

FORUM REVIEW ARTICLE

Targeting Histone Deacetylases in Diseases: Where Are We?

Rosaria Benedetti,¹ Mariarosaria Conte,¹ and Lucia Altucci^{1,2}

Abstract

Significance: Epigenetic inactivation of pivotal genes involved in cell growth is a hallmark of human pathologies, in particular cancer. Histone acetylation balance obtained through opposing actions of histone deacetylases (HDACs) and histone acetyltransferases is one epigenetic mechanism controlling gene expression and is, thus, associated with disease etiology and progression. Interfering pharmacologically with HDAC activity can correct abnormalities in cell proliferation, migration, vascularization, and death. **Recent Advances:** Histone deacetylase inhibitors (HDACi) represent a new class of cytostatic agents that interfere with the function of HDACs and are able to increase gene expression by indirectly inducing histone acetylation. Several HDACi, alone or in combination with DNA-demethylating agents, chemopreventive, or classical chemotherapeutic drugs, are currently being used in clinical trials for solid and hematological malignancies, and are, thus, promising candidates for cancer therapy. **Critical Issues:** (i) Non-specific (off-target) HDACi effects due to activities unassociated with HDAC inhibition. (ii) Advantages/disadvantages of non-selective or isoform-directed HDACi. (iii) Limited number of response-predictive biomarkers. (iv) Toxicity leading to dysfunction of critical biological processes. **Future Directions:** Selective HDACi could achieve enhanced clinical utility by reducing or eliminating the serious side effects associated with current first-generation non-selective HDACi. Isoform-selective and pan-HDACi candidates might benefit from the identification of biomarkers, enabling better patient stratification and prediction of response to treatment. *Antioxid. Redox Signal.* 23, 99–126.

Shaping the Epigenome

EPIGENETIC MECHANISM(S) ALLOW genetically identical cells to adopt different phenotypes regulating transcriptional availability of the genome through differential chromatin marking and packaging (137), creating a network of mutually reinforcing or counteracting signals (192). A key aspect of epigenetics is that chromatin marks can be preserved and/or changed according to environmental, developmental, or pathological needs. These very complex and plastic steps are achieved via the activity of *initiators* (such as long noncoding RNA), *writers* (which establish the epigenetic mark, such as histone acetyltransferases), *readers* (which interpret the epi-mark), and *erasers* (which remove the epi-mark, such as histone deacetylases, or HDACs) (41, 232). In concert, *remodelers* (which reposition nucleosomes) and *insulators* (which build boundaries between epi-domains) create, maintain,

and modulate the three-dimensional structure of networking within a cell (223). It is now clear that genetic and epigenetic mechanisms influence each other, cooperating to enable the acquisition of hallmarks of human cancer (89). The frequency of epi-target mutations seen in cancers underlines the relevance of mutations in epigenetic modifiers in cancer (213) and corroborates the concept that deregulation of epigenetic control is a common characteristic of cancer (105). Conversely, these findings confirm and strengthen the crucial role of epigenetic-based drugs (so-called *epidrugs*) (3) in reverting the malignant phenotype. This review aims at providing a comprehensive overview of current knowledge about HDACs and histone deacetylase inhibitors (HDACi), from biochemical data to development of clinical trials. It summarizes ongoing efforts to design epi-based treatments against cancer and other human diseases. The review analyses the chemical, biochemical, and therapeutic aspects of the use of HDACi in a clinical setting

¹Department of Biochemistry, Biophysics, and General Pathology, Seconda Università degli Studi di Napoli, Napoli, Italy.

²Istituto di Genetica e Biofisica “Adriano Buzzati-Traverso,” Napoli, Italy.

(highlighting the advantages and disadvantages), guiding the reader toward a better understanding of the complex balance between acetylation/deacetylation.

HDAC Classification

HDACs catalyze the removal of acetyl groups from the amino-terminal lysine residues of histone and non-histone proteins, such as transcription factors (TFs), hormone receptors, signaling proteins, chaperones, and DNA damage response proteins (10, 47, 68, 78). Four classes of human HDACs have been defined, based on their homology to yeast HDACs (13, 108, 228), enzymatic activities, and cellular localization (Fig. 1). HDAC1, 2, 3, and 8 belong to class I and are localized in the nucleus, presenting similarity to the yeast Rpd3 (47). Class II HDACs (229) are present in both the cytoplasm and nucleus, and they shuttle between these compartments (113, 147, 234). Class II HDACs (grouped for homology to Hda1 in yeast) are divided into two subclasses: class IIa (HDAC4, 5, 7, and 9) and class IIb (HDAC6, 10). Class IV HDACs includes one member (HDAC11) (49, 71), which can be considered a “hybrid” sharing similarities to both class I and II HDACs. Sirtuins, related to Sir2 in yeast (42), have also been categorized as class III HDACs (165) (Fig. 1, lower part) and grouped as SIRT1-7 (209). These enzymes display an NAD function that is associated to a nuclear, mitochondrial, and cytoplasmic localization (31, 149). HDAC1 and 2 are highly similar with an overall identity of ~82%. The catalytic domain on the N-terminus constitutes the main part of the proteins. Moreover, HDAC1 and 2 only act in complex with proteins required for modulating their deacetylase activity and binding to DNA (87), mediating the recruitment of HDACs to promoters (163, 190). Three protein complexes containing both HDAC1 and 2 have been characterized: Sin3, NuRD, and Co-REST (251). Both Sin3 and NuRD complexes consist of a core containing HDAC1 and 2, Rb-associated protein 48 and RbAp46 (123, 258). The core alone does not possess maximal HDAC activity, and further cofactors are needed. In addition to functioning through these complexes, HDAC1 and 2 can also bind directly to DNA-binding proteins such as Yin and Yang 1 (YY1) (249), Rb-binding protein-1 and Sp1 (51). Both activity and complex formation are regulated by phosphorylation. HDAC1 and 2 are phosphorylated (189) at a low steady-state level in resting cells, and their hyper-phosphorylation leads to a slight, but significant, increase in deacetylase activity, and, simultaneously, to disruption of the complex between HDAC1/2 and HDAC1/mSin3/YY1 (67). When hypophosphorylation occurs, the activity of HDAC1 and 2 decreases, although complex formation is increased (67, 173). HDAC3 (56) is closely related to HDAC8, with 34% identity, displaying the canonical domain of all class I HDACs. HDAC3 can co-precipitate with HDAC4, 5, and 7 through complex formation with SMRT and N-CoR (86). HDAC8 (69, 224, 225) consists largely of the catalytic domain with a nuclear localization signal. HDAC4, 5, and 7 represent a subgroup within class II HDACs (143). HDAC4 and 5 share an overall similarity of 70%. HDAC6 is evolutionarily most related to HDAC10. In general, however, the identity of HDAC6 with other human HDACs is low, with some resemblance to yeast Hda1, suggesting an early separation from other HDACs. HDAC6 (228) is a unique enzyme (23), in that it

contains two catalytic domains in tandem, and an HDAC6-, USP3-, and Brap2-related zinc finger motif (HUB) domain at the C-terminus (264). This domain is a signal for ubiquitination, implying an involvement in degradation pathways. The catalytic domains of HDAC6 are most similar to those of HDAC9. HDAC6 and the enzyme functions as a tubulin deacetylase (98, 144, 257). Although it resides predominantly in the cytoplasm (having a nuclear export signal [NES]) to exert its function (227), HDAC6 can also be found in the nucleus along with HDAC11. HDAC9 (and its splice variants) belongs to class IIa and has a catalytic domain on the C-terminus (172, 263). HDAC10 is the most recently discovered class II HDACs (61). HDAC10 (112) is closely related (37% overall similarity) to HDAC6. HDAC10 has a catalytic domain on its N-terminus, as well as both an NES and a putative second catalytic domain on the C-terminus. HDAC10 might function as a recruiter rather than as a deacetylase (85), interacting with HDAC1, 2, 3, 4, 5, and 7, but not with HDAC6, with some contradictions present in literature. HDAC11, containing a catalytic domain at the N-terminus, is similar to HDAC3 and 8, suggesting that it might be more closely related to class I than to class II HDACs (71). In mammals, seven Sir2 homologues (SIRT1-7) have been identified, which possess primarily HDAC (SIRT1, 2, 3, and 5) or mono-ribosyltransferase activity (SIRT4 and 6) (64), targeting histone and various non-histone proteins in distinct subcellular localizations. SIRT1 has a wide range of substrates and cellular functions (171). In contrast to SIRT1, SIRT2 is predominantly cytoplasmic, deacetylating several substrates such as α -tubulin (20). For a detailed classification of SIRT3-7 (31).

HDACs and Sirtuins in Cancer: Quantitative and Functional Abnormalities

Cancer has traditionally been considered a disease of genetic defects such as gene mutations and deletions as well as chromosomal abnormalities, resulting in the loss of function of tumor-suppressor genes and/or gain of function or hyperactivation of oncogenes (121, 218). Nevertheless, the epigenetic regulation of genes and, therefore, the plasticity of the epigenome play a key role in cancer development and progression (93, 214). HDACs can act directly on chromatin, modifying histone acetylation and changing the characteristics of transcriptional factor recruitment on promoters. Consequently, HDAC deregulation frequently leads to aberrant expression of genes regulating cell proliferation, cell cycle, and apoptosis. Moreover, HDACs can promote the acetylation of structural proteins, chaperones, and TFs with a significant impact on physio-pathological pathways. Current knowledge of HDAC involvement in cancer is summarized next according to HDAC family groups.

Class I HDACs

All bonafide class I HDACs are deregulated in cancer (156). HDAC1 overexpression has been found in gastric (35, 155), breast (115), pancreatic (70, 262), hepatocellular (58, 244), lung (186), and prostate (200) malignancies, and overexpression levels correlate with prognosis and survival. High expression levels of HDAC1, 2, and 3 are associated with renal cancer (63) and Hodgkin's lymphoma (1). Interestingly, HDAC2 frame-shift mutation is the only genetic mutation identified in HDAC genes, leading to loss of

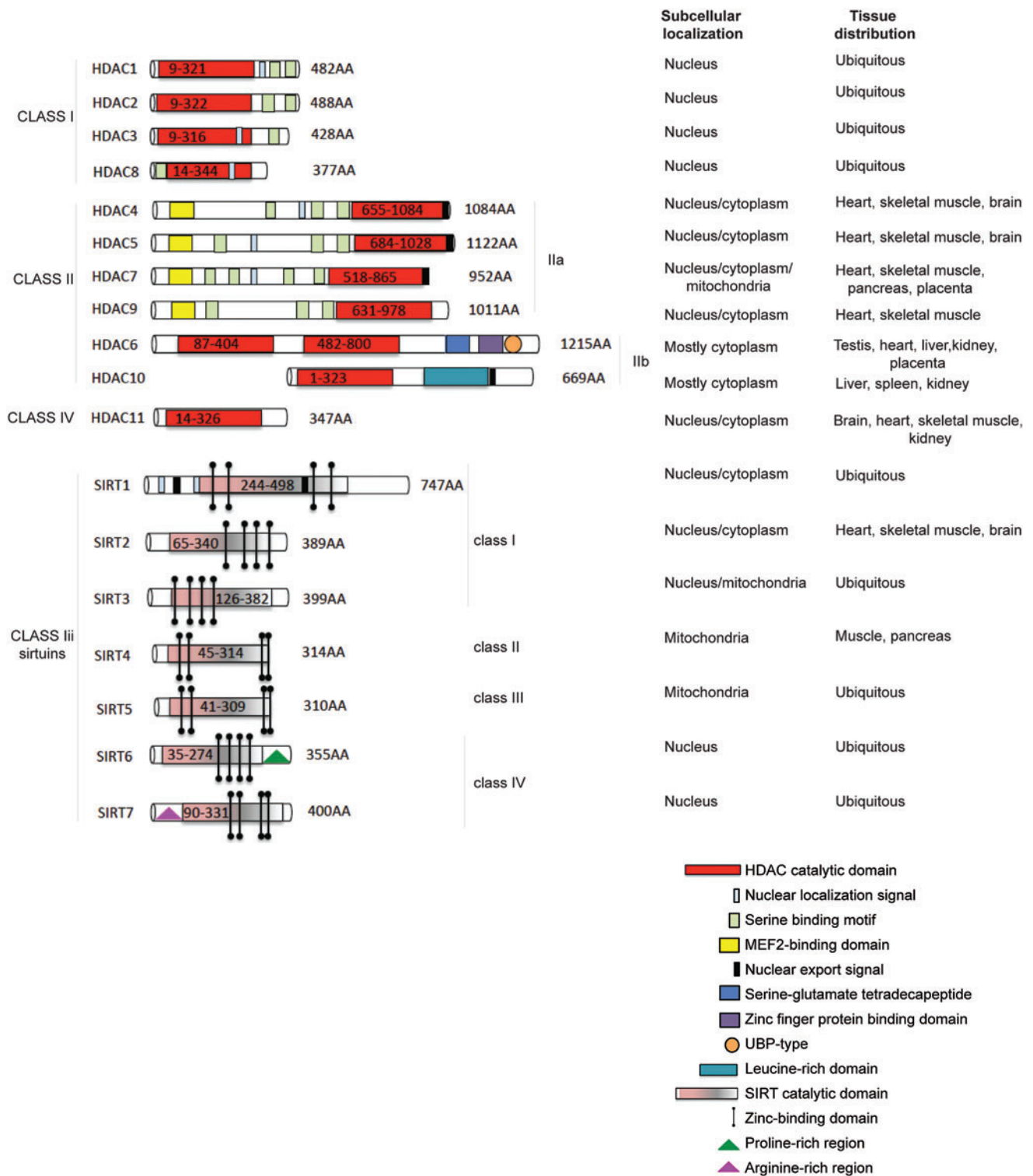


FIG. 1. HDAC and SIRT classification. Enzymes are divided into classes and sub-classes; subcellular localization and tissue distribution are reported for each member. HDAC, histone deacetylase; SIRT, sirtuin. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

HDAC2 protein and activity in human microsatellite instability (MSI)-high endometrial and MSI colon cancer cells. Unlike other class I members, HDAC8 expression appears to be cancer-type specific, as to date it has been found altered in childhood neuroblastoma (166, 167) and pancreatic ductal adenocarcinoma (48).

Class IIa HDACs

HDAC4 exerts an atypical role in cancer being overexpressed (*e.g.*, in breast cancers) and/or down-regulated (*e.g.*, in chondrosarcoma (204) and melanoma (252)) differently in different malignancies HDAC5 and 9 may be considered markers for

medulloblastoma risk stratification (150). Their overexpression correlates with poor survival in affected patients. HDAC5 is also overexpressed in hepatocellular carcinoma (125), while HDAC7 overexpression is associated with pancreatic cancer and acute lymphoblastic leukemia (152, 169).

Class IIb HDACs

HDAC6 is implicated in breast cancer (184), diffuse large B-cell lymphomas, and peripheral T-cell lymphomas (142). Increased HDAC6 expression has been observed in more advanced—compared with early-stage cancers (239). HDAC10 expression is stronger in patients with chronic lymphocytic leukemia, and it has recently been linked to gastric cancers and reactive oxygen species (ROS) production in many cancers (130, 233).

Class III (sirtuins)

SIRT1, 4, and 7 are up-regulated in certain cancers (myeloid leukemia, prostate and non-melanoma skin cancer) (20), whereas SIRT2 is down-regulated in gliomas (94) and gastric carcinoma, as well as in melanomas (118). In melanomas, a mutation in the catalytic domain of SIRT2 (P199L) eliminates its enzymatic activity (131). Evidence suggests that SIRT2 acts as tumor suppressor and that its loss compromises the mitotic checkpoint, contributing to genomic instability and tumorigenesis. SIRT3 is, instead, up-regulated or down-regulated in different types of breast cancer (254). The quantity of the enzyme may be relevant in tumorigenesis for both loss and gain. Some studies also suggest a role for SIRT7 in breast cancer maintenance (8).

Class IV HDACs

Overexpression of HDAC11 has been found in myeloproliferative disorders as well as in Hodgkin's lymphoma (29, 198).

To date, important studies (179) have focused on aberrant HDAC recruitment to promoters through their physical association with oncogenic DNA-binding fusion proteins resulting from chromosomal translocations, or overexpression of repressive TFs that physically interact with HDACs. PML-RAR α and AML1-ETO fusion proteins induce acute promyelocytic leukemia and acute myeloid leukemia (AML) by recruiting HDAC-containing repressor complexes to stably repress the expression of target genes (HDAC1 and 2) (73). HDAC6 plays a key role, by physically interacting with non-histone proteins such as HSP90, β -catenin, and epidermal growth factor (in Wnt signaling pathway) (134, 248). Altered levels of acetylation of these targets can disrupt the balance of c-Myc activation, enabling cells to escape from apoptosis. The same is true for p53 and its level of acetylation, which can induce its activity as TF. Taken together, these findings support the development of HDACi to restore the physiological pattern of gene expression and function in tumors and, possibly, other diseases.

HDAC and SIRT Modulators

HDACi represent a class of cytostatic agents that interfere with the function of HDACs and are able to modulate gene expression through indirect induction of histone acetylation (14). In particular, these compounds inhibit the proliferation

of tumor cells *in vivo* and *in vitro* by inducing cell-cycle arrest, differentiation, and/or apoptosis with different kinetics and activities depending on chemical structures. Surprisingly, normal cells are often less sensitive to HDACi than are tumor cells (87). HDACi derive from natural or synthetic sources and can be classified into five main groups (16):

- (i) Hydroxamates, including trichostatin A (TSA), suberoylanilide hydroxamic acid (also called Vorinostat), LAQ824, LBH589 (Panobinostat), or PXD101 (Belinostat), M344, CR2408, abexinostat hydrochloride (PCI-24781)
- (ii) aliphatic acids, including sodium butyrate (NaB), valproic acid (VPA), and phenylbutyric acid
- (iii) benzamides, including MS-275 (Entinostat)
- (iv) tetrapeptides/depsipeptides, including Apicidin, Romidepsin, and Trapoxin B
- (v) sirtuin inhibitors (SIRTi), including the pan-inhibitor nicotinamide and the specific SIRT1 and 2 inhibitors sirtinol, cambinol, and EX-527.

TSA inhibits HDAC1, 4, and 6 with IC_{50} =6, 38, and 8.6 nM, respectively [for further information, the reader is referred to ref. (39)]. The pan-inhibitor Vorinostat inhibits (177) HDAC1 and 3 with IC_{50} =10 and 20 nM, respectively. Vorinostat also induces marked hyperacetylation of histone H4 and inhibits growth of three prostate cancer cell lines, LNCaP, PC-3, and TSU-Pr1, at 2.5–7.0 μ M concentrations. Vorinostat treatment in MCF-7 breast cancer cells inhibits cell proliferation at IC_{50} =0.75 μ M, resulting in the accumulation of cells in G1 and G2-M phase. Vorinostat treatment at 1 μ M for 8 h or more is sufficient to irreversibly induce the apoptosis of human multiple myeloma (MM) cells. LAQ824 (6, 80) activates the expression of p21 cell-cycle inhibitor by activating the p21 promoter with AC_{50} =0.3 μ M. LAQ824 inhibits cell growth of H1299 and HCT116 with IC_{50} =0.15 and 0.01 μ M, respectively. The antiproliferative effect of LAQ824 is selective toward tumor cell lines while inducing only growth arrest in normal fibroblasts. Furthermore, LAQ824 induces a dose-dependent increase of p21 in A549 cells and an increase in the hypo-phosphorylated state of Rb tumor suppressor. Panobinostat (5, 174) is a broad-spectrum HDACi with IC_{50} =5 and 20 nM in MOLT-4 and Reh cells, respectively. Panobinostat induces acetylation of histones H3K9 and H4K8 as well as p21 expression while decreasing levels of c-Myc in a dose-dependent manner. Belinostat displays (77) IC_{50} =27 nM in HeLa extracts. *In vitro* Belinostat inhibits the growth of tumor cells such as A2780; HCT116 induces apoptosis through PARP cleavage and acetylation of histones H3/H4, and shows enhanced tubulin acetylation in ovarian cancer cell lines. M344 (110, 178, 235) is toxic at concentrations above 10 μ M, while a maximum of only 20% of the surviving cell population are induced to differentiate. *In vitro*, M344 shows significant antiproliferative activities against Ishikawa and SK-OV-3 cancer cell lines with EC_{50} =2.3 and 5.1 μ M, respectively, while it displays little effect on normal human endometrial epithelial cells. In addition, M344 leads to a decreased proportion of cells in the S phase and an increased proportion in G0/G1 phases of the cell cycle, induces apoptosis, and decreases the trans-membrane potential of mitochondria. PCI-24781 (28) exhibits potent antitumor activity against a variety of cell lines with 0.15μ M < IC_{50} < 3.09 μ M.

PCI-24781 treatment causes dose-dependent accumulation of both acetylated histones and acetylated tubulin in HCT116 or DLD-1 cells, induces p21 expression, and leads to PARP cleavage and accumulation of γ H2AX. Sodium butyrate (44) induces differentiation and prevents cell proliferation. Mechanistic studies suggest that its action is often mediated through Sp1/Sp3-associated HDAC activity, leading to transcriptional activation of the p21 gene. In addition, this inhibitor down-regulates numerous genes that are associated with cytokine signaling, specifically the interferon- γ (IFN- γ) pathway. This compound has HDAC1, 7, and 8 with $IC_{50} = 140 \mu M$. VPA has $IC_{50} \approx 0.4 mM$ and exhibits anticancer, anti-inflammatory, and neuro-protective effects. MS-275 (181, 183), class I HDACi, induces the accumulation of p21 and gelsolin in K562 cells and decreases the expression of cyclin D1 and the anti-apoptotic proteins Mcl-1 and XIAP. MS-275 inhibits the proliferation of human tumor cell lines, including A2780, Calu-3, HL-60, K562, St-4, HT-29, KB-3-1, Capan-1, 4-1St, and HCT-15 with $41.5 nM < IC_{50} > 4.71 \mu M$. MS-275 strongly inhibits human leukemia and lymphoma cells, including U937, HL-60, K562, and Jurkat. MGCD0103 (22) inhibits at nM or low μM concentrations only a subset of the nine HDACs. MGCD0103 is active against HDAC1 and 2 *in vitro* and in whole cells, but it does not inhibit class II HDACs. The exocyclic amino group in MGCD0103 is necessary for inhibitory activity, as HDAC-inhibitory activity against HDAC1 and 2 is completely abolished with the desamino analogue. Apicidin (88, 219), which contains an electrophilic ketone, is a potent HDACi with $IC_{50} = 0.7 nM$. An *in vitro* activity assay demonstrates Apicidin-mediated inhibition of HDAC3/NcoRat at a much higher potency than for HDAC6 ($IC_{50} = 15.8$ and $665.1 nM$, respectively). Romidepsin (157, 161) is an HDAC1 and 2 inhibitor with $IC_{50} = 36$ and $47 nM$, respectively. Romidepsin treatment in HeLa cells induces histone acetylation and p21 expression with $EC_{50} = 3.0 nM$, G2/M arrest, cyclin D1 down-regulation, and p53-independent p21 induction, leading to inhibition of CDK and dephosphorylation of Rb (resulting in early G1 phase arrest). Although SIRTi are classified along with HDACi, they act *via* a nicotinamide-dependent mechanism, suggesting that they should have their own class based on their chemical functionalities. To date, a number of specific SIRT inhibitors (mainly SIRT1 and 2) have been proposed for cancer therapy. Moreover, both activators and inhibitors of sirtuins might act beneficially against different types of neurodegenerations and cancers (127). In addition to nicotinamide, some other specific inhibitors have been characterized, including splitomicin and its analogues, tenovins, AGK2, sirtinol, suramin, the indole derivative EX-257, salermide, and UVI5008. Phenol derivatives, including quercetin, piceatannol, and resveratrol, possess SIRT1-activating properties. Many other compounds have subsequently been developed such as SRT1720, SRT2183, and SRT1460. For further information on SIRTi (42, 205).

Figure 2 presents a list of well-known HDACi. Many of these compounds are in clinical trials alone or in combination with other agents for the treatment of leukemias or solid tumor malignancies. Further information with regard to compounds that are currently being used in clinical evaluation is provided in the section “HDACi and SIRTi in clinical trials against cancer.” Our understanding of the mechanistic effects of HDACi will probably change significantly, estab-

lishing a new paradigm in the field of so-called “smart drugs.” New insights will have profound implications for the design of targeted therapies in cancer and other diseases.

Biology of HDACs in Cancer and Effects of HDACi

Many HDACs exist as components of multi-protein complexes, such as the transcriptional co-repressors mSin3, N-CoR, and SMRT (97, 215, 217, 245, 256, 259). These are then targeted to specific genomic regions by interactions with DNA-binding factors, including TFs, nuclear receptors, and other epigenetic modifiers, such as methyl-binding proteins, DNA methyltransferases, and histone methyltransferases. Along with their interactors, HDACs are able to modulate the expression of genes coding for signaling proteins and thus the function of key pathways, including apoptosis, Wnt pathway, cell-cycle progression and differentiation, regulation of the proteasome system, activation of kinases, and the unbalanced reaction between DNA damage repair and surveillance. At the chromatin level, therefore, HDACs work as transcriptional repressors in most, though not all, cases. Indeed, protein acetylation can have many different effects, as listed next (graphically represented in Fig. 3), underscoring how a defect in HDAC expression or function might affect biological processes. The readout of treatment with HDACi is a change in the expression level of about 10% of cellular proteins, leading to a global effect.

Protein degradation

Protein stability depends on the balance between synthesis and degradation (128). Protein degradation is mediated by ubiquitination. Both acetylation and ubiquitination occur on the same lysine residues, and a direct cross-talk exists between the two (82). Thus, HDACs can decrease the half life of several substrates by exposing the lysine residue for ubiquitination (76, 82, 104, 151). Modified lysine residues mediate many interactions, and an aberrant HDAC function could, therefore, alter the assembly of multi-complexes. HDAC6 is known to interact with Hsp90, and HDACi-mediated hyperacetylation of Hsp90 induces the activation of proteasome degradation of oncogenic client proteins such as Akt, Bcr-Abl, and c-Raf (24). One of the client proteins of HSP90 is androgen receptor, which is degraded after HDACi treatment, suggesting the use of HDACi in hormone-dependent or refractory cancers. All these findings reveal that the inhibition of HDAC6 makes cells more sensitive to induced stresses, which are useful in therapeutic strategies.

Protein-protein interactions

Acetylation/deacetylation balance regulates a growing number of non-histone proteins (as p53, nuclear factor- κ B [NF- κ B], p65, CBP, p300, STAT3, tubulin, PC4, GATA factors, nuclear receptors, c-Myc, hypoxia-inducible factor [HIF]-1 α , FOXO1, HSP-90, HMG, E2F, MyoD, Bcr-Abl, the FLT3 kinase, c-Raf kinase, and others), affecting their interactions. HDACi modulate gene regulatory activities of TFs such as E2F1, p53, STAT1, STAT2, and NF- κ B *via* a hyperacetylated status (75). HDACi can also regulate the activity of p53 and correlated genes to induce a p53-mediated pro-apoptotic signal in cancer treatment. Thus, many genes regulated by HDACi are likewise a target of p53, suggesting

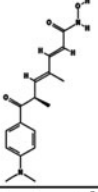
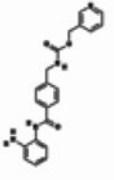
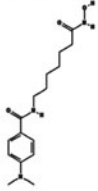
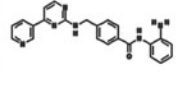
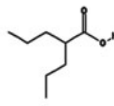
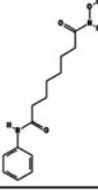

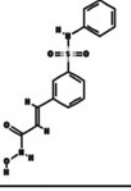
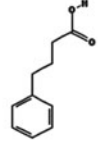
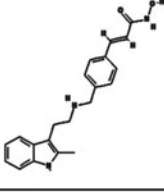
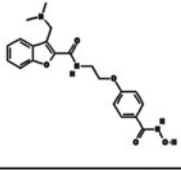
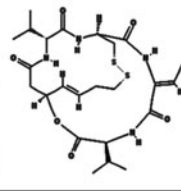
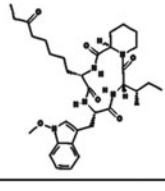
Hydroxamic acids		Benzamides	
	Trichostatin A M.F= C ₁₇ H ₂₂ N ₂ O ₃ M.W= 302.36818 [g/mol]		Entinostat M.F: C ₂₁ H ₂₀ N ₄ O ₃ M.W: 376.4085 [g/mol]
	M-344 M.F= C ₁₆ H ₂₅ N ₃ O ₃ M.W: 307.388 [g/mol]		Mocetinostat M.F= C ₂₃ H ₂₀ N ₆ O M.W= 396.4445 [g/mol]
Aliphatic acids			Valproic Acid M.F: C ₈ H ₁₆ O ₂ M.W: 144.21144 [g/mol]
	SAHA M.F: C ₁₄ H ₂₀ N ₂ O ₃ M.W= 264.3202 [g/mol]		Butyric Acid M.F: C ₄ H ₈ O ₂ M.W: 88.10512 [g/mol]
	Belinostat M.F: C ₁₅ H ₁₄ N ₂ O ₄ S M.W: 318.34766 [g/mol]		Phenylbutyric acid M.F: C ₁₀ H ₁₂ O ₂ M.W: 164.20108 [g/mol]
	Panobinostat M.F: C ₂₁ H ₂₃ N ₃ O ₂ M.W: 349.42622 [g/mol]	Tetrapeptides/depsipeptides	
	PCI-24781 M.F: C ₂₁ H ₂₃ N ₃ O ₅ M.W: 397.42442 [g/mol]		Romidepsin M.F: C ₂₄ H ₃₆ N ₄ O ₆ S ₂ M.W: 540.69584 [g/mol]
			Apicidin M.F: C ₃₄ H ₄₉ N ₅ O ₆ M.W: 623.78276 [g/mol] electrophilic ketone

FIG. 2. Structures of major HDACi belonging to classes of hydroxamic acids, benzamides, aliphatic acids, and cyclic tetrapeptides/depsipeptides. HDACi, histone deacetylase inhibitors.

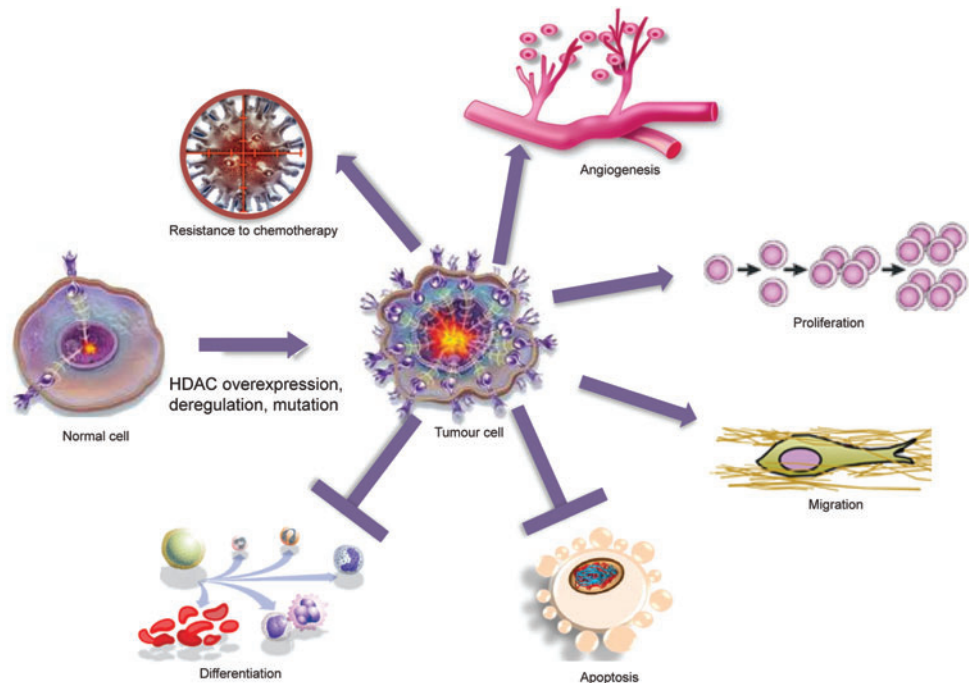
the use of HDACi in tumors due to p53 (activity or expression) loss (196, 207).

Cell-cycle progression (proliferation)

Crucial stages of cell cycle are generally controlled through the transcriptional regulation of a subset of genes, which are, in turn, regulated by acetylation/deacetylation of histone and non-histone proteins (231). HDAC and sirtuin overexpression

has been observed in multiple cancers (see section “HDACs and sirtuins in cancer: quantitative and functional abnormalities”). HDACi cause cell growth arrest in the G1 phase in normal and transformed cells through the activation of and promiscuous interference with many processes, and they also induce p21, which binds to and inhibits the activity of cyclin-CDK2 or -CDK1 complexes. The expression of this gene is tightly controlled by p53, which competes with HDAC1 for a binding site (SP1) on p21 promoter region (260).

FIG. 3. HDACs control many aspects of cancer biology. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars



Apoptosis induction

HDACs act as regulators in intrinsic and extrinsic apoptosis processes (180). The evasion of this pathway is the principal hallmark of tumor growth and progression, as confirmed by the fact that resistance to apoptosis induction has been recognized in many cancers. In the extrinsic pathway, HDACs negatively affect the transcriptional regulation of death receptors such as tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and TNF receptor superfamily member (Fas) (38, 139, 159). In the intrinsic pathway, the overexpression or aberrant function of HDACs interferes with the expression of pro-apoptotic proteins such as Bcl-family members, with the concomitant up-regulation of anti-apoptotic proteins. HDACi are capable of inducing apoptosis in tumor cells by regulating the expression of pro and anti-apoptotic genes through the intrinsic or extrinsic pathway. In the extrinsic mechanism, HDACi enhance the sensitivity of cancer cells to TRAIL by modulating expression of the protein and its receptors (65). Relaxed chromatin, promoted by treatment with HDACi, increases the accessibility of transcriptional machinery to the TRAIL promoter region, as well as the acetylated forms of many TFs (*e.g.*, p53), which are strongly implicated in the up-regulation of TRAIL. In the intrinsic (mitochondrial) pathway, HDACi cause the down-regulation of anti-apoptotic proteins (Bcl-2, Bcl-x1, and Mcl-1) and a change in the expression of pro-apoptotic proteins (Bax, Bak, and Bid). Oligomerization of Bax/Bak leads to mitochondrial membrane permeabilization and, consequently, the release of cytochrome *c* by the activation of caspases (in particular, caspase 9) and apoptosome formation (19, 191). In the intrinsic cell pathway that is induced by HDACi, an important effect is mediated by ROS production, which is increased and regulated by Bax, Hsp90, TBP-2, and Trx (220). HDACi can also induce the cell death of transformed cells by causing mitotic defects. Extremely high histone acetylation levels can disrupt centromere

structure and function with the concomitant loss of heterochromatin-binding proteins. Moreover, the level of acetylation interferes with histone phosphorylation, and, consequently, the action of proteins (many of which are spindle checkpoint proteins such as BubR1, CENP-F) recognizing phospho groups is itself inhibited. Levels of Aurora A and B are also decreased after HDACi treatment, contributing to transient arrest in prometaphase after aberrant mitosis and so, the activation of apoptosis (32).

Differentiation

The deacetylated state of histones is required for the maintenance of the undifferentiated cellular state, as genes involved in lineage progression are unexpressed. A shift to an acetylated state interferes with the proper execution of the differentiating process and contributes to cancer development. Particular attention has been focused on the role of HDACs in AML. In these systems, the presence of oncofusion proteins affects the release of HDACs from promoters (*e.g.*, thus containing RAREs), maintaining cells in an undifferentiated state (by silencing genes required for hematopoietic differentiation) (221). HDACi can induce the differentiation in haematological malignancies by interfering not only with the HDAC(s)-oncofusion proteins complex, but also in breast cancer (154), hepatoma cells (246), endometrial stromal sarcoma (96), glioblastoma (206), and thyroid carcinoma cells (253).

Metastasis and migration

HDACs (in particular, class I HDACs) play an important role in metastasis and migration pathways, as they repress the expression level of RECK and Rho- β , and decrease the level of Kangai-1 (a metastasis suppressor factor) through β -catenin pathway (55). HDACs work in multi-complexes with metastasis-associated protein 1 and 2 (247). HDACs have also been linked to extracellular matrix formation and its

remodeling, thus increasing cancer cell invasion and migration (237). In human cancer tissues, reduced histone acetylation is significantly correlated with advanced tumor stage and the depth of tumor invasion (199, 216). Treatment with HDACi up-regulates a set of metastasis-suppressor genes and down-regulates a set of metastasis-promoting genes (107), as well as represses cancer cell invasion and metastasis both *in vitro* (120) and *in vivo* (148). Many cases are reported in literature (236), though the role played by HDACi remains controversial (136).

Angiogenesis

Solid tumor metastasis and progression require the formation of new blood vessels. One of the key initiators of angiogenesis is the protein HIF-1 α , which is degraded once acetylated. Deacetylation of HIF-1 α is mediated by HDACs (classes I, II, III) and induces activation of the angiogenic process (by the expression of vascular endothelial growth factor [VEGF]) (74). Anti-angiogenic effects seem to be related to the down-regulation of pro-angiogenic genes, such as VEGF and endothelial nitric oxide synthase (eNOS). The mechanism by which HDACi act is still unclear, but the destabilization of mRNA coding for these genes such as protein degradation may be involved. The degradation of VEGF and eNOS seems to increase after treatment with HDACi (182) as a consequence of HSP90 hyperacetylation (25, 170). The same effect is observable on the expression level and stability of the protein HIF-1 α (255).

DNA damage and oxidative stress

Hyperacetylation of histones causes chromatin relaxation and, thus, increases TFs binding to DNA. HDACi have a negative impact on biological mechanisms, ensuring genome integrity and stability, such as DNA recognition and repair pathway and regulation of ROS concentration. In open chromatin, sites usually packaged and unexposed are not protected from damage (induced by radiation, drugs, and unbalanced ROS scavengers) and, therefore, act as signals for genome integrity failure, culminating in cell death pathway activation. Thus, targeting genome integrity in rapidly cycling cells (such as tumor cells), combining HDACi and radiotherapy, could be considered a preferred strategy in cancer (discussed in section “Examples of HDACi in Combination Treatments”). ROS, such as superoxide anion, hydrogen peroxide, and hydroxyl radicals, are a major source of endogenous DNA damage. Normal cells control ROS concentration through the action of enzymatic (thioredoxin 1/thioredoxin-binding receptor-2 cross-talk) and non-enzymatic ROS scavengers (220). The activity of thioredoxin-1 is modulated by its receptor, which binds the enzyme interfering with its activity. In response to HDACi, levels of thioredoxin-binding receptor-2 increase; while thioredoxin-1 is reduced (30). In particular, the inhibition of HDAC10 causes an accumulation of ROS, increased release of cytochrome C, and activation of proapoptotic molecules such as caspase 3, caspase 9, and Bid in SNU-620 gastric cancer cells (130).

HDACs and Sirtuins in Non-Cancer Human Diseases

The therapeutic potential of HDACi has also been investigated for a wide range of other diseases. Here, we focus on cardiac, cognitive, immunological, and metabolic disorders.

Cardiac disorders

Recent evidence has suggested that different HDACs are implicated in heart diseases such as arrhythmia, heart failure, acute coronary syndromes, and hypertrophy (117). Cardiac hypertrophy is characterized by increased cell size, enhanced protein synthesis, and heightened organization of the sarcomere. In this state, fetal genes such as natriuretic peptide precursor type A (Nppa), myosin heavy polypeptide 7 (Myh7), and skeletal α -actin are reactivated; whereas cardiac contractile proteins such as myosin heavy polypeptide 6 (Myh6) and calcium-handling proteins are repressed. In addition, immediate-early genes encoding c-fos, c-jun, and heat shock proteins are up-regulated. In many of these processes, HDACs play a critical role in changing the accessibility of chromatin to transcriptional machinery and deregulating levels of acetylation in non-histone proteins (thus interfering with intracellular cross-talk). One line of evidence (103) suggests that class IIa HDACs interact with MEF2, one of the early activators of hypertrophy. HDACi-based therapy could, thus, help prevent heart failure. The mechanism by which HDACi suppress cardiac hypertrophy is still being investigated, but overexpression of Krüppel-like factor 4 (KLF4) seems to be involved (116). Conversely, much evidence suggests that treatment with HDACi causes QT prolongation and heart diseases (see section “Side effects of HDACi”) (203).

Neurodegenerative disorders

Neurodegeneration is an umbrella term for progressive loss of structure and/or function of neurons. For an in-depth description of this topic, we refer the reader to Fischer *et al.* (60) and the references therein. Despite the relatively recent discovery of sirtuins, the role of SIRT1 (and marginally SIRT2) in neurodegenerations seems to be clearer than that played by other HDACs (52). In Alzheimer’s disease (AD) models, SIRT1 performs a protective function, as demonstrated by the fact that its overexpression or activation by small molecules (SIRT activators) reduces β -amyloid peptide content, a hallmark of AD, and ameliorates cognitive capabilities and memory. In Huntington’s disease, SIRT1, a mediator of the beneficial metabolic effects of calorie restriction, protects neurons against mutant htt toxicity. The mechanisms subtending these effects are still not fully understood. A possible underlying defect in transcriptional regulation has been described for the diseases cited earlier, strongly suggesting the use of HDACi. By way of an example, VPA is able to decrease A- β production plaques in AD transgenic mice by inhibiting GSK-3 β -mediated γ -secretase cleavage of APP protein. At the cellular level, transcriptional regulatory proteins such as CBP, NF- κ B, p53, brain-derived neurotrophic factor, and PGC1 α , a key regulator of mitochondrial gene expression, are each implicated in these diseases. These proteins are potentially amenable to modulation by HDACi and conceivably with SIRT1 activators or inhibitors (222).

Inflammation and immune disorders

Recently, several HDACi have also been recognized as promising anti-inflammatory agents (135). The most exciting field of application is the treatment of rheumatoid arthritis (26, 185, 230). The anti-inflammatory capability of HDACi seems to be due to the inhibition of transcriptional functions

of important proteins, such as NF- κ B, leading to a reduction in the expression of pro-inflammatory cytokines (TNF- α , interleukin-1 β , and IFN- γ). HDACi treatments are currently under investigation for other immunological disorders, including inflammatory bowel diseases, systemic lupus erythematosus, and psoriasis. Growing evidence shows that HDACi exert immune-modulatory effects, causing an up-regulation of surface antigens on tumoral cells (193), leading to their enhanced recognition. HDACi up-regulate the expression of major histocompatibility complex class I and II proteins (4, 197).

Metabolic syndromes

HDACs may be considered a promising target for insulin sensitization, as demonstrated by several studies (37) in which PPAR- γ ligands are compared with canonical HDACi. HDAC3, 4, 5, and 7 work together to enhance glucose production in hepatocytes by the activation of FOXO1. This pathway leads to an increase in hepatic gluconeogenesis by the expression of G6Pase. Thus, interfering with HDACi could be beneficial for controlling obesity and diabetes (162, 238). In the context of metabolic and energetic status, the involvement of SIRT1 is more clear, because many of its deacetylation targets are key metabolic regulators. By shuttling between the nucleus and the cytosol, SIRT1 can interact with eNOS (145) and Atgs (critical components of autophagy machinery) (129), as well as with the proteins involved in mitochondrial respiration (p53 and PGC-1 α) (101), gluconeogenesis and oxidative stress (FOXOs and CRTC2), and lipid anabolism (LXR α) (133) in the nucleus.

HDACi and SIRTi in Clinical Trials Against Cancer

Based on published reports, at least 20 structurally different HDACi are currently being used in clinical trials as mono- and combination therapy for hematological and solid cancers. In this review, the focus has been limited to FDA-approved HDACi or those at an advanced stage of experimentation. All information regarding clinical trials comes from *clinicaltrials.gov*.

Vorinostat

Vorinostat is at the most advanced stage of clinical development and is studied worldwide, as shown in Figure 4. A

second-generation polar compound, Vorinostat binds to the catalytic domain of HDACs. This enables the hydroxamic moiety to chelate zinc ion located in the catalytic pockets of HDACs, thereby inhibiting deacetylation and leading to an accumulation of both hyperacetylated histones and TFs. Vorinostat was the first HDACi approved by FDA (2006) for clinical use in treating patients with advanced cutaneous T-cell lymphoma. Table 1 reports a list of trials (completed with and without results) having Vorinostat as a single drug. Although Vorinostat has shown strong antitumor activity against hematological malignancies, it is also currently being investigated for solid tumors. As an example, Vorinostat is most efficacious against glioblastoma, demonstrating the validity of HDACi-based therapies for brain tumor (66). Interestingly, four different trials employing Vorinostat against glioblastoma are currently being recruited (NCT00555399, NCT01378481, NCT01266031, and NCT01738646). Similar to many HDACi, Vorinostat has been used in a wide range of trials in combination with other drugs (demethylating agents and/or Bortezomib) and/or radiation (Table 2).

Panobinostat

Panobinostat (LBH-589), developed by Novartis, is a non-selective HDACi that is currently being used in Phase I and II clinical trials as mono- and combination therapy for hematological tumors such as non-Hodgkin's lymphoma, acute leukemia myeloblasts, AML, MM, advanced solid tumors, and breast and lung cancer (46, 54, 208) (Table 3). Table 4 presents trials in which Panobinostat is used in combination with other drugs. Panobinostat is coupled with chemicals that are able to interfere with the methylated status of DNA (NCT00946647) and with tyrosine kinase inhibitor Lapatinib (NCT00632489) (124). No results are available for any of the trials listed in Table 4, many of which are recruiting or still active, highlighting scientific interest in the development of combinatorial therapies that improve the efficacy of HDACi. In many of these interventional studies, Panobinostat is being evaluated to determine the maximum tolerated dose in different pathologies and administration regimes (NCT00686218 and NCT01496118, not present in Table 4). Panobinostat is currently used in a Phase I/II

FIG. 4. World map showing distribution of Vorinostat currently in clinical trials. Colors indicate the number of studies with locations in that region. Some trials are carried out simultaneously in different countries. Downloaded and modified from *clinicaltrials.gov*. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

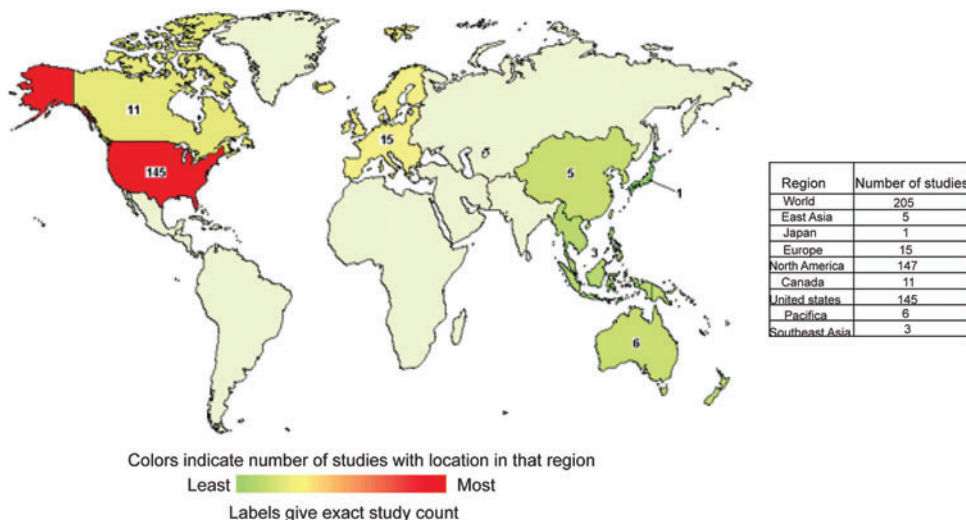


TABLE 1. CLINICAL TRIALS WITH VORINOSTAT AS SINGLE DRUG

<i>NCT number</i>	<i>Study results</i>	<i>Conditions</i>	<i>Phases</i>	<i>Funded by</i>	<i>Start data</i>
NCT00305773	Has results	AML (different Fab classifications)	2	NIH	January 2006
NCT00128102	No results available	Mesothelioma Lung cancer	3	Industry	June 2005
NCT00278330	No results available	BLCML Recurrent ALL Recurrent ALL RAEB Relapsing CML ALL AML	1	NIH	January 2006
NCT00132067	No results available	Primary peritoneal cavity cancer Recurrent ovarian epithelial cancer	2	NIH	October 2005
NCT00330161	No results available	Recurrent prostate cancer Stage IV prostate cancer	2	NIH	March 2006
NCT00363883	No results available	Localized, metastatic, recurrent, regional TCC of the renal pelvis and ureter TCC of the bladder	2	NIH	June 2006
NCT00641706	No results available	Adult giant cell glioblastoma Adult glioblastoma Adult gliosarcoma Brain tumor	2	NIH	July 2008
NCT00238303	No results available	Adult giant cell glioblastoma Adult glioblastoma Adult gliosarcoma Brain tumor	2	NIH	September 2005
NCT00132002	No results available	Male breast cancer Recurrent breast cancer Stage IV breast cancer	2	NIH	June 2005
NCT00776503	No results available	Myelodysplastic syndromes	1/2	Other Industry	May 2008
NCT00134043	No results available	Thyroid cancer	2	NIH	December 2005
NCT00479232	Has results	Leukemia, myelocytic, acute MDS MDS	1	Industry	June 2007
NCT00632931	Has results	Advanced cancer relapsed Advanced cancer refractory	1	Industry	June 2007
NCT00907738	Has results	Advanced cancer	2	Industry	August 2005
NCT00132028	Has results	Lymphoma	2	NIH	September 2005
NCT00771472	Has results	Lymphoma	1	Industry	August 2008
NCT00336141	No results available	Colorectal cancer	1	Other Industry	June 2006
NCT00373490	Has results	Tumors	1	Industry	July 2006
NCT00127127	No results available	Tumors	1	Industry	June 2005
NCT00138203	No results available	Lung cancer	2	Other NIH	January 2006
NCT00045006	No results available	Cancer	1	Other NIH	July 2001
NCT00455351	No results available	Pelvic cancer	1	Other	February 2007
NCT00005634	No results available	Leukemia Lymphoma MM and PCN Prostate cancer Small intestine cancer	1	Other NIH	January 2000
NCT00127140	No results available	Lymphoma	1	Industry	June 2005
NCT00097929	No results available	B-cell lymphoma	2	Industry	May 2005
NCT00091559	No results available	Cutaneous T-cell lymphoma Sezary syndrome Mycosis fungoides	2	Industry	February 2005
NCT00750178	No results available	Cancer, Advanced	1	Industry	November 2004
NCT00858234	No results available	Multiple myeloma	1	Industry	March 2009

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BLCML, blastic phase of chronic myeloid leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; MM, multiple myeloma; PCN, plasma cell neoplasm; RAEB, refractory anemia with excess blasts; TCC, transitional cell carcinoma.

clinical trial (NCT01680094), assessing its use in HIV-affected patients on highly active antiretroviral therapy (175).

Belinostat

Belinostat (PXD101) is a novel hydroxamic acid-type HDACi in late-stage clinical development with close to 1050 patients treated (March 2012). Belinostat is well tolerated, enabling combination with traditional chemotherapy without causing further bone marrow toxicity. Belinostat has been tested in a number of Phase I/II clinical trials in hematological cancers and solid tumors (Table 5). Data from these trials have provided evidence of the antitumor

effect of Belinostat as monotherapy in peripheral T-cell lymphoma (PTCL) (95) and cutaneous T-cell lymphoma (CTCL), liver cancer (250), and thymoma. In addition, Belinostat has beneficial effects in combination with other anticancer drugs for the treatment of multiple types of cancer, including ovarian cancer (50), cancer of unknown primary, MM, AML, and bladder cancer (Table 6).

Givinostat

Givinostat (originally ITF2357) was developed by Italfarmaco of Milan (Italy) patented in 1997 (WO 97/43251, US 6034096) and first described in 2005. It is in numerous

TABLE 2. CLINICAL TRIALS WITH VORINOSTAT IN COMBINATION WITH OTHER DRUGS

NCT number	Recruitment	Study results	Conditions	Interventions	Phases	Funded by	Start date
NCT00875745	Active, not recruiting	No results available	AML APL MDS	Sorafenib-Vorinostat	1	Other Industry	April 2009
NCT00838929	Active, not recruiting	No results available	Brain metastases	Vorinostat Radiation: radiation therapy	1	Other Industry	March 2009
NCT01023737	Active, not recruiting	No results available	Malignant solid tumor	Hydroxychloroquine (HCQ) Vorinostat	1	Other Industry	November 2009
NCT01110876	Active, not recruiting	No results available	Brain cancer Glioblastoma multiforme	Vorinostat Erlotinib Temozolomide	1/2	Other Industry	June 2011
NCT00821951	Completed	Has results	NSCLC	Vorinostat Radiotherapy	1	Other Industry	May 2009
NCT00948688	Active, not recruiting	No results available	Pancreatic cancer Adenocarcinoma of the pancreas	Radiation therapy 5-FU Vorinostat	1/2	Other Industry	August-2009
NCT00937495	Active, not recruiting	No results available	Tissue sarcoma	Vorinostat Bortezomib	2	NIH	June 2009
NCT00839956	Active, not recruiting	No results available	Multiple myeloma	Bortezomib Vorinostat	2	Other NIH	February 2009
NCT01038388	Active, not recruiting	No results available	Multiple myeloma	Vorinostat, Lenalidomide, Bortezomib, Dexamethasone	1	Other Industry	January 2010
NCT00939991	Active, not recruiting	No results available	Brain tumor Glioblastoma	Vorinostat Bevacizumab/Temozolomide	1/2	Other Industry	October 2009
NCT01132911	Completed	No results available	Lymphoma Sarcoma Wilms Tumor Neuroblastoma	Vorinostat Velcade (PS-341, Bortezomib)	1	NIH	May 2010
NCT00858234	Completed	No results available	Multiple myeloma	Vorinostat Bortezomib	1	Industry	March 2009

Table reports a list of trials started in 2009 and now closed (2013). APL, acute promyelocytic leukemia; NSCLC, non-small cell lung cancer.

Phase II clinical trials for relapsed leukemias and myelomas (NCT01761968) (79), and has been granted orphan drug designation in the European Union for the treatment of systemic juvenile idiopathic arthritis (NCT01261624, NCT01557452) and polycythaemia vera (NCT00928707). One interesting study involves assessing the use of Givinostat for Duchenne's disease (NCT01761292) (40).

PCI-24781

PCI-24781 (formerly CRA-024781) is currently being used in Phase I clinical trials for the treatment of neoplasia. The assessment of *in vitro* activity against tumor cell lines revealed the growth inhibition of multiple solid tumor lines, including colon, breast, lung, prostate, and ovarian cancers, as well as Hodgkin's and non-Hodgkin's lymphoma (15, 28, 210). Six different clinical interventional trials are now ongoing for PCI-24781, mainly for hematological diseases (NCT01149668, NCT00473577) and for testing the tolerability of the molecule in patients with hematological malignancies (NCT00562224, NCT00724984). A trial is now being recruited for the same drug (NCT01543763) in combination with PZP115891 (a tyrosine kinase inhibitor) (62).

Mocetinostat

Mocetinostat (MGCD0103) has shown clinical activity in several Phase I and II clinical trials both as a single agent (18) and in combination with Azacitidine and Gemcitabine (153) (Fig. 5). It has received orphan drug designation from FDA and has been designated an orphan medicinal product by the European Medicines Agency for the treatment of Hodgkin's lymphoma (NCT00543582) and AML (NCT00323934, NCT00324129).

Entinostat

Entinostat (MS-275, SNDX-275) is a synthetic benzamide derivative shown to inhibit HDACs, and it displays antitumor activity in many preclinical models. Due to its relatively long half life, weekly and biweekly dosing schedules are explored in the clinic. A clinical trial with this agent was first performed in patients with advanced solid tumors or lymphoma in 2005. Since then, scientific interest in Entinostat has increased, and it is currently being used in Phase I/II clinical trials for recurrent advanced non-small cell lung cancer (NSCLC) combined with 5-azacytidine, or as a single drug for breast cancers (11, 109) (see Fig. 6 for disease-type classification).

Romidepsin

Romidepsin (INN, trade name Istodax) (90), a natural product obtained from the bacteria *Chromobacterium violaceum*, is an anticancer agent undergoing clinical trials for the treatment of CTCL (119), peripheral TCL, and other cancers (pancreatic, colorectal, renal, prostate, bladder, brain, thyroid, and ovarian) (106). In 2009, FDA granted approval for the use of Romidepsin in the treatment of CTCL in patients who had received at least one previous systemic therapy. Romidepsin is currently being used in more than 50 interventional trials, alone or in combination regimens (see *clinicaltrials.gov*). Romidepsin is often coupled with canonical chemotherapies (NCT00379639),

TABLE 3. CLINICAL TRIALS WITH PANOBINOSTAT (LBH-589) AS SINGLE DRUG

<i>NCT number</i>	<i>Recruitment</i>	<i>Study results</i>	<i>Conditions</i>	<i>Phases</i>	<i>Funded by</i>	<i>Primary completion date</i>
NCT01802879	Not yet recruiting	No results available	Hematologic neoplasms	2	Industry	July 2018
NCT01451268	Recruiting	No results available	MDS AML	1/2	Other	January 2016
NCT00663832	Completed	No results available	Prostate cancer	1	Industry	December 2011
NCT01242774	Recruiting	No results available	AML	1	Industry	November 2013
NCT01245179	Recruiting	No results available	Sickle cell disease	1	Other Industry	December 2013
NCT01034163	Completed	No results available	Hodgkin's lymphoma	3	Industry	May 2012
NCT01065467	Active, not recruiting	No results available	Melanoma Malignant melanoma	1	Other Industry	December 2012
NCT00840346	Recruiting	No results available	AML	1/2	Other	September 2011
NCT00878436	Recruiting	No results available	Prostate cancer Prostatic neoplasms	1/2	Other	December 2013
NCT00985946	Completed	No results available	Neuroendocrine tumors	2	Other Industry	October 2012
NCT01613976	Recruiting	No results available	MDS CMML AML	1	Industry	September 2013
NCT01136499	Completed	No results available	Soft tissue sarcoma	2	Other	January 2012
NCT00742027	Active, not recruiting	No results available	Classical Hodgkin's lymphoma	2	Industry	July 2013
NCT01680094	Active, not recruiting	No results available	HIV infection	2	Other Industry	March 2013
NCT01007968	Completed	No results available	Advanced solid tumors	1/2	Industry	November 2012
NCT00670553	Completed	No results available	Prostate cancer Head and neck cancer Esophageal cancer	1	Industry	January 2010
NCT00532389	Active, not recruiting	No results available	Multiple myeloma	1	Industry	December 2012
NCT00739414	Completed	No results available	Cancer Advanced solid tumor	1	Industry	November 2009
NCT00570284	Completed	No results available	Cancer	1	Industry	February 2011
NCT00550277	Completed	Has results	Renal cell carcinoma	2	Other Industry	March 2010
NCT01055483	Completed	No results available	Acute myeloid leukemia	1	Industry	February 2012
NCT00412997	Completed	No results available	Tumors Cutaneous T-cell lymphoma	1	Industry	May 2008
NCT00936611	Active, not recruiting	No results available	Waldenstrom's macroglobulinemia	2	Other Industry	December 2012
NCT00667862	Completed	No results available	Prostate cancer	2	Industry	November 2010
NCT01032148	Recruiting	No results available	Hodgkin's lymphoma Non-Hodgkin's lymphoma	1	Other Industry	July 2013
NCT00490776	Completed	No results available	Cutaneous T-cell lymphoma	2/3	Industry	September 2009
NCT00594230	Completed	Has results	MDS	2	Other Industry	March 2011
NCT00880269	Completed	No results available	Refractory leukemia AML	2	Industry	February 2012
NCT01523834	Recruiting	No results available	Diffuse large B-cell lymphoma	2	Other	January 2014
NCT00535951	Completed	No results available	Carcinoma, non-small-cell lung Mesothelioma	1	Industry	January 2009
NCT00690677	Completed	No results available	Colorectal cancer	2	Other Industry	December 2011
NCT00449761	Completed	No results available	Leukemia, myeloid, chronic	2/3	Industry	August 2008
NCT00532675	Active, not recruiting	No results available	Multiple myeloma	1	Industry	October 2013
NCT00621244	Completed	No results available	Lymphoma Leukemia Multiple myeloma	1/2	Industry	August 2009
NCT01222936	Completed	No results available	Small cell lung carcinoma	2	Other Industry	June 2009
NCT00451035	Completed	No results available	Chronic myeloid leukemia in chronic phase	2/3	Industry	September 2008
NCT00777049	Active, not recruiting	No results available	Breast cancer	2	Other	November 2012
NCT00939159	Active, not recruiting	No results available	Myelodysplastic syndrome	2	Other Industry	December 2014
NCT00425555	Active, not recruiting	No results available	Cutaneous T-cell lymphoma	2/3	Industry	June 2013

CMML, chronic myelomonocytic leukemia.

TABLE 4. CLINICAL TRIALS WITH PANOBINOSTAT IN COMBINATION WITH OTHER ANTICANCER DRUGS

<i>NCT number</i>	<i>Recruitment</i>	<i>Conditions</i>	<i>Interventions</i>	<i>Phases</i>	<i>Funded by</i>	<i>Start data</i>
NCT01321346	Recruiting	AML/ALL/Hodgkin's disease Non-Hodgkin's lymphoma	Panobinostat Cytarabine Panobinostat	1	Other Industry	March 2011
NCT01169636	Recruiting	Hodgkin's lymphoma	Panobinostat Fosfamide Mesna Carboplatin Etoposide Pegfilgrastim	1/2	Other Industry	January 2011
NCT01238692	Recruiting	DLBCL	LBH589 Rituximab	2	Other Industry	November 2010
NCT01245179	Recruiting	Sickle cell disease	Panobinostat	1	Other Industry	November 2010
NCT01651039	Recruiting	MM	Panobinostat, lenalidomide and dexamethasone	2	Other Industry	July 2012
NCT01460940	Recruiting	Hodgkin lymphoma	Panobinostat Lenalidomide	2	Other Industry	October 2011
NCT00967044	Active, not recruiting	Lymphoma	Panobinostat Everolimus	1/2	Other Industry	November 2009
NCT00925132	Recruiting	Metastatic melanoma	Temozolomide, decitabine, panobinostat	1/2	Other Industry	January 2010
NCT01549431	Recruiting	MM	Panobinostat Carfilzomib Panobinostat Carfilzomib Dexamethasone	1	Other Industry	January 2012
NCT01336842	Recruiting	Solid tumors NSCLC	Panobinostat, cisplatin, pemetrexed	1	Other Industry	April 2011
NCT01463046	Recruiting	AML/Advanced MDS	Panobinostat Cytarabine Daunorubicin	1	Other Industry	January 2012
NCT00788931	Completed	HER-2 positive breast cancer Metastatic breast cancer	LBH589 trastuzumab paclitaxel	1	Industry	December 2008
NCT01023308	Active, not recruiting	MM	Panobinostat Bortezomib	3	Industry	December 2009
NCT00632489	Active, not recruiting	Breast cancer	LBH589 Capecitabine Lapatinib	1	Other Industry	May 2008
NCT01282476	Recruiting	DLBCL	Panobinostat with Rituximab	2	Other Industry	June 2011
NCT01301807	Recruiting	Myeloma	Carfilzomib Panobinostat	1	Other Industry	August 2011
NCT00691938	Active, not recruiting	AML MDS	LBH589 Decitabine	1/2	Other	June 2008
NCT00891033	Active, not recruiting	MM	Bortezomib (Velcade) Panobinostat	1	Other Industry	April 2011
NCT01440582	Recruiting	Myeloma	Panobinostat Bortezomib Lenalidomide Dexamethasone	1/2	Other Industry	February 2013
NCT01159418	Active, not recruiting	Advanced solid tumors	Panobinostat (LBH589), carboplatin and paclitaxel	1	Other Industry	June 2008
NCT00946647	Recruiting	MDS CMML AML	Panobinostat (LBH589) and 5-Azacytidine 5-Azacytidine	2	Industry	December 2009
NCT01693601	Recruiting	Myelofibrosis	Panobinostat Ruxolitinib	1/2	Other	January 2013
NCT01005797	Recruiting	Renal cancer NSCLC	Panobinostat (LBH589), sorafenib	1	Other Industry	November 2009
NCT00567879	Completed	Breast cancer	Panobinostat, trastuzumab	1	Industry	April 2008
NCT00859222	Recruiting	Malignant glioma	LBH589 Bevacizumab	1/2	Other Industry	March 2009
NCT00907179	Recruiting	NSCLC	LBH 589 Pemetrexed	1/2	Other Industry	July 2009
NCT01055795	Completed	Advanced solid tumors	Bevacizumab, everolimus and LBH589	1	Other Industry	March 2010
NCT01504776	Recruiting	Mantle cell lymphoma	Panobinostat Bortezomib	1	Other Industry	April 2011
NCT00556088	Completed	Solid tumors	LBH589, paclitaxel, carboplatin, bevacizumab	1	Other Industry	December 2007
NCT00880269	Completed	Refractory leukemia AML	Panobinostat LBH589	2	Industry	August 2009
NCT00738751	Active, not recruiting	Lung cancer Head and neck cancer	LBH589 Ertotinib	1	Other Industry	November 2008
NCT01341834	Recruiting	Nasopharyngeal carcinoma, lymphomas, any EBV + solid tumor	LBH589 and RAD001	1	Other Industry	January 2011
NCT01194908	Recruiting	Breast cancer Breast tumors Breast neoplasms	Decitabine, LHB589, tamoxifen	1/2	Other Industry	July 2010
NCT00962507	Completed	Lymphoma MM and plasma cell neoplasm	Everolimus Panobinostat	1	Other NIH	July 2009
NCT00978432	Recruiting	DLBCL	RAD001 LBH589 RAD001 and LBH589 as a doublet	2	Other Industry	February 2012
NCT01175109	Active, not recruiting	Chordoma	Imatinib + LBH589	1	Other	October 2011

DLBCL, diffuse large-B cell lymphomas; EBV, Epstein-Barr virus.

TABLE 5. CLINICAL TRIALS WITH BELINOSTAT (PXD101) AS SINGLE DRUG

<i>NCT number</i>	<i>Recruitment</i>	<i>Study results</i>	<i>Conditions</i>	<i>Phases</i>	<i>Funded by</i>
NCT00413075	Completed	No results available	Solid tumor Lymphoma	1	Industry
NCT00274651	Completed	No results available	Cutaneous T-cell lymphoma Peripheral T-cell lymphoma Non-Hodgkin's lymphoma	2	Industry
NCT00865969	Active, not recruiting	No results available	Peripheral T-cell lymphoma	2	Industry
NCT00301756	Active, not recruiting	No results available	Fallopian tube cancer Primary peritoneal cavity cancer Ovarian tumor Ovarian epithelial cancer	2	NIH
NCT01583777	Not yet recruiting	No results available	Advanced cancer	1	Industry
NCT00321594	Completed	No results available	Adult primary hepatocellular carcinoma Liver cancer	1/2	NIH
NCT00589290	Active, not recruiting	Has results	Thymoma Thymic carcinoma	2	NIH
NCT01273155	Recruiting	No results available	Neoplasms Lymphomas	1	NIH
NCT00357162	Completed	Has results	<i>de Novo</i> , previously, secondary MDS	2	NIH
NCT00357032	Completed	No results available	AML	2	NIH
NCT00365053	Completed	No results available	Mesothelioma	2	NIH
NCT00303953	Completed	Has results	Recurrent adult Burkitt lymphoma Recurrent adult DLCL	2	NIH

DLCL, diffuse large cell lymphoma.

demethylating agents (NCT01537744), and proteasome inhibitors (PIs; NCT00765102) (91, 194, 202).

Valproic acid

VPA has found clinical use as an anticonvulsant and mood-stabilizing drug, primarily in the treatment of epilepsy, bipolar disorder, and, less commonly, major depression. In addition, it is used to treat migraine headaches and schizo-

phrenia (around 300 interventional and/or observational trials). VPA is also an HDACi and is under investigation for the treatment of HIV and various cancers (26 studies are reported for VPA used as HDACi) (43). Phase I and II clinical trials have tested VPA, alone or in combination, for lymphocytic leukemia, AML and myelodysplastic syndrome, melanoma, HIV infection, autoimmune lymphoproliferative syndrome, and human T-lymphotropic virus type-1-associated myelopathy/tropical spastic paraparesis. Some Phase II clinical

TABLE 6. CLINICAL TRIALS WITH BELINOSTAT IN COMBINATION WITH OTHER DRUG/REGIMENS

<i>NCT number</i>	<i>Recruitment</i>	<i>Conditions</i>	<i>Interventions</i>	<i>Phases</i>	<i>Funded by</i>
NCT01075425	Recruiting	ALL AML MDS CML	Bortezomib Belinostat	1	Other NIH
NCT00421889	Completed	Ovarian cancer Epithelial ovarian cancer Fallopian tube cancer	Belinostat Paclitaxel Carboplatin	1/2	Industry
NCT00873119	Completed	Occult primary	Belinostat, carboplatin, paclitaxel Carboplatin, paclitaxel	2	Industry
NCT01317927	Active, not recruiting	Solid tumor Hematological malignancy	Belinostat, warfarin	1	Industry
NCT01310244	Recruiting	NSCLC	Belinostat, carboplatin, paclitaxel	1/2	Industry
NCT01090830	Terminated	NSCLC	Belinostat, carboplatin, paclitaxel and bevacizumab	1/2	Other
NCT01839097	Recruiting	Peripheral T-cell lymphoma	Belinostat CHOP	1	Industry
NCT00878800	Completed	Soft tissue sarcomas	PXD101 + doxorubicin	1/2	Industry
NCT01100944	Active, not recruiting	Thymoma Thymic carcinoma	Belinostat Cisplatin Cyclophosphamide Doxorubicin	1/2	NIH
NCT00413322	Completed	Tumor	Belinostat 5-Fluorouracil (5-FU)	1	Industry
NCT01686165	Recruiting	Large cell lymphoma	Belinostat Biological: rituximab Radiation: yttrium Y 90 ibritumomab tiuxetan	2	Other NIH
NCT00334789	Active, not recruiting	Unspecified adult solid tumor	Belinostat Isotretinoin Other: pharmacological study	1	NIH
NCT01188707	Terminated	NSCLC	Belinostat and Erlotinib	1/2	Other Industry
NCT00926640	Recruiting	SCLC Malignant epithelial neoplasms	(Belinostat) (PDX101) Cisplatin Etoposide	1	NIH
NCT00131261	Completed	Multiple myeloma	PXD101 Dexamethasone	2	Industry

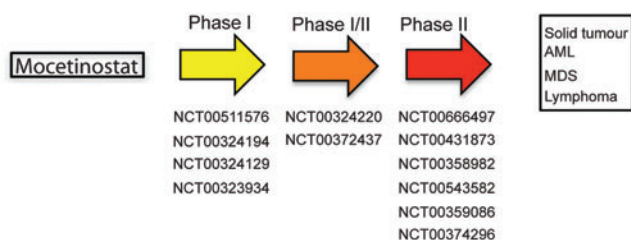


FIG. 5. Clinical trials with Mocetinostat (MGCD0103). Studies are divided according to phase progression. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

trials have involved combination therapy with hydralazine, a DNA demethylating agent, and magnesium valproate for the treatment of cervical and breast cancers, and refractory solid tumors. Other clinical trials are ongoing for HD and amyotrophic lateral sclerosis.

Clinical trials with SIRT inhibitors or activators are still in early phases, and many of these involve resveratrol. For an in-depth description of this topic, we refer the reader to Nebbioso *et al.* (158) and references therein, such as trials NCT01521832, NCT01485952, NCT01485965, and NCT01521585.

Limits in the Current Use of HDACi

Tumor cells are extremely plastic, and one of the key aspects of the malignant transformation process is the capability to escape normal checkpoints. Consequently, tumor cells are generally more “independent” from the environment and they are also able to modify. In many cases, tumor cells develop mechanisms of resistance to HDACi caused by epigenetic and genetic characteristics, contributing to the generation and maintenance of a neoplastic phenotype. Cellular factors implicated in resistance phenomena to HDACi include drug efflux, target status (such as overexpression, mutation, and desensitization), chromatin alteration, up-regulation of oxidative stress response mechanism, defects in pro-apoptotic pathways, and up-regulation of anti-apoptotic signals/stimuli (Fig. 7).

Effect of HDACi efflux in cells

Multidrug resistance (MDR), mediated by multiple drug efflux ATP-binding cassette (ABC) transporters, is a critical issue in the treatment of many cancers (hematological and solid), with permeability (P)-glycoprotein (P-gp), multidrug

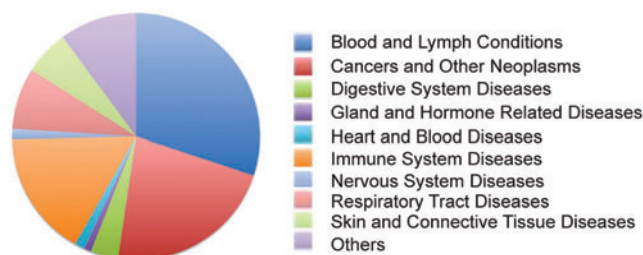


FIG. 6. Clinical trials with Entinostat (MS-275) by topic classification. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

resistance-associated protein 1 (MRP1), and breast cancer resistance protein (ABCG2), which are consistently shown to be the key effectors of MDR in cell line studies (132, 241). Pumps conferring MDR are often overexpressed in many types of cancer, requiring the use of HDACi at a higher dose or in combination with inhibitors of transporter systems (132). Overexpression of these cellular pumps is also a side effect in treatment with HDACi. Hyperacetylation of the promoter regions encoding for these genes is often observed after HDACi treatment. To date, some information is available for Romidepsin, which has been found to interact with P-gp and MRP1 (168, 242, 243). The variability of HDACi structures can help overcome problems with cell intake. Changing permeability properties of the molecules using a combinatorial or rational approach could, therefore, improve the efficacy of available drugs; while further studies into pharmacokinetics and pharmacodynamics could reduce required doses and aspecific interactions.

HDAC status

As previously discussed (see section “HDACs and sirtuins in cancer: quantitative and functional abnormalities”), many HDACs are overexpressed in cancer. To date, various studies have investigated the aberrant expression of HDACs in tumorigenesis as well as the progression to metastatic/refractory phenotypes. Although overexpression and mutation of HDACs contributes to inducing tumorigenesis (41), this represents a serious drawback in HDACi-based therapies. In many cases, overexpression of HDAC1 alone is able to influence the outcome of epigenetic therapy (156), without considering the compensative effects always present when a member of a specific enzymatic class is targeted. In some cases, mutation of a specific HDAC causes resistance to treatment with specific inhibitors (HDAC2 is mutated in colon cancers) (140). Resistance acquired by alterations to HDACs requires further investigation with regard to the possibility of using pan- or selective inhibitors against the specific HDAC involved in a specific cancer (see next section).

Apoptotic/pro-survival mechanisms

Altered levels of anti-apoptotic proteins in cancer cells drive resistance against HDACi-mediated apoptosis. More specifically, it has been observed that by inhibiting the transcriptional activity of the JAK/STAT pathway, resistant CTCL cells can be re-sensitized to Vorinostat-induced apoptosis (53, 240). Consistently, overexpression of anti-apoptotic proteins such as Bcl-2, Bcl-XL is sufficient to render transformed cells resistant to HDACi. This observation is common in leukemias and lymphomas, in which the expression level of Bcl-2 protein is increased (211). The same is true for NF-κB, which is able to interfere with apoptosis in NSCL cancer lines, after treatment with HDACi. A different trend is observed for p21, which is overexpressed after treatment with HDACi (in particular, specific class I HDACi), acts as a negative regulator of cell-cycle progression in a p53-dependent or -independent manner, and has been implicated in the regulation of cell death. Overexpression of p21 also correlates with the start of biochemical processes, leading to DNA repair (12, 100). Since one of the mechanisms by which HDACi induce cell death is the creation of DNA damage (and genome instability), and since these

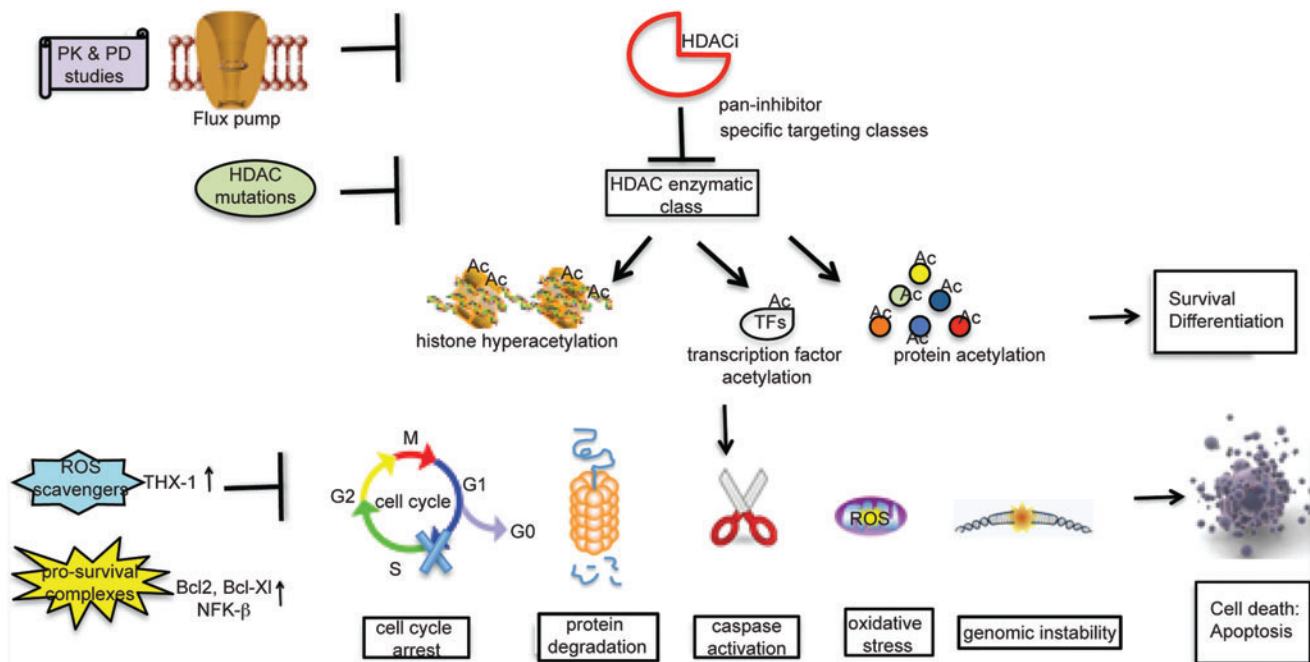


FIG. 7. Mechanisms leading to cell death and possible interfering causes in response to HDACi treatment in cancer cells. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

phenomena contribute to the cytotoxic effects of HDACi, p21 may act as a resistance-inducing factor.

Chromatin and epigenetic modifications

DNA methylation contributes to malignant transformation (45), such as histone acetylation/deacetylation. The balance between the acetylated status of histones and the methylated status of DNA determines expression levels of onco-suppressors and oncogenic effectors. In many reports, the treatment of cells with HDACi alone is able to restore the expression of tumor suppressor genes, while other studies claim that a hierarchy in methylation and acetylation exists, with the dominance of DNA methylation as repressive marks in cancer cells. These findings suggest combining HDACi treatment with DNA demethylating agents (see section “Examples of HDACi in Combination Treatments”).

ROS scavengers as determinants of resistance

Redox pathways play an important role in the resistance of malignant transformed cells to HDACi. Many studies hypothesize a correlation between sensitivity to HDACi and levels of ROS scavengers (36, 72). A large body of information is available for thiol reductase (Trx), which is up-regulated in half of the tumors that are resistant to HDACi treatment (141, 220). Vorinostat is able to increase the level of Trx in normal cells (an important finding that explains HDACi selectivity for cancer cells) and, conversely, in transformed cells, Vorinostat induces up-regulation of the negative regulator of thioredoxin-binding protein. Increased levels of thioredoxin-binding protein lead to ROS accumulation and so, to the activation of pro-death pathways. In many pathological systems, such as myeloid leukemias, the level of Trx is up-regulated and normal HDACi-based therapies, consequently, fail. In this scenario, not only Trx but also other proteins participating in stress

response and oxidative damage recognition could act as determinants of HDACi-resistance mechanisms.

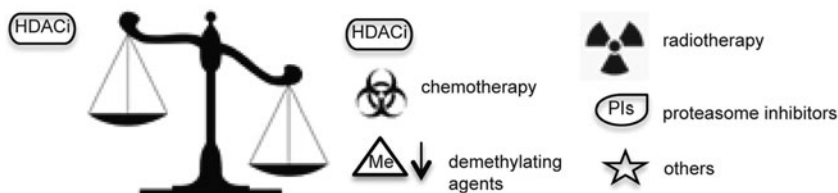
Side Effects of HDACi

As with any class of anticancer agents, some HDACi in clinical trials, such as Romidepsin (2, 146, 164), Panobinostat (46), and Vorinostat (261), have been associated with serious cardio-toxicity in the treatment of solid cancers. The most important/frequent signal in cardio-toxicity status induced after HDACi administration is the prolongation of QT interval. The biological mechanism causing this side effect is not fully understood, but it may involve the aberrant expression/function of ion channels [e.g., (hERG)K⁺ channel]. (hERG)K⁺ channel is expressed in the nervous system and heart, where it determines the timing of electrical repolarization of the action potential in ventricular myocytes. Genetic mutations in and inhibition of (hERG)K⁺ channel can result in long QT syndrome. The function of (hERG)K⁺ channel *in vivo* is associated with other proteins such as MinK and MiRP1, and defects in their expression levels could, therefore, cause QT prolongation syndromes. To date, it is still unclear as to whether HDACi can react directly with K⁺ channels or whether their effect is due to an altered gene expression pathway. The former hypothesis is not corroborated by the fact that many HDACi induce QT prolongation independently of their structures. The greatest cardio-toxicity is, however, observed after treatment with hydroxamic-derivative HDACi. Again, it is unclear as to whether hydroxamic derivatives interact preferentially with (hERG)K⁺ channels or whether their broad-spectrum activity against all HDACs could exacerbate cardiac side effects (195).

Examples of HDACi in Combination Treatments

Although HDACi show promise as single anticancer agents, in particular with regard to their low-molecular range of

FIG. 8. Schematic representation of the unbalanced number of trials with HDACi as a single drug and in combination regimens.



activities and minimal toxicity, many studies are investigating the use of HDACi in combination with other drugs (21, 212). In this section, we present the most frequently used combinatorial therapeutic approaches. Figure 8 shows a comparison between the number of single and multiple agents in ongoing clinical trials.

HDACi and death receptor agonists

HDACi can increase the expression and/or activity of proteins that directly transmit an apoptotic signal through death receptor pathways such as Fas and TRAIL ligands and death receptors, and downstream caspases (caspase-8 and -3), and can down-regulate proteins negatively regulating death-receptor signaling (*e.g.*, c-FLIP, XIAP, survivin, IAP1/2). These findings provide a strong rationale for combining HDACi with death receptor stimuli. Since HDACi do not sensitize normal cells to TRAIL-mediated apoptosis, their combination with TRAIL is the most attractive option for cancer therapy (57, 99, 176, 226).

Combination of HDACi with irradiation

HDACi such as Vorinostat, TSA, VPA, and PCI-24781 enhance the radiosensitivity (γ -radiation) of tumoral cells. HDACi-mediated radiosensitization has been observed in several cancer cell lines such as lung, colon, prostate, breast, cervical, head, and neck. Preclinical studies have demonstrated that pre-treatment with HDACi followed by ionizing radiation is more effective (9, 83). Pre-treatment with Vorinostat in colorectal xenograft models produced a significant reduction in tumor volume compared with treatment with either therapy alone. Similar results have been observed in glioma and prostate tumors using other HDACi (34). At the molecular level, the synergistic antitumor effects of HDACi combined with radiotherapy enhance ATM, p53, and BRCA1 expression (114). Although the synergistic mechanisms are not fully understood, some findings suggest that two different biological pathways are involved. First, concomitant treatment with HDACi and radiotherapy induces the activation of ATM, culminating in the phosphorylation of p53 and, thus, triggering p53-mediated apoptotic cell death. ATM is activated by DNA damage induced by γ -radiation. Many authors report that treatment with HDACi exacerbates γ -radiation-mediated DNA damage, because chromatin is in a more open configuration (9, 122). HDACi also influence DNA repair mechanisms, with the down-regulation of proteins involved (such as Ku70, Ku86, and kinases). The capability of HDACi to induce a DNA damage response while suppressing the cellular repair mechanisms that are able to overcome it, therefore, provides a molecular rationale for combining HDACi and radiotherapy. This synergic approach could also improve the efficacy of anti-cancer therapies by controlling cutaneous radiation syndrome, a side effect of radiotherapy.

HDACi and regulators of proteasomal degradation

HDACi have recently been coupled with proteasome degradation regulators to enhance their efficacy against many cancers (92). Among all the PIs, Bortezomib is the best characterized. Proteasome inhibition results in the accumulation of mis-folded and damaged proteins, which, in turn, triggers a heat-shock protein response leading to apoptosis. The exact mechanism by which HDACi and PIs exert anti-cancer activity is not completely clear, but it is known to involve the regulation of Hsp90 and its client targets, such as the inhibition of NF- κ B activity. ROS production and accumulation also very likely plays a role (59).

HDACi in combination with demethylating agents

Accumulating evidence has shown that the inhibition of aberrant epigenetic modifications caused by altered function/expression of HDACs could be more effective if the target strategy involving HDACi is coupled with demethylating agents. In many studies, HDACi treatment alone is insufficient to reactivate genes that are silenced by promoter hypermethylation, and is, therefore, used in combination with Decitabine, Azacitidine, and Zebularine. HDACi frequently used in combination regimens are VPA, sodium phenylbutyrate, and TSA for the treatment of AML, as well as colon, breast, endometrial, and thyroid cancers. Synergistic effects of HDACi and demethylating agents on gene expression might prolong the therapeutic time window. Although most clinical trials are still ongoing and concentrate predominantly on dosing schedules and safety of combination therapies, some studies have shown encouraging results. In patients with advanced hematological or solid malignancies, the combination of DNA-methyltransferase inhibitors and HDACi is well tolerated and induces biological effects such as DNA hypo-methylation and histone acetylation (33, 81, 102, 111, 188, 201).

HDACi with chemotherapeutic drugs

Combinatorial chemotherapeutic treatment is frontline therapy for many cancer types. Taxanes, which include paclitaxel and docetaxel, have also been used with HDACi such as TSA, Vorinostat, and PCI-24781. Since taxanes promote the stabilization of microtubules and interfere with transition from metaphase to anaphase during mitosis, the pre-treatment of cancer cells with HDACi enhances therapy by increasing tubulin acetylation. In particular, by combining class I/II HDACi with taxanes, a reduction in the proliferation of prostate, breast, ovarian, and gastric cancer cells has been observed (265). Other chemotherapeutic combinations, including DNA damage and microtubule-targeting agents, have also been used for cancer treatment. The addition of escalating doses of Belinostat in co-treatment with carboplatin and paclitaxel has been well tolerated for solid tumors

(27, 50, 126). Vorinostat is also used in the carboplatin-paclitaxel chemotherapeutic regimen against NSCL. HDACi co-treatment with chemotherapeutic agents has also been employed in patients affected by anaplastic thyroid cancer. The combination of VPA and cisplatin-doxorubicin with radiotherapy decreases the volume of this tumor. However, clinical evaluation has, thus, far been limited to early-phase trials, making it difficult to draw conclusions regarding any added clinical benefit from the addition of HDACi. In conclusion, combining drugs that target different signaling pathways often lessens adverse side effects, while increasing the efficacy of treatment and reducing patient morbidity (187). In addition, multi-drug regimens using a variety of drugs and time schedules of drug administration may offer patients a more tailored therapy based on clinical status.

Open Questions: Specific or Pan-HDACi?

Unselectively inhibiting HDAC activity elicits not only therapeutic responses but also unexpected side effects. Considering the diversity of HDAC targets, selective HDACi (active against a specific HDAC class or a single isoform) may cause fewer adverse reactions and, thus, be more clinically useful. For example, class II HDACs are abundant in the heart and repress cardiac gene expression by direct or indirect association with TFs (7). As discussed in the section “Side effects of HDACi,” HDACi administration commonly causes QT interval prolongation. A selective HDACi, inactive against class II HDACs, could eliminate or reduce unwanted cardiac responses by preserving the activity of HDAC4, 5, 7 (class IIa), 6, and 10 (class IIb). To date, although considerable effort has been made toward their development, a few class-specific HDACi are available (for instance, MC1568, active on HDAC4 and 6 with $IC_{50} \approx 220 \text{ nM}$) (160). The difficulty in designing this kind of HDACi is essentially due to structural homology between HDACs and the way in which these inhibitors are developed. HDACi reported thus far are structurally diversified but, nevertheless, act as competitive inhibitors, targeting zinc ion in HDAC active sites. These compounds generally belong to the pan-inhibitor class. Sequence comparison indicates that the main differences between individual HDACs are found near the catalytic core, in the regions forming loops. Thus, inhibitors directed against this region, acting as non-competitive modulators, may display HDAC selectivity. Another way to develop non-competitive HDACi is to look at enzymatic regions that are responsible for interactions with DNA-binding proteins and/or transport proteins (for class IIa HDACs) (138). Non-competitive modulators have the advantage of being inactive (or less active) against other enzymes inside the cell requiring zinc ion as cofactor (such as RNA polymerase II, metalloprotease). The design of a new selective HDACi usually starts by taking a known active HDACi and modifying some of the structural features of the molecule without reducing its activity. Known inhibitors comprise a capping group, metal-binding moiety, and a linker region. While the capping group has been successfully modified for the design of several class-selective inhibitors (17), a combination of modifications at all three regions likely enhances their selective function. Modifications of the capping group are also useful in targeting HDACi to specific tissues. To confer tissue-selectivity properties to HDACi, they can be equipped with a surface recognition capping group that is

capable of binding unique biological targets, such as expressed or overexpressed membrane receptor-binding molecules. One example of this strategy is compound CHR-2845, which is hydrolyzed by macrophage membrane receptor hCE-1 (carboxylesterase 1 receptor). The hydrolysis of CHR-2845 gives CHR-2847, the active compound accumulated only in cells expressing hCE-1 (84).

Concluding Remarks

In this review, we summarize studies on the classification, mechanisms of action, and chemistry of HDACs and their inhibitors. Several HDACi are currently being used in clinical trials for both solid and hematological malignancies. In combination with DNA-demethylating agents, chemopreventive, or classical chemotherapeutic drugs, HDACi are promising candidates for cancer therapy. Many questions regarding efficacy, tumor specificity, and gene regulation patterns still remain to be answered. A key issue is the identification of biomarkers that are predictive of positive outcome (*i.e.*, are we aiming at the right “enemies” with the right “weapons”?). Is it possible to identify mRNAs and/or proteins that increase in response to a particular HDACi and which might also be used as indicators of drug efficacy? This is an ongoing discussion in epigenetics, and future research may help provide insights into response-based patient stratification. Due to the huge number of newly discovered epi-enzymes and their interconnection with tumorigenesis and cancer progression, a key task in future diagnostic, therapeutic, and prognostic strategies will focus on characterizing the interplay between genome and epigenome, and, thus, designing personalized epi-based single or combinatorial treatments.

Acknowledgments

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Address correspondence to:

Prof. Lucia Altucci
Department of Biochemistry, Biophysics,
and General Pathology
Seconda Università degli Studi di Napoli
Vico L. De Creschio 7
Napoli 80138
Italy

E-mail: lucia.altucci@unina2.it

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Abbreviations Used

ABC = ATP-binding cassette
AD = Alzheimer's disease
ALL = acute lymphoblastic leukemia
AML = acute myeloid leukemia
APL = acute promyelocytic leukemia
DLBCL = diffuse large B-cell lymphomas
eNOS = endothelial nitric oxide synthase
HDAC = histone deacetylase
HDACi = histone deacetylase inhibitors
HIF-1 α = hypoxia-inducible factor
IFN- γ = interferon- γ
MDR = multidrug resistance
MDS = myelodysplastic syndrome
MM = multiple myeloma
MRP1 = multidrug resistance-associated protein 1
MSI = microsatellite instability

NES = nuclear export signal
NF- κ B = nuclear factor- κ B
P-gp = permeability-glycoprotein
PIs = proteasome inhibitors
ROS = reactive oxygen species
SIRT = sirtuin
SIRTi = sirtuin inhibitors
TF = transcription factors
TNF = tumor necrosis factor
TRAIL = tumor necrosis factor-related apoptosis-inducing
ligand
Trx = thiol reductase
TSA = trichostatin A
VEGF = vascular endothelial growth factor
VPA = valproic acid
YY1 = Yin and Yang 1