number of downstream components, such as activation of caspase-3, are shared in the extrinsic and intrinsic pathways [76]. The extrinsic apoptotic pathway is initiated by binding of death receptor, including Fas, TNF receptor-1 (TNFR-1), TNF-related apoptosis-inducing ligand (TRAIL) receptor (DR-4 and −5), DR-3 (Apo3) and DR-6, to their ligands leading to activation of caspase-8 and −10 [80]. HDACi can upregulate expression of both the death receptors and their ligands *in vitro* and *in vivo* in transformed cells, but not in normal cells. For example, *M*-carboxycinnamic acid, bishydroxamide (CBHA) induced Fas and Fas ligand in neuroblastoma cells [79]. TNF was upregulated by depsipeptide in HL-60 and K562 cells, and C-FLIP, an inhibitor of the death receptor pathway, was downregulated. Sequential treatment with vorinostat followed by TRAIL was shown to target multiple pathways in tumor progression, angiogenesis and metastasis. HDACi can cause TNF-related apoptosis inducing ligand protein degradation by inhibiting the ubiquitin-dependent pathway, an effect that may be the basis of the effectiveness of the combination of HDACi with a proteasome inhibitor in inducing transformed cell death [80]. Taken together, these studies indicate that the extrinsic apoptotic pathway can account for HDACi-induced cell death in many transformed cells. Combination therapy of HDACi with factors inducing the extrinsic apoptotic pathway have a potential for effective therapeutic strategies.

Intrinsic apoptotic pathway is mediated by disruption of mitochondria with the release of mitochondrial intermembrane proteins, including cytochrome c, AIF and Smac, leading to activation of caspases [6,8,11,81,82]. HDACi induce intrinsic apoptotic pathway by inactivation or suppression of antiapoptotic proteins and activation of pro-apoptotic proteins. HDACi can induce the pro-apoptotic cleavage of Bid, which initiates the intrinsic pathway leading to mitochondrial disruption in transformed cells. High levels of expression of Bcl-2 or Bcl-X_L, which protect mitochondria, have been found in some transformed cells resistant to HDACi-induced cell death [77]. Inhibition of Bcl-2 by a chemical inhibitor, such as HA14 – 1, can increase sensitivity to HDACi-induced cell death. HDACi upregulate pro-apoptotic proteins of the Bcl-2 family, such as Bim, Bmf, Bax and Bik, and decrease antiapoptotic proteins of the Bcl-2 family, such as Bcl-2, Bcl-$X_{L,}$ Bcl-w and Mcl-1, as well as the pro-survival gene inhibitor of apoptosis, XIAP; it also induces degradation of survivin.

The HDACi, vorinostat or butyrate, induced autophagic cell death with vacuoles in the cytoplasm of transformed cells with Apaf-1 knockout or overexpression of Bcl-X_L [83]. A senescence phenotype with polyploidy was induced by vorinostat in HCT1165 colon cancer cells [84].

4.5 Mitotic disruption

HDACi can cause aberrant accumulation of acetylated histones in heterochromatin and centromere domains with consequent death of transformed cells [85–88]. In transformed cell cultures with TSA, histones in newly synthesized chromatin remain acetylated, and disrupt the structure and function of the centromere and the pericentric heterochromatin with loss of

binding to heterochromatin binding proteins. Histone acetylation also blocks histone phosphorylation, disrupting the function of mitotic spindle checkpoint proteins, such as BubR1, hBUB1, CENP-F and CENP-E, causing transient arrest at pro-metaphase and aberrant mitosis with chromosomal disruption resulting in cell death.

4.6 ROS and altered redox pathways

HDACi cause an accumulation of ROS in transformed cells but not in normal cells [55,56,89–91]. Increased cellular ROS can occur within 2 h of culture with HDACi, before disruption of mitochondria. Free radical scavengers such as N-acetylcysteine decrease HDACi-induced apoptosis, indicating that the generation of ROS is a factor in facilitating transformed-cell death. Thioredoxin (Trx) is a hydrogen donor that is required for activation of several proteins, including, ribonucleotide reductases, essential for DNA synthesis, and transcription factors, such as NF-κB. Reduced Trx is an antioxidant scavenger of ROS [92]. Vorinostat upregulates the expression of TBP-2 [56,58], which binds and inhibits reduced Trx activity, causing a downregulation of Trx in transformed but not in normal cells. Trx is an inhibitor of apoptosis signal-regulating kinase 1 (ASK1). Inhibition of Trx by binding to TBP2 activates ASK1 which, in turn, promotes apoptosis by inducing SET1-JNK and MKK3/MKK6-p38 signaling cascades, and enhancing the expression of pro-apoptotic protein Bim [93].

4.7 Inhibition of HDAC6 and target proteins

HDAC6 is unique among zinc-containing HDACs in having two catalytic sites: a ubiquitin binding site and cytoplasmic localization where it associates with non-histone substrates, such as HSP90 and α-tubulin [18–22,94–96]. Overexpression of HDAC6 leads to deacetylation of α-tubulin and an increase of cell motility. HDAC6 can bind both mono- and poly-ubiquitinated proteins and promotes its own ubiquitination. Specific inhibition of HDAC6 activity with tubacin or downregulation by SiRNAs cause accumulation of acetylated α-tubulin, HSP90, peroxiredoxins and other client proteins. HSP90 acetylation causes loss of chaperone function and exposes its client proteins, such as pro-survival and pro-proliferation proteins, Akt, Bcr-Abl, c-Raf and ErB2 to poly-ubiquitination and degradation via proteasome pathway [22,23,97]. HSP90 chaperone function is essential for the stability and function of several proteins, such as steroid hormone receptors and protein kinases involved in cell signaling pathways and cellular homeostasis. Recent studies have demonstrated both a direct physical interaction between HDAC6 and HSP90 and HDAC6 as a regulator of HSP90 activity through its deacetylation [22,95–98]. HDAC6 can bind directly to protein phosphatase (PP1) and cause simultaneous changes in cellular protein phosphorylation and acetylation; given the number of client proteins of HSP90, many molecular alterations can be a result of HSP90 inactivation through HDAC6 inhibition by HDACi.

HDAC6 is a component of the aggresome, a cellular structure that constitutes the major site of degradation for misfolded protein aggregates, both non-ubiquitinated and ubiquitinated misfolded proteins [22,23]. Misfolded proteins are susceptible to forming cytotoxic aggregates that can interfere with normal cell function. HDAC6 acts as a bridge between the dynein motors and the ubiquitination process, directing the poly-ubiquitinated proteins to the

aggresome. The BUZ domain of HDAC6 has high affinity for ubiquitin molecule and is involved in the transport of poly-ubiquitinated proteins. Loss of HDAC6 function increases the sensitivity of transformed cells to misfolded protein stress induced by proteasome inhibitor.

Taken together, these findings have implications for developing therapeutic strategies combining HDACi and proteasome inhibitors and/or HSP90 inhibitors in the treatment of certain malignancies. Indeed, there is an ongoing substantial effort to develop HDAC6 isoform selective inhibitors that may be clinically useful in treating cancers and possibly other diseases [50].

4.8 Antiangiogenesis

HDACi can exert anticancer activity by inhibiting tumor angiogenesis [99]. Solid tumors are frequently angiogenesis-dependent. Tumor angiogenesis can be mediated by hypoxia secondary to tumor growth or by increased oncogenic signaling and, as a consequence, induce hypoxia-inducible factor-1α (HIF-1α) and its transcriptional target, VEGF. HDACi inhibit angiogenesis via the suppression of HIF-1α and its target, VEGF, in animal models [100–102]. Under normoxic conditions, HIF-1α binds to von Hippel–Lindau protein (pVHL) and is inactivated by ubiquitination-proteasome degradation. Hypoxic conditions can increase transcription of HDACs 1, 2, and 3 in transformed cells, causing decreased expression of pVHL and, as a consequence, increased expression of HIF-1α, which promotes angiogenesis – a sequence of events that may be clocked by HDACi. HDACi can also induce HIF-1α degradation in a VHL-independent mechanism. Class II HDACs, HDAC4 or HDAC6, physically associate with HIF-1α and their selective inhibition by siRNA induces HIF-1α degradation. HIF-1α binds to HSP90 and disrupts the HSP90 chaperone function by acetylation, exposing HIF-1α to proteasomal degradation. These observations support the development of combination therapies of HDACi with antiangiogenesis drugs.

4.9 Antimetastatic

HDACi upregulate metastasis suppressor genes, such as Kangai (KAII), Ras homologue genes, RhoB, reversion-inducing cysteine-rich protein with KAZAL motifs (RECK) and tissue inhibitor of metalloproteinases (TIMP-1) [103]. Metastasis-promoting genes can be downregulated by HDACi, including genes for MMPs, integrin-α5 and collagen proteins. These findings suggest that HDACi may be effective in reducing the metastatic potential of primary tumors.

4.10 Glucose metabolism

HDACi target glucose transporter 1 (GLUT1-mediated glucose transport) and hexokinase I (HXK1) enzymatic activity, inhibiting glucose utilization in transformed cells [104]. This HDACi effect may have importance in selectively ‘starving’ transformed cells, contributing to cell growth arrest and death.

The present evidence, based primarily on studies with transformed cells in culture, indicates that HDACs have multiple targets that are involved in almost every cellular pathway critical

to cell survival, differentiation, proliferation, migration, and death (Figure 1). In cultured cell studies, HDACi induce cell death of transformed but not normal cells, which may reflect the capacity of normal but not transformed cells to 'recover' from exposure to reversible HDACi.

5. HDACi in combination with other anticancer agents

In preclinical studies with cultured cells and tumor-bearing animal models, and in clinical trials, HDACi have been used in combination with radiation; antimetabolites, such as 5-flurodeoxyuridine and gemcitabine; antitubulin agents, such as docetaxel, paclitaxel and epothilone B; topoisomerase (Topo) II inhibitors, doxorubicin, epirubicin, VP-16 (etoposide) and ellipticine; DNA cross-linking agent, cisplatin; HSP90 antagonist, 17-ally-amino-demethoxy gelda-namycin; and targeted agents, such as rituximab, trastuzumab, and EFGR inhibitor, erlotinib. Synergy or additive effects have been found with many of these combinations in inducing cancer cell death [6,8,105–112]. Romidepsin is synergistic with several conventional antileukemia/lymphoma drugs in leukemia and lymphoma cell line studies [103,109]. In preclinical studies, vorinostat was found to be synergistic with cytosine arabinoside and etoposide in treatment of acute leukemias [106]. HDACi have also been reported to have synergy with the transcription modulator, all-trans retinoic acid (ATRAL): DNA demethylating agent, 5-aza-2′ deoxycytidine, and the Bcr-Abl kinase inhibitor, imatinib. Upregulation of death receptors, and/or reducing the inhibitory regulators of death receptor pathway by HDACi, sensitizes tumor cells to TRAIL. HDACi can achieve synergy with TRAIL by simultaneous activation of the intrinsic and the extrinsic apoptotic pathways without changing the expression of TRAIL receptors or the inhibitory protein c-FLIP. Kinase inhibitors, including CDK inhibitor flavopiridol, phosphatidylinositol 3 kinase inhibitor, LY294002, FLT3 inhibitor, PKC412 and MEK1/2 inhibitor PD184352, potentiate the cell-killing effect of HDACi. Blocking NF-κB activation by the IκBα phosphorylation inhibitor, BAY 11-7082, markedly increased HDACi-induced apoptosis.

The synergistic effects of HDACi in combination with other drugs may depend on the sequence of drug administration. For example, prior treatment with HDACi, which induced chromatin decondensation, followed by Topo II inhibitors resulted in synergistic antitumor cell activity. The reverse order of administration of the drugs resulted in antagonistic effects, or had no more effect than each drug alone. Pretreatment with HDACi had greater antitumor effect than the reverse, in combination with cisplatin. These studies have guided, in part, clinical development of HDACi in combination therapeutic clinical trials.

A better understanding of the biological roles of the HDACs, the mechanisms of action of HDACi, and the development of selectively targeted HDACi should lead to increasingly effective combination HDACi therapeutic strategies.

6. Development of HDACi for therapy of non-oncologic diseases

Several studies have shown that HDACi have therapeutic potential in several non-oncologic diseases including inflammatory lesions, autoimmune disorders, neurological disorders, sickle cell anemia and malaria [6,8,113–120]. Vorinostat slowed the progression of Huntington-like syndrome in trans-genic mice [119] and increased expression of SNM

protein in spinal muscular atrophy fibroblasts [117]. HDAC2 modulates synaptic plasticity and long-lasting changes of neural circuits and, in turn, plays a role in regulating learning and memory. HDAC2-selective inhibition may be useful in memory impairment [118]. HDACi have been reported to be potent inducers of γ-globin gene expression with therapeutic value in the treatment of sickle cell anemia [120]. HDACi have anti-rheumatic activity in rodent models [121]. HDACi modulate stem cell survival and mobilization in *in vitro* studies [122]. HDACi have been shown to decrease multilineage differentiation potential of human mesenchymal stem cells [123]. HDACi have been found to improve animal survival after hemorrhagic shock [124].

7. Clinical development of HDACi as anticancer drugs

Over a dozen structurally different HDACi are in clinical trials either as monotherapy or in combination therapy for various hematologic and solid tumors (Table 2). Four major chemical classes of HDACi are currently in clinical trials, including short-chain fatty acid (butyrates and valproic acid), hydroxamates (vorinostat, panobinostat, belinostat, givinostat, PCI24781 and JNJ26481585), benzamides (entinostat and MGCD-103), and cyclic tetrapeptide (romidepsin). There are ongoing clinical trials with HDACi in combination therapy with radiation, cytotoxic agents, and different targeted anticancer agents (ClinicalTrials.gov [6,8,11,105–112,125]). These clinical trials include patients with cancer of lung, breast, pancreas, renal and bladder, melanoma, glioblastoma, leukemias, lymphomas, and multiple myeloma.

Vorinostat was the first of the HDACi to be approved for clinical use in the therapy of CTCL by the US FDA. In a Phase II study, orally administered vorinostat in 33 previously treated patients with refractory CTCL achieved partial response in eight patients (24.2%); 14 of 31 evaluable patients (45.2%) had pruritus relief. More recently, romidepsin received FDA approval for the therapy of CTCL [109,110].

Vorinostat is being evaluated in Phase II and III clinical trials as monotherapy and in combination with various anticancer agents for both hematologic and solid tumors [47,105,126,127]. Ongoing clinical trials in combination therapy for vorinostat include azacitidine, decitabine, the proteasome inhibitor, bortezomib, and taxanes.

Panobinostat (LBH589) is more potent than vorinostat in preclinical models [107,128]. It is in clinical trials for hematologic and solid tumors as monotherapy and various combination therapy protocols, including with proteasome inhibitors as well as with the DNA methylase inhibitor, azacitidine.

Other hydroxamic acid-based HDACi in clinical trials include belinostat (PDX101), givinostat (ITF2357) and JNJ26481585 (Table 2). Belinostat is in Phase I and II clinical trials for hematological and solid malignancies, including metastatic and refractory ovarian cancer. Givinostat is an orally administrated hydroxamate that is being investigated in a clinical trial in patients with pretreated refractory Hodgkin’s disease. Each of the hydroxamic acid-based HDACi in clinical trials has shown antitumor activity, including stable disease, partial response and in a few cases, complete responses of transient duration at doses generally well tolerated by the patients.

Adverse effects observed with the hydroxamic class of HDACi include fatigue, nausea, dehydration, diarrhea, and thrombocytopenia. With certain hydroxamic acid-based HDACi, electrocardiogram changes have occurred. These side effects have been reversible upon cessation of the administration of the drug.

Two benzamide HDACi are in clinical trials, entinostat (MS275, Sndx-275) and MGCD103 (Table 2). These agents are being evaluated as monotherapy and in combination with other anticancer drugs. Recently, clinical trials with MGCD103 were suspended owing to the development of pericarditis as a possible adverse effect. Entinostat is in clinical trials in patients with advanced acute leukemia and in patients with solid tumors, including Phase II clinical trials in patients with refractory metastatic melanoma.

Romidepsin, a cyclic peptide HDACi, is in clinical trials as monotherapy as well as in combination with gemcitabine. Romidepsin, FDA-approved for CTCL, is being evaluated in a Phase II study with patients with high-risk myelodysplastic syndrome and acute myelogenous leukemia [109,110]. Another Phase II clinical trial with depsipeptide is ongoing in patients with refractory lung cancer.

The fatty acids, including valproic acid, are relatively weaker HDACi than hydroxamic acids, benzamides or cyclic peptides, and are in clinical trials as monotherapy and combination therapy with various cancer agents (Table 2).

8. Biomarkers predicting response to HDACi

In essentially all the clinical trials with HDACi in which anti-cancer activities is observed, only a portion of patients respond. The identification and development of assays for biomarkers that can predict resistance or sensitivity to HDACi is a major challenge in development of these agents [129]. High levels of expression of class I HDACs (HDACs 1, 2, and/or 3) have been found in colon and rectal cancers and prostate cancer in patients with a poor prognosis. Evaluation of HDAC expression in tumors in relation to the therapeutic response to HDACi is an ongoing area of investigation.

The accumulation of acetylated histones in peripheral mononuclear cells is a marker for the biological activity of HDACi but does not correlate with therapeutic response. The level of acetylated histones in peripheral mononuclear cells returns to base levels within 8 – 10 h following a single oral dose of the HDACi, vorinostat [126].

A biomarker that may inform on the therapeutic response to the HDACi is HR23B, a protein that shuffles ubiquitinated cargo proteins to the protostome and has been validated as sensitivity determinate of HDACi induced apoptosis [129]. HR23B is found expressed at high levels in CTCL that respond favorably to the HDACi, vorinostat.

9. Factors associated with resistance to HDACi

Development of resistance to HDACi is a major concern, as with any new antitumor therapy [130]. The mechanism of resistance to HDACi are not well understood. Based on pre-clinical studies, resistance to HDACi has been found to be associated with epigenetic

alterations, altered stress response mechanisms and antiapoptotic survival mechanisms. High levels of Bcl-2 [77], Trx [131] and peroxiredoxins [132] have been associated with resistance of transformed cells to chemotherapy, and may play a role in resistance to HDACi-induced cell death. Overexpression of Bcl-2 blocks HDACi-induced cell death [71]. Peroxiredoxins reduced H_2O_2 and may protect transformed cells from HDACi-induced cell death, which is associated with ROS production.

Pharmacokinetic properties of the HDACi, such as poor solubility and relatively short half-life, are factors limiting therapeutic efficacy.

HDACi treatment of cancer cells can induce P-glycoprotein (P-gp), resulting in multidrug resistance of cancer cells to other chemotherapy agents [133]. Romidepsin is the only HDACi in clinical trials where P-gp may be a factor in resistance to this agent. It has been reported that the specific downregulation of HDAC1 but not HDAC2 by RNA silencing caused an induction of P-gp expression in HeLa cells, suggesting that HDAC1 inhibition by HDACi can induce P-gp.

10. Expert opinion

HDACi are a promising group of targeted anticancer agents with potential applications in the therapy of hematologic and solid neoplasms, as well as in several non-oncologic diseases. There is a great deal of competition in the HDACi field; several new and, hopefully, more effective compounds are being developed and entering clinical trials.

Histones and many non-histone proteins are targets of HDACs. This group of enzymes are more properly designated *lysine deacetylases.* The target proteins include factors regulating gene expression, cell proliferation, cell migration and cell death, and have a role in angiogenesis and immune response. While not completely understood, it is clear that the mechanisms of HDACi-induced transformed cell death may involve more than one pathway.

Normal cells are relatively resistant to HDACi-induced cell death. A possible explanation for this therapeutic window is that the majority of cancer cells have multiple genetic and molecular defects. Normal cells, compared with transformed cells, have the capacity to reverse the adverse effects of HDACi. This suggests that an intermittent regimen of exposure to the drugs may minimize occurrence of toxic effects on normal cells.

Understanding the biological activities of the 11 zinc-dependent HDACs is evolving. An important question is whether pan HDACi – such as vorinostat, which inhibits class I HDACs and class IIb HDAC6 – are potentially more effective therapeutic agents than an HDAC-selective inhibitor. The development of HDAC isoform-selective inhibitors will certainly be useful in further dissecting their biological functions.

HDACs inhibitors developed for clinical use to date are structurally diverse and inhibit more than a single zinc-dependent HDAC. HDACi can have antitumor activity across a broad variety of hematologic and solid tumors, but only a proportion of patients with a given diagnosis show a therapeutic response. This highlights the need to identify markers of potential response or resistance to one or another HDACi.

Optimization of the pharmacokinetic properties, including water solubility and oral availability, is a challenging area in the future development of HDACi.

Preclinical and clinical studies to date indicate that HDACi may be most therapeutically effective in combination with other anticancer agents including radiation and cytotoxic and targeted drugs. As we gain more understanding of the mechanisms of action of the zinc-dependent HDACs, and the different HDACi, we should be able to develop more effective therapeutic strategies. Current evidence indicates that HDACi can not only block the catalytic activity of the enzymes but also affect the protein–protein interactions of specific HDACs with various critical protein partners. The large number of defects present in most cancer cells suggests that therapeutic strategies targeting multiple biological pathways are likely to be more effective than a drug targeted at a single pathway. This concept supports the need for continued clinical development of HDACi in combination with other anticancer agents.

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Article highlights

- There are 11 zinc-dependent histone deacetylases in humans.
- HDACs have histone and many non-histone substrates.
- HDAC inhibitors (HDACi) can selectively induce cancer cell death.
- HDACi can induce cancer cell death by one or more pathways.
- HDACi are in clinical trials as monotherapy and combination therapy for hematologic and solid tumors.
- HDACi, vorinostat and romidepsin are approved for therapy of cutaneous T-cell lymphoma.

This box summarizes key points contained in the article.

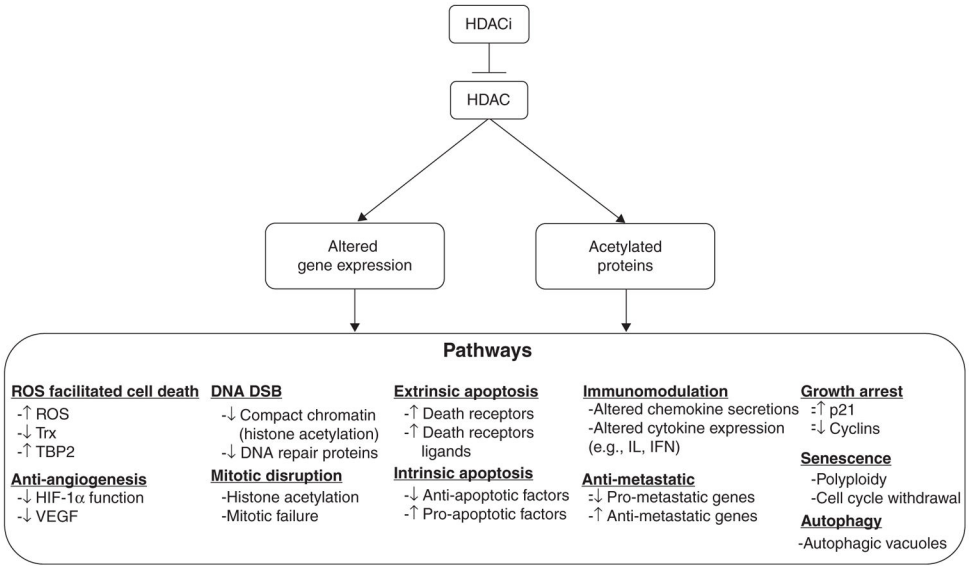


Figure 1.
HDACi can affect one or more pathways, leading to transformed cell death.

Table 1

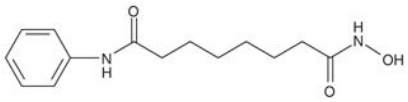
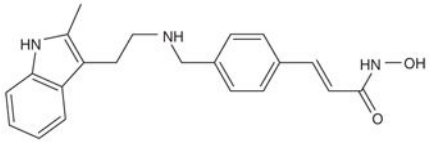
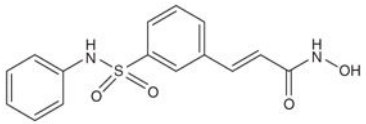
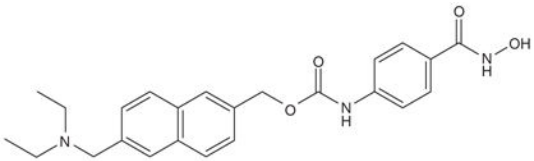
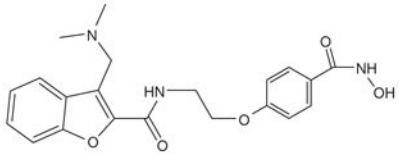
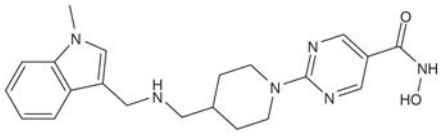
Zinc-dependent histone deacetylases.

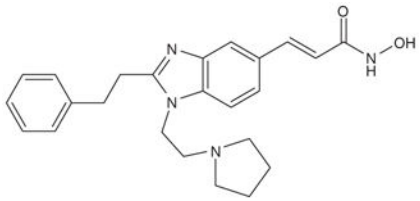
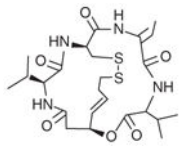
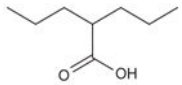
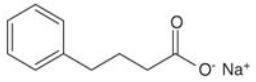
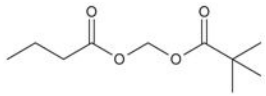
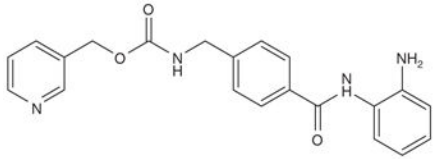
HDAC	Yeast homology	Localization	Size (AA)	Catalytic sites	Chromosomal site
Class I	RPD3				
HDAC1		Nucleus	483	1	1p34.1
HDAC2		Nucleus	488	1	6p21
HDAC3		Nucleus	423	1	5q31
HDAC8		Nucleus	377	1	Xq13
Class IIa	HDA1				
HDAC4		Nuc/Cyt	1084	1	2q372
HDAC5		Nuc/Cyt	1122	1	17q21
HDAC7		Nuc/Cyt	855	1	12q13
HDAC9		Nuc/Cyt	1011	1	7p21-p15
Class IIb					
HDAC6		Mainly Cyt	1215	2*	Xp11.22 – 33
HDAC10		Mainly Cyt	669	2	22q13.31 – 33
Class IV					
HDAC11		Nuc/Cyt	347	1	3p25.2

*Ubiquitin binding site toward C terminal end.

Table 2

Histone deacetylase inhibitors in clinical trials as monotherapy and combination (partial list)*.

Drug name	Structure	Dose range	Clinical trials	Ref.
Hydroxamates				
Vorinostat (SAHA, zolinza)		μmol/l	FDA approved CTCL Phll-Ill (p.o.)	[6,45,105,126,127,134–146]
Panobinostat (LBH589)		nmol/l	Phl-ll (p.o.), Phll (i.v.)	[107,128]
Belinostat (PXD101)		μmol/l	Phll (i.v.) Phl (p.o.)	[109,147–154]
Givinostat (ITF-2357)		nmol/l	Phl-ll (p.o.)	[48,150]
PCI-24781 (CRA-024781)		nmol/l	Phl (p.o.)	[48,151]
JNJ-26481585		μmol/l	Phl-ll (p.o.)	[48,152]

Drug name	Structure	Dose range	Clinical trials	Ref.
SB-639		μmol/l	Phl (p.o.)	[48,153]
Cyclic peptide				
Romidepsin (depsipeptide, FK228)		nmol/l	FDA approved CTCL Phl-ll (i.v.)	[109,110,154–158]
Aliphatic acids				
Valproic acid (baceca) (savicol)		mmol/l	Ph ll-lll (p.o) Ph II (top)	[159]
Phenylbutyrate (VP-101, EI-532)		mmol/l	Ph II (p.o.)	[160–163]
Pivanex (AN-9)		mmol/l	Phl-ll (i.v.)	[164]
Benzamides				
Entinostat (MS 275, SNDX 275)		μmol/l	Phl-ll (p.o.)	[165,166]

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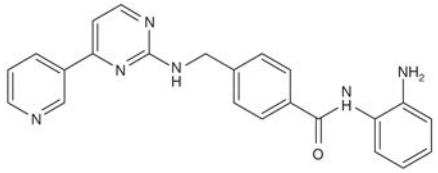
Drug name	Structure	Dose range	Clinical trials	Ref.
MGCD0103		μmol/l	Phl-ll (p.o.)	[166,167]

* For reviews of clinical trials with HDACi see references in text.

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