Annu. Rev. Pharmacol. Toxicol. 2005. 45:495–528
doi: 10.1146/annurev.pharmtox.45.120403.095825

First published online as a Review in Advance on September 27, 2004

CLINICAL DEVELOPMENT OF HISTONE DEACETYLASE INHIBITORS AS ANTICANCER AGENTS*

Daryl C. Drummond,[1] Charles O. Noble,[2,3] Dmitri B. Kirpotin,[1] Zexiong Guo,[2] Gary K. Scott,[4] and Christopher C. Benz[4]

[1]*Hermes Biosciences, Inc., South San Francisco, California 94080*
[2]*California Pacific Medical Center-Research Institute, San Francisco, California 94115*
[3]*University of California at San Francisco, San Francisco, California 94143*
[4]*Buck Institute for Age Research, Novato, California 94945*

Key Words HDAC inhibitors, targeting chromatin structure and epigenetic mechanisms, transcription regulation, hydroxamic acids

■ **Abstract** Acetylation is a key posttranslational modification of many proteins responsible for regulating critical intracellular pathways. Although histones are the most thoroughly studied of acetylated protein substrates, histone acetyltransferases (HATs) and deacetylases (HDACs) are also responsible for modifying the activity of diverse types of nonhistone proteins, including transcription factors and signal transduction mediators. HDACs have emerged as uncredentialed molecular targets for the development of enzymatic inhibitors to treat human cancer, and six structurally distinct drug classes have been identified with in vivo bioavailability and intracellular capability to inhibit many of the known mammalian members representing the two general types of NAD^+-independent yeast HDACs, Rpd3 (HDACs 1, 2, 3, 8) and Hda1 (HDACs 4, 5, 6, 7, 9a, 9b, 10). Initial clinical trials indicate that HDAC inhibitors from several different structural classes are very well tolerated and exhibit clinical activity against a variety of human malignancies; however, the molecular basis for their anticancer selectivity remains largely unknown. HDAC inhibitors have also shown preclinical promise when combined with other therapeutic agents, and innovative drug delivery strategies, including liposome encapsulation, may further enhance their clinical development and

*Nonstandard abbreviations: AOE, 2-amino-8-oxo-9,10-epoxy-decanoic acid; ATRA, all-*trans*-retinoic acid; CBHA, m-carboxylcinnamic acid bis-hydroxamide; CDK, cyclin-dependent kinase; DAC, 5-aza-2′-deoxycytidine; HAT, histone acetyltransferase; HDAC, histone deacetylase; HMT, histone methyltransferase; IMID-1, immunomodulatory thalidomide derivative 1; PB, phenyl butyrate; SAHA, suberoylanilide hydroxamic acid; SB, sodium butyrate; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TSA, trichostatin A.

anticancer potential. An improved understanding of the mechanistic role of specific HDACs in human tumorigenesis, as well as the identification of more specific HDAC inhibitors, will likely accelerate the clinical development and broaden the future scope and utility of HDAC inhibitors for cancer treatment.

INTRODUCTION

The packaging of DNA into the higher order and dynamic structure of chromatin provides a pivotal point of control for gene expression by regulating access of transcription factors to DNA. Chromatin is composed of multiple repeating units termed nucleosomes, which are comprised of 146 base pairs of DNA wrapped around a core of eight histone proteins composed of two copies each of H2A, H2B, H3, and H4. Posttranslational modifications play a prominent role in the regulation of gene expression and signal transduction pathways. Phosphorylation, methylation, acetylation, ubquitination, and sumoylation are the known modifications thought to influence chromatin architecture and regulate gene transcription. The composition and consequences of these various histone modifications are often referred to as the histone code, orchestrating an intricate regulation of nucleosomal structure, DNA accessibility, and gene transcription (Figure 1). To date, acetylation is the most thoroughly studied of these modifications; and while the acetylation state of chromatin proteins is unquestionably very dynamic, it seems to depend on the net local balance between histone acetyltransferase (HAT) and histone deacetylase (HDAC) activities. Empirically observed is the fact that HDAC activity is invariably increased in cancer cells, resulting in altered gene transcription, impaired differentiation, increased cell survival, and dysregulated proliferation (1).

Transcriptional Regulation and the Histone Code

Early models of how acetylation regulates transcription focused on the physical interactions of the basic histone proteins with negatively charged DNA. The addition of charge-neutralizing acetyl groups to lysine residues on histones disrupts interactions with DNA, resulting in decompaction of chromatin, greater access of the DNA to transcription factors, and the presence of a transcriptionally active genomic locus. However, there is considerable evidence that these models are oversimplified. In cell culture studies, less than 10% of transcriptionally active genes appear to be altered in response to treatment with HDAC inhibitors, with a near equal proportion of these being induced as repressed (2, 3). This suggests that regulation of gene expression by acetylation is more highly selective than would be expected by a simple and unregulated physical disruption of histone-DNA structure, and also likely involves chromatin-associated nonhistone proteins.

Nonetheless, the complex network of interdependent and site-specific histone modifications associated with restricted and sequence-specific DNA binding by transcription factors has resulted in a histone code hypothesis for gene-specific

transcriptional control (4, 5). The code is set by a variety of histone tail–derivatizing enzymes, including HATs for the acetylation of lysine residues (6), histone methyltransferases (HMTs) for methylation of histone lysine and arginine residues (7, 8), serine kinases for the phosphorylation of specific histone serine residues (9), ubiquitin ligase for the addition of the 76-amino-acid 9-kDa protein ubiquitin to specific lysine residues (10), and the sumoylation of lysine by the 11-kDa small ubiquitin-related modifier (SUMO) (11). In addition, the activities of modification destabilizing enzymes such as HDACs, methylases, phosphatases, and ubiquitin and ULP-related proteases help shape the status of the code. The complexity of transcriptional regulation by histone modifications is further enhanced by the interaction of HATs and HDACs with other proteins involved in chromatin modification, including methyl CpG-binding proteins and ATP-dependent chromatin-remodeling complexes, which can lead to replication propagated and more enduring epigentic modifications of DNA, such as the gene silencing cytosine methylation of specific CpG dinucleotides (12–14).

The setting of the histone code involves establishing defined patterns of histone tail modifications, whereupon a particular modification in turn affects subsequent modifications. For example, histone deacetylation has been shown to activate lysine (K) methylation, resulting in relatively stable transcriptional silencing (15). In an eloquent experiment demonstrating sequential histone modifications, Kouzarides and colleagues showed that upon estrogen stimulation, H3 is acetylated initially at K18, then at K23, and finally methylated at R17 (16). A specific set of histone modifications was proposed to direct DNA methylation (17). The reading of the code can be accomplished through recognition of particular modifications or groups of modifications (18, 19). The bromodomain of proteins such as BRG1 and TAFII250 and the chromodomain of HP1 recognize acetylated lysines and methylated lysines, respectively (20–22). Certain combinations of modifications can also dictate the recruitment of various *cis*- or *trans*-acting regulatory proteins. The role of the particular modification in transcriptional signaling may also be influenced by the degree and stability of the modification. Lysine residues may be modified with one, two, or three methyl groups, and the degree of methylation determines if transcription of certain genes is activated or repressed (23, 24). The methylated lysines, and more so the methylated cytosines in DNA, are more stable modifications than the relatively dynamic modifications of histone tail acetylation and phosphorylation. Thus, with the lack of any known histone or DNA demethylases, methylation may be more important in epigenetic memory, whereas the acetylation status of histones may be more of a switch that can be rapidly reset and allow transcription to respond more rapidly to changes in the cell's environment.

The histone code is just beginning to be deciphered and thus its complexity and its role in carcinogenesis are far from understood. Although it is obvious that a wide variety of posttranslational protein modifications are responsible for regulating transcription of any given gene and as such can play important roles in human cancer cell behavior, the remainder of this review focuses specifically on the preclinical and clinical development of HDAC inhibitors as potential anticancer

agents. In this regard it is important to note that the activity of a wide variety of nonhistone transcription factors and co-regulators of transcription are known to be modified by acetylation, and both are structurally and functionally affected by HDAC inhibitors. Acetylation may enhance or inhibit the function of transcriptional activators as well as transcriptional repressors; therefore, enhancing their degree of acetylation by cell treatment with an HDAC inhibitor can either increase or repress the transcription of genes regulated by such nonhistone proteins (Table 1). TFIIE (25), TFIIF (25), p53 (26), androgen receptor (27), estrogen receptor-α (28), and GATA-1 (29, 30) are promoter-binding and transcription-regulating proteins shown to be acetylated in response to HDAC inhibition. In addition, other DNA binding nonhistone proteins are functionally affected by acetylation. For example, HMG-17 is a nucleosomal binding protein responsible for unfolding the higher order structure of chromatin and thus exerts indirect control over gene transcription; and acetylation of HMG-17 has been shown to reduce its binding to chromatin (31).

Classification of HDACs

There are three major groups or classes of mammalian HDACs based on their structural homologies to the three distinct yeast HDACs: Rpd3 (class I), Hda1 (class II), and Sir2/Hst (class III). Class III HDACs consist of the large family of sirtuins (SIRs) that are evolutionarily distinct, with a unique enzymatic mechanism dependent on the cofactor NAD^+, and are virtually unaffected by all HDAC inhibitors currently under development (32, 33). This review focuses on the NAD^+-independent class I and II HDACs (Figure 3), as they are evolutionarily similar, contain an active site zinc as a critical component of their enzymatic pocket, have been more thoroughly described in association with cancer, and are thought to be comparably inhibited by most currently available HDAC inhibitors. The Rpd3 homologous class I HDACs include HDAC1, HDAC2, HDAC3, and HDAC8. They are widely expressed in a variety of tissues and are primarily localized in the nucleus. The Hda1 homologous class II HDACs include HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 (a and b isoforms), and HDAC10, and are structurally much larger in size. Class II HDACs can shuttle between the nucleus and cytoplasm (34–37), suggesting different functions and cellular substrates from Class I HDACs. HDAC6 in particular is predominantly localized in the cytoplasm (38). Class II HDACs also display a more limited tissue distribution (39–41). HDACs 4, 8, and 9 are expressed to a greater extent in tumor tissues than in normal tissues, with HDAC 4 demonstrating the greatest difference in this regard (39). Class II enzymes have also been shown to be specifically involved in differentiation (40). Finally, HDAC6 and HDAC10 are unique among class II HDACs in having two catalytic domains (37, 40). Although there is some evidence that certain HDAC inhibitors display different degrees of HDAC specificity, considerable research must still be performed to delineate differences in HDAC function, their roles in cancer, and their sensitivities to drugs. Some of these differentiating features are reviewed

TABLE 1 Nonhistone proteins whose acetylation may be increased by HDAC inhibitors

Protein	Intracellular function	Reference(s)
p53	Tumor suppressor	(26, 145, 146)
c-Myb	Protooncogene—regulates proliferation and differentiation	(147)
GATA-1	Differentiation of blood cells	(29, 30)
Estrogen receptor-α	Stimulates growth of certain breast cancers	(28)
TFIIE	General transcription factor	(25)
TFIIF	General tanscription factor	(25)
Androgen receptor	Androgen-dependent transcription factor	(27)
hsp90	Chaperone—targets proteins for degradation by proteasome	(82)
α-tubulin	Microtubule component	(61, 148)
HMG-17	Unfolds higher order chromatin structure	(31)
HMGI	Essential architectural component for enhancesome assembly	(149)
TCF $\downarrow$	Transcriptional regulator	(150)
PCNA	DNA repair and replication, cell cycle control, chromatin remodeling	(151)
EKLF	Red cell–specific transcriptional activator	(152)
ACTR	Nuclear receptor coactivator, HAT	(153)
HNF-4	Transcriptional activation	(154)
Importin-α	Nuclear import factor	(155)
NF-κB	Regulates antiapoptotic responses	(156)
ER81	Downstream effector of HER2/neu and Ras	(157)
SF-1	Transcription factor—expression of steroidogenic proteins	(158)
Ku70	Suppresses apoptosis	(159)
UBF	Structures DNA in ribosomal enhancesome	(160)
Sp3	Transcriptional activator or repressor	(161)
TAL1	Regulator of normal and leukemic hematopoiesis	(162)
YY1	Multifunction transcription factor	(163)
E2F1	Cell cycle activator—required for progression	(164)
MyoD	Stimulates cdk inhibitor p21	(165)

PCNA, proliferating cell nuclear antigen; SF-1, steroidogenic factor-1; UBF, architectural upstream binding factor.

in detail elsewhere (39). Unraveling specific roles by these HDAC isozymes during human tumorigenesis will further incentivize development of more specific HDAC inhibitors (42), potentially enhancing their clinical activity as well as decreasing their nonspecific toxicities, while also optimizing potential interactions with other rationally designed and integrated therapeutic agents.

STRUCTURAL CLASSES AND MECHANISTIC ACTIONS OF HDAC INHIBITORS

The six structurally distinct classes of HDAC inhibitors (Figure 2) act by binding to various portions of the catalytic domains within class I and II HDACs (Figure 3*A*). Although reviewed here briefly, a detailed examination of the medicinal chemistry and activity relationships for these structurally varied inhibitors is beyond the scope of this review, and the reader is directed to several excellent reviews on this subject (43–45). Hydroxamic acid–type chelators, including TSA, SAHA, and LAQ824, have three basic components (Figure 3*B*): (*a*) a hydroxamic acid moiety that chelates the active zinc in a bidentate manner, hydrogen bonds with residues composing the charge relay systems, and displaces the nucleophilic water molecule present in the active site; (*b*) a hydrophobic spacer that has a length optimal for spanning the length of the hydrophobic pocket and dimensions capable of navigating the narrowest segment of the cavity; and (*c*) a hydrophobic cap that blocks the entrance to the active site. Design and understanding of the enzymatic inhibitory mechanisms for various HDAC inhibitors was aided by solving the crystal structure of an HDAC homologue that shares significant homology with class I and class II HDACs, including all critical active site residues (46). The active site of class I and class II HDACs includes critical zinc and water molecules; two charge relay systems, where aspartate residues act to increase the basicity of histidine residues by polarizing the epsilon nitrogen; and an active site tyrosine residue that coordinates to the acetyl oxygen during the transition state (Figure 3*C*). The zinc ion acts by polarizing the acetyl carbonyl to make the carbonyl carbon a better electrophile for attack by the activated water molecule. Substitution of other divalent cations, or chelation of the zinc cation by a small-molecular-weight chelator, abolishes enzymatic activity. A hydrophobic pocket high in aromatic and glycine residues leads to the active site, with the narrowest point having a distance of 7.5 Å marked by two opposing phenylalanine residues. A depiction of the predicted transition state interaction between HDAC1 and the hydroxamic acid–type inhibitor LAQ824 is shown in Figure 3*C*. Hydroxamates with five or six carbon spacers were found to be the most active inhibitors (47), and replacement of the hydroxamic acid with a carboxylate was found to eliminate inhibitory activity (48).

Epoxyketone-based HDAC inhibitors, such as trapoxin B, HC-toxin, or 2-amino-8-oxo-9,10-epoxydecanoic acid (AOE), may act by chemically modifying an active site nucleophile with the epoxy group (49) and forming important

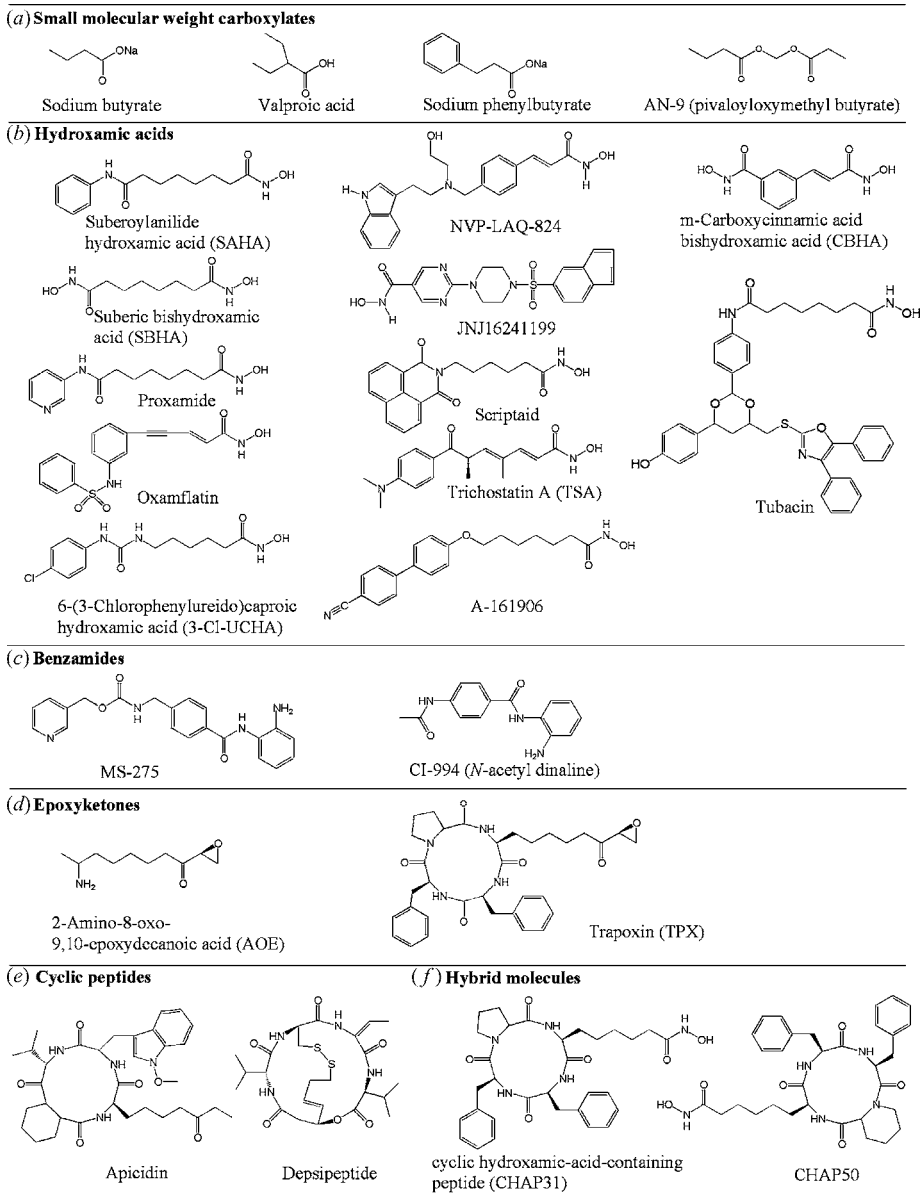


Figure 2 Structural classes of HDAC inhibitors. Six basic classes of HDAC inhibitors are shown: (*a*) small-molecular-weight carboxylates, including sodium butyrate, valproic acid, and sodium phenylbutyrate; (*b*) hydroxamic acids, including CBHA, TSA, SAHA, and LAQ824; (*c*) benzamides, including MS-275 and CI-994; (*d*) epoxyketones, including AOE and trapoxin B; (*e*) cyclic peptides, including depsipeptide and apicidin; and (*f*) hybrid molecules, such as CHAP31 and CHAP50.

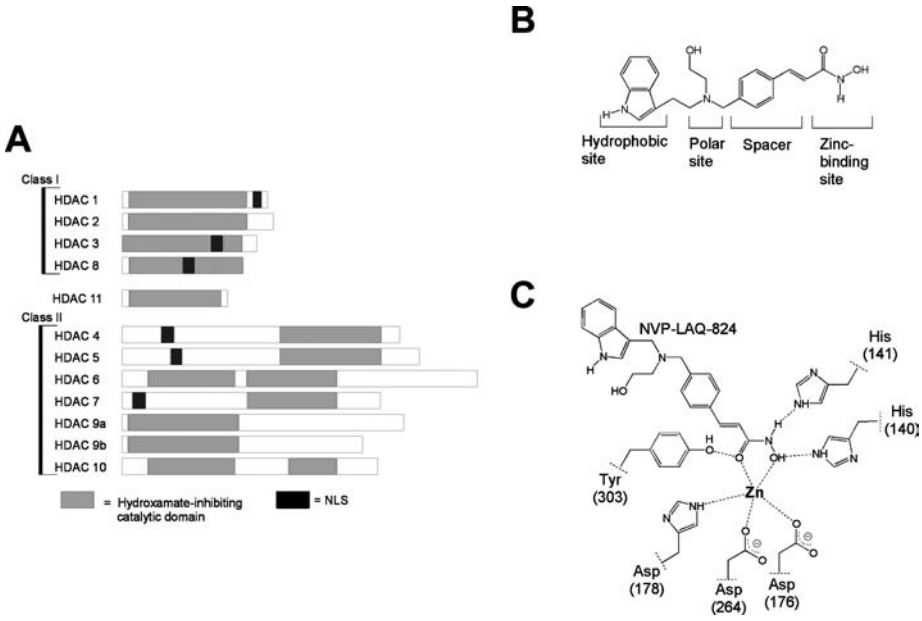


Figure 3 Structural basis for hydroxamic acid inhibition of HDACs. (*A*) Structural homology of class I and II HDACs showing hydroxamate-inhibiting catalytic domains. (*B*) Functional components of hydroxamic acid–type HDAC inhibitors (LAQ824). Hydroxamic acid–based HDAC inhibitors are composed of four primary functional components: (*a*) a zinc-chelating hydroxamic acid, (*b*) a linker region, (*c*) a polar site, and (*d*) a hydrophobic cap that blocks the active site. (*C*) Predicted transition state inhibition of HDAC1 by LAQ824.

hydrogen bond contacts with the ketone. Elimination of the ketone, or reduction of the ketone to an alcohol, abolishes the activity of these molecules (50). Trapoxin B and HC-toxin also contain a five-carbon linker for transversing the cavity and a cyclic tetrapeptide capable of acting as a hydrophobic cap for the cavity. Trapoxin B is a hybrid molecule and can also be listed with the cyclic peptide HDAC inhibitors. The combination of cyclic peptide and epoxyketone resulted in nanamolar HDAC inhibitory activity.

The carboxylates or short-chain fatty acids, including sodium butyrate, valproic acid, and sodium phenylbutyrate, have much weaker HDAC inhibition constants (K_is), commonly in the millimolar range. In spite of their weak activity, several of these agents have been studied clinically (51) owing in part to their clinical use for alternative medical indications. The most commonly studied members of this class are simple molecules with alkyl or phenylalkyl carboxylates. The carboxylate is thought to coordinate with the zinc ion in the active site, albeit more poorly than in the case of hydroxamates.

Cyclic peptide HDAC inhibitors have been discovered or developed that either contain an epoxyketone group (HC-toxin, trapoxin B) or are devoid of it (Apicidin,

Depsipeptide). In general, these inhibitors have nanamolar HDAC inhibitory activity and can have either irreversible (epoxyketone-based) or reversible mechanisms of action. The macrocyclic peptide portion of the inhibitor binds tightly to the rim or opening of the channel to the active site, whereas an aliphatic linker navigates the channel to the active site (44). Depsipeptide, also known as FK228, is a prodrug that requires intracellular reduction to liberate a sulfhydryl-containing aliphatic group that enters the active site and binds the active site zinc and water molecule (44, 52). Hybrid molecules, including CHAP31 and CHAP50, that possess both a cyclic peptide and an aliphatic hydroxamate have been prepared and shown to have a reversible mechanism of action and remarkable inhibitory activity when optimized in the range of 1–5 nanamolar (53, 54). The optimal linker in these studies was found to have five methylenes, similar to that described previously for other hydroxamates (47).

Inhibitors of the benzamide class, such as CI-994 (55) and MS-275 (56), are in general less active than members of the hydroxamate or cyclic peptide classes, with K_is in the micromolar range (44, 56). The mechanism of HDAC inhibition for benzamides remains uncertain at present. In addition to the structural classes of HDAC inhibitors described thus far, a variety of inhibitors have been prepared that are not readily classified into one of the above mentioned five classes. Brosch and colleagues have recently described 3-(4-Aroyl-1-methyl-1H-2-pyrrolyl)-N-hydroxy-2-alkylamides containing a range of different metal chelating groups with IC_{50}s in the micromolar range (57, 58). Another series of Psammaplin derivatives containing novel metal chelating groups have demonstrated considerably greater inhibitory activity, with the most active of these compounds having nanomolar K_is (59).

Little is presently known about the potential selectivity of various HDAC class I or II isoforms for structurally different inhibitors. HDAC6 and HDAC10 both possess two catalytic domains that appear to be differentially inhibited by drugs that preferentially bind near the entrance of the catalytic site (37, 54, 55). These class II HDAC isoforms appear relatively resistant to trapoxin when compared to class I HDACs. Despeptide, MS-275, and several of the hybrid CHAP derivatives also appear considerably more selective for HDAC1 over HDAC6 (54). TSA is generally considered a nonspecific HDAC inhibitor, as it has a similar K_i for all isoforms examined. Recently, Schrieber and colleagues described an HDAC6-specific inhibitor, tubacin (Figure 2), responsible for the deacetylation of tubulin, as well as another "histacin," which appears to be a histone-selective deacetylase (60, 61). The continued development of isoform-specific inhibitors will undoubtedly remain a major emphasis of HDAC inhibitor development.

ANTITUMOR MECHANISMS OF HDAC INHIBITORS

There is an everexpanding body of evidence supporting the involvement of, as well as structural alterations in, various HATs and HDACs with development of cancer (62, 63). Broadly speaking, this includes evidence for their genetic disruption

(e.g., translocation, amplification, mutation, overexpression) in a subset of hematological and epithelial malignancies, as well as the aberrant genomic recruitment of otherwise normal HDACs in conjunction with oncogenic transcription factors. Such observations have led to the conclusion that defects and/or imbalances in the genome's acetylation machinery accompany changes in local chromatin structure and oncogenic dysregulation of genes controlling cell cycle progression, differentiation, and apoptosis. Despite these observations and conclusions, there are as yet no specific HAT or HDAC measurements devised that can predict the sensitivity of any given tumor to any class of HDAC inhibitor.

It has also been generally accepted that more actively transcribed chromatin regions are associated with histone hyperacetylation and recruitment of HATs (although HDACs are also known to be recruited), and histone deacetylation associated with recruitment of HDACs often restores these genomically active regions to a more repressed and condensed chromatin state. Thus, an attractive paradigm for the antitumor action of HDAC inhibitors has been the induction of histone acetylation producing transcriptional activation of critical genes needed for tumor growth arrest (1, 43, 44, 64–67). Unquestionably, HDAC inhibitors produce a global increase in histone acetylation within hours of treatment of many different malignant and nonmalignant tissue types, including those showing little if any biological consequences upon treatment with HDAC inhibitors. Thus, while a global increase in the level of histone acetylation by itself cannot explain selective changes in gene expression or specific patterns of antitumor activity following HDAC inhibition, assaying for enhanced histone acetylation in readily sampled cells or tissues (e.g., peripheral white blood cells) is being routinely employed to demonstrate HDAC inhibitor bioavailability and activity. Greater attention is currently being given to the expanding list of nonhistone proteins acetylated in direct response to HDAC inhibition (Table 1), especially because many of these are tissue/development-specific (EKLF, GATA-1, ERα, MyoD), oncogenic (c-Myb), tumor-suppressing (p53), or even rather ubiquitous (TFIIE, TFIIF, TCF, HNF-4) transcription factors.

Virtually all HDAC inhibitors currently in clinical development show some degree of preclinical activity against malignant cells proliferating in culture and also tumors growing in animal models; this antitumor activity may be characterized as either inducing cytostasis (cell cycle arrest), differentiation, or apoptosis. However, the HDAC-dependent mechanisms accounting for the observed and rather selective modulation of gene expression, as well as specific patterns of antitumor activity, remain poorly understood. Several studies have now revealed that fewer than 10% of expressed genes in a given malignant cell population are affected by an antitumor dose of an HDAC inhibitor, with a near equal number of transcriptionally active genes being repressed as those being stimulated; structurally different HDAC inhibitors can similarly modulate expression of a relatively limited set of core genes (2, 3, 68). As shown in Table 2, among the commonly up- and down-modulated gene transcripts identified in these expression microarray studies, as well as in numerous single-gene expression studies (66–78), are

TABLE 2 Tumor-associated proteins whose transcriptional expression is altered in response to HDAC inhibitor treatment of cells

Regulated protein	Function (oncogenic or antioncogenic/tumor supressing)	Reference(s)
Downregulated by HDAC inhibitors (e.g., oncogenic)		
HER2/neu	Growth factor receptor (EGFR class)	(81)
TGF-β	Regulates cell proliferation and differentiation through TGF-β type II receptor	(166, 167)
Thioredoxin	Disulfide reductase, cytokine activity, can inhibit apoptosis	(168)
Telomerase	Prevents telomere erosion	(97)
RECK	Regulates matrix metalloproteinases	(86)
VEGF	Angiogenic factor	(87, 169)
bFGF	Angiogenic factor	(87)
Myb/c-MyBL2	Oncogenic transcription factor–regulation of transformation and differentiation	(68)
raf-1	Effector of Ras	(68)
cyclin A	Cell cycle regulator	(111)
cyclin B	Cell cycle regulator	(111)
DAF	Complement inhibitory protein	(170)
abl	Growth factor receptor, component of bcr/abl chimeric kinase	(68)
DEK	Putative role in regulating chromatin structure and postsplicing events	(68)
Proteasome	Degradation of misfolded or oxidized proteins	(68)
Upregulated by HDAC inhibitors		
Fas/Fas ligand	Proapoptotic	(76)
Bcl2	Proapoptotic	(78)
p53	Proapoptotic	(169)
Bak, Bax, Bim	Proapoptotic	(171)
c-myc	Inhibitor of differentiation	(100)
Caspase 3	Cysteine protease involved in apoptosis, proapoptotic	(125, 172)
Carboxypeptidase A3 (CPA3)	Carboxypeptidase, putative role in regulating differentiation	(173)
RECK	Negatively regulates matrix metalloproteinases	(86)
$p21^{WAF1/Cip1}$	Cell cycle regulation	(66, 70)
Gelsolin	Regulation of cell morphology	(70)

(*Continued*)

TABLE 2 (*Continued*)

Regulated protein	Function (oncogenic or antioncogenic/tumor supressing)	Reference(s)
ERα	Estrogen-activated nuclear receptor regulates transcription of estrogen responsive genes	(174)
TSSC3	Regulates Fas-mediated apoptosis	(68)
IGFBP-3	Augments IGF actions, promotes apoptosis, and inhibits cell growth	(175)
TBP-2	Inhibits thiol-reducing activity of thioredoxin	(168)

Bak, Bcl2 antagonist killer; Bax, Bcl2-associated X protein; DAF, decay-accelerating factor; TBP-2, thioredoxin binding protein; TSSC3, tumor supressing subtransferable candidate.

those encoding known tumor-associated proteins that mediate proliferation and cell cycle progression, survival factors, growth factor receptors, kinases and signal transduction intermediates, DNA synthesis/repair enzymes, shuttling proteins, transcription factors, and proteases.

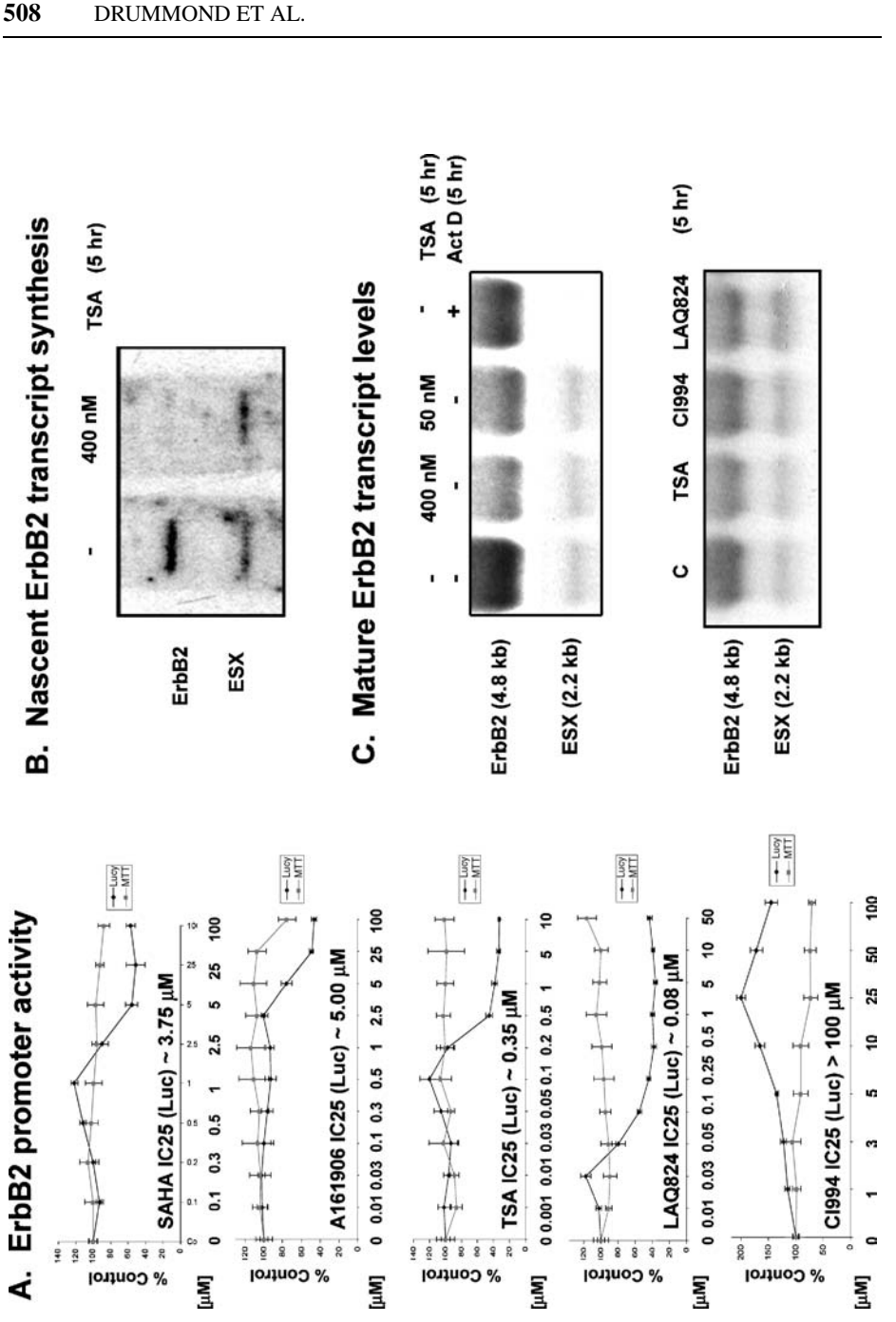
Some study has gone into the question of how HDAC inhibitors actually relieve transcriptional repression and reverse the differentiation arrest in malignancies such as acute leukemia, where differentiation arrest and the malignancy phenotype induced by such chimeric oncoproteins as PLZF-RARα, PLZF-RARα, or AML1/ETO can be reversed, at least in part, by HDAC inhibitors (69, 79). In other types of malignancies, HDAC inhibitors induce differentiation and/or apoptosis by activating transcription of CDKN1A through a p53-independent mechanism, producing increased levels of the cyclin-dependent kinase (CDK) inhibitor, $p21^{WAF1/CIP1}$ (66). Likewise, HDAC inhibitors have been observed to induce transcription of other tumor suppressor genes such as gelsolin and maspin (70, 71). When administered in combination with DNA demethylating agents such as 5-aza-2′-deoxycytidine, HDAC inhibition can fully restore transcriptional expression to various genes, including MLH1, TIMP3, CDKN2A, and CDK2NB, that have been epigenetically silenced by promoter methylation during the course of tumorigenesis (72, 73).

Apart from the upregulation of epigenetically silenced tumor suppressor proteins or induction of caspases and other proapoptotic proteins (26, 68, 74–78), there are emerging data showing HDAC-induced repression of critical transforming growth factor mechanisms, such as those involving oncogenic tyrosine kinases like bcr/abl and ErbB2 (80–82). We recently reported that HDAC inhibitors can selectively repress ErbB2 transcript levels by two distinct HDAC-dependent mechanisms: repression of new ErbB2 transcript synthesis and the accelerated decay of mature ErbB2 mRNA (81). The hydroxamic acid TSA was identified in a high-throughput cell-based chemical screen for its ability to repress ErbB2 promoter activity (81). Figure 4 (panel *A*) compares the potency of TSA against several

other HDAC inhibitors for their ability to inhibit ErbB2 promoter function. Of interest, the rank order of potency for the HDAC inhibitors shown in this screening assay (LAQ824 > TSA > A1616906 > SAHA ≫ CI994) is comparable to their relative antitumor activity against several ErbB2 overexpressing breast cancer cell lines. When evaluated further against SkBr3 and other ErbB2-dependent breast cancer cell lines (e.g., BT-474, MDA-453), HDAC inhibitors were shown to inhibit the synthesis and elongation of nascent ErbB2 transcripts as well as destabilize and accelerate the decay of mature cytoplasmic ErbB2 transcripts (Figure 4, panels *B* and *C*). Although ongoing preclinical studies are confirming that ErbB2-dependent cancers appear somewhat more sensitive to HDAC inhibitors than ErbB2-independent cancers, molecular studies are attempting to define the drug-sensitive HDAC-dependent nuclear and cytoplasmic mechanisms that differentially regulate ErbB2 transcription and ErbB2 transcript stability, respectively. The presence of multiple distinct HDAC-dependent mechanisms capable of controlling ErbB2 transcript levels suggests that even among ErbB2-dependent cancers, there will be differential sensitivity to structurally different classes of HDAC inhibitors. Other investigators have identified HDAC-dependent posttranslational mechanisms that can also downregulate the expression of oncoprotein kinases like ErbB2 and bcr/abl (80, 82). Acetylation of the chaperone protein, Hsp90, induced by HDAC inhibition, results in the enhanced proteasomal degradation of ErbB2 and bcr/abl kinases. These examples of multiple mechanisms by which HDAC inhibitors potentially downregulate critical oncogenic pathways also suggest new combinatorial strategies for possible clinical evaluation, including HDAC inhibitor treatment in conjunction with tyrosine kinase inhibitors (80, 82–84) or Hsp90 antagonists (85).

Apart from directly affecting transformed cells, HDAC inhibitors have also been shown to inhibit tumor angiogenesis, suggesting additional therapeutic mechanisms for the observed in vivo activity of these antitumor drugs (76, 86–88). Depsipeptide was shown to suppress the expression of pro-angiogenic factors, including vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) (87). VEGF and bFGF mRNA levels were significantly reduced in prostate tumor xenografts sensitive to this cyclic peptide HDAC inhibitor. The hydroxamic acid HDAC inhibitor TSA was shown to upregulate the RECK protein responsible in part for inhibiting tumor metastasis and angiogenesis through its action on matrix metalloproteases (86). The carboxylate and short-chain fatty acid HDAC inhibitor, valproic acid, was also shown to inhibit angiogensis both in vitro and in vivo via a mechanism involving diminished expression of endothelial nitric oxide synthase (88). Additional miscellaneous or less well-studied tumor-associated mechanisms may prove to be important in determining the ultimate clinical utility of some HDAC inhibitors.

Last, various drug-resistance phenotypes have been shown to be modulated by HDAC inhibitors (89–95). Treatment of different multidrug-resistant cell lines with TSA or SAHA was shown to downregulate P-glycoprotein (93), helping



reverse the multidrug-resistant phenotype. SAHA and oxamflatin were shown in separate studies to overcome multidrug resistance (89, 94), whereas in one study depsipeptide was shown to be a substrate for P-glycoprotein (94). In another study, depsipeptide was shown to inhibit cell growth in irinotecan-, etoposide-, and cisplatin-resistant cell lines in conjunction with its ability to inhibit telomerase expression and activity (90). Telomerase is responsible for adding telomeric repeats to the ends of chromosomes and is required for the relative immortality of cancer cells; other investigators have also shown an inhibitory effect of HDAC inhibitors on telomerase activity (96, 97). These diverse examples illustrate the immense need for further studies to understand the relative importance of the many potential in vitro and in vivo mechanisms by which HDAC inhibitors can produce antitumor responses.

Figure 4 Transcriptional repression of ErbB2 induced by hydroxamic HDAC inhibitors is caused by a combination of both ErbB2 promoter repression and transcript destabilization. (*A*) Employing our previously described high-throughput screening assay (81), an ErbB2-independent subline of MCF-7 breast cancer cells (MCF/R06pGL-4) bearing a chromatin-integrated ErbB2 promoter-driven luciferase construct was used to compare the ErbB2 promoter repressing potency of four structurally different hydroxamic acid–type HDAC inhibitors (SAHA, A1616906, TSA, LAQ824) and a benzamide-type HDAC inhibitor (CI994). After 24 h culture exposure to the indicated drug doses, cell viability as measured by MTT assay (*squares*) shows little change, whereas specific repression of ErbB2 promoter activity is detected by luciferase expression (*diamonds*). The benzamide inhibitor (CI994) shows slight ErbB2 promoter stimulation with no evidence of promoter repression; in contrast, the hydroxamic acid inhibitors show ErbB2 promoter repression at different potencies as indicated by the 25% luciferase inhibitory concentration (μM IC25) values. (*B*) When ErbB2-dependent SkBr3 breast cancer cells in culture are treated for 5 h with an ErbB2 promoter–repressing dose of TSA, nascent ErbB2 transcript synthesis and elongation, as measured by nuclear run-off assays (81), appears completely inhibited, whereas nascent transcript synthesis of the Ets transcription factor ESX appears marginally increased. (*C*) Total RNA extracted and Northern blotted after 5-h treatment of cultured SkBr3 breast cancer cells shows treatment effects on mature long-lived ($\sim$8 h half-life) ErbB2 transcripts (4.8 kb) in comparison to short-lived ($<$2 h half-life) ESX transcripts (2.2 kb). Treatment for 5 h with an RNA polymerase inhibiting dose of Actinomycin D (10 μg/ml) demonstrates the expected absence of ESX transcripts and partial decline in total ErbB2 transcripts. In contrast, and after 5-h treatment with comparable doses of the HDAC inhibitors TSA, CI994, and LAQ824, ESX levels appear marginally increased, whereas ErbB2 transcript levels are reduced below levels caused by Act D treatment, demonstrating the independent ability of HDAC inhibitors to destabilize and accelerate the decay of mature ErbB2 transcripts.

IN VIVO BIOLOGICAL AND CLINICAL CHARACTERISTICS OF HDAC INHIBITORS

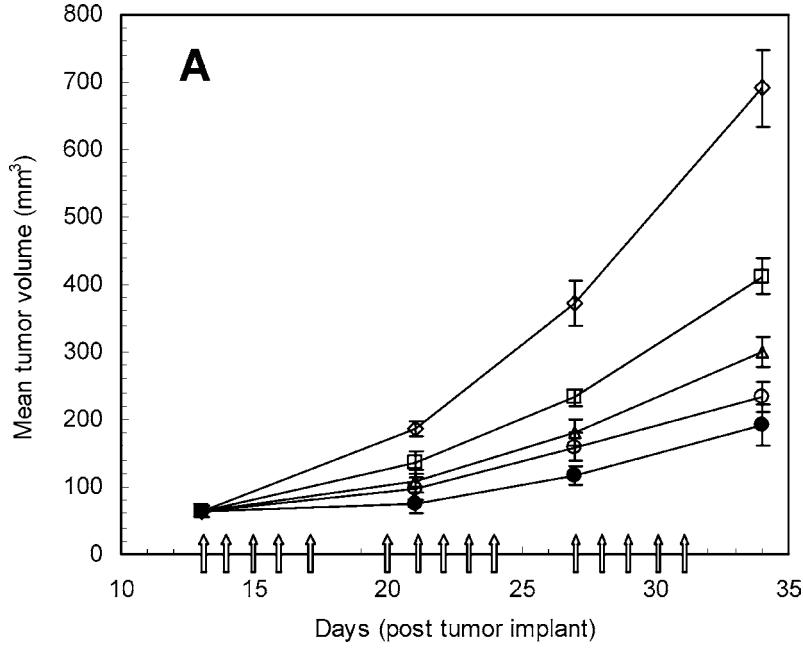
In Vivo Preclinical Antitumor Activity

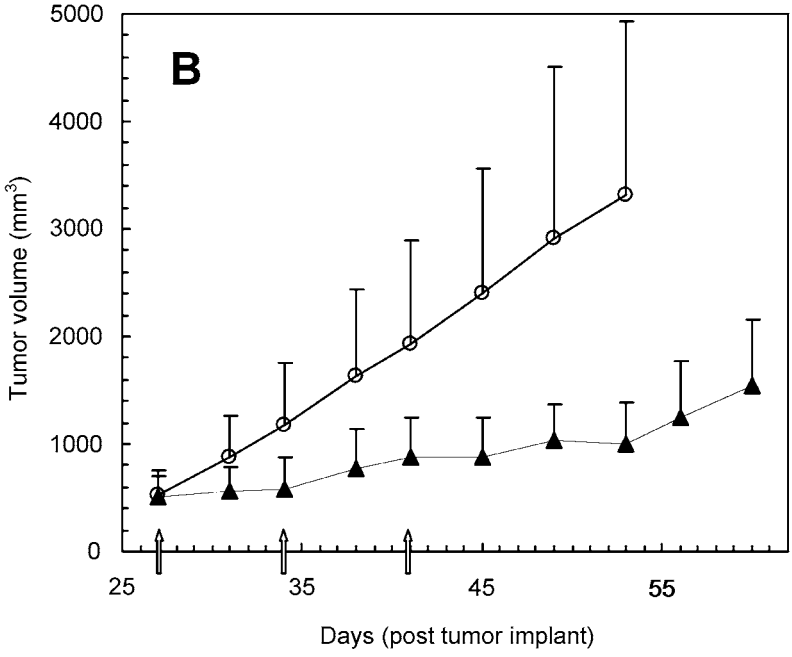
Numerous animal model studies have demonstrated significant antitumor efficacy for HDAC inhibitors from virtually every structural class (71, 87, 98–106). One of the newest clinical candidates, the hydroxamate JNJ1641199 (Figure 2), exhibited nanomolar HDAC inhibition and antitumor activity against lung, ovarian, and colon cancer xenograft models, along with excellent oral bioavailability (106). Another hydroxamate now in clinical trials, NVP-LAQ824 (Figure 2), shows potent antitumor activity against human colon (Figure 5*A*) and lung cancer xenograft models at submicromolar concentrations when administered parenterally every day and with a maximal tolerated dose (MTD) that exceeds 100 mg/kg (101). Likewise, TSA, SAHA, and pyridoxamide hydroxamates were previously shown to have in vivo antitumor activity with daily parenteral dosing associated with little systemic toxicity (98, 104, 105).

The cyclic peptide prodrug depsipeptide (FK228) demonstrates efficacy in leukemia and lymphoma models (100, 103), which can be further enhanced in combination with the cell-differentiating retinoid, ATRA (103). Depsipeptide was also recently shown to have clinical activity in treating T cell lymphoma in early Phase I/II trials (107).

Although most of the carboxylated short-chain fatty acid HDAC inhibitors have displayed limited potency in vivo owing to their lack of specificity and high drug-concentration requirements (51, 65), the prodrug AN-9 has shown good activity

Figure 5 In vivo antitumor efficacy of hydroxamate-type HDAC inhibitor, LAQ824, is dependent on dose, schedule, and formulation. Insert arrows in both panels show treatment points. In vivo antitumor activity of LAQ824 was determined in (*A*) a human colon (HCT116) tumor xenograft model and (*B*) liposomal LAQ824 in an ErbB2-dependent human breast (BT474) tumor xenograft model. The drugs in both studies were administered intravenously. For the free LAQ824 study (*A*), treatments started when HCT116 tumors reached a mean size of 50 mm^3 and nude mice were injected 5 times per week for 3 weeks, for a total of 15 doses. The treatment groups were as follows: (*diamonds*) 10% DMSO/D5W (control), (*squares*) 10 mg/kg/dose LAQ824, (*triangles*) 25 mg/kg/dose LAQ824, (*open circles*) 50 mg/kg/dose LAQ824, and (*closed circles*) 100 mg/kg/dose LAQ824. For the liposomal LAQ824 study (*B*), BT474 breast tumor xenografts were allowed to grow to a size of approximately 250–300 mm^3. Mice were then injected with either saline (*open circles*) or conventional liposomal LAQ824 (*closed triangles*) at a dose of 25 mg/kg weekly, for a total of 3 weeks, beginning on day 27. The data are expressed as mean tumor volume $\pm$ standard error. (*A*) was adapted from Remiszewski et al. (101) with permission from the American Chemical Society.





in several murine tumor and human tumor xenograft models (108), and has shown some encouraging results in early clinical trials (109).

The benzamide HDAC inhibitors, MS-275 (56, 71, 110, 111) and CI-994 (55), have also shown in vivo activity against various tumor models. MS-275 was shown to inhibit the growth of three different orthotopic pediatric tumor xenografts (110). In another study, MS-275 administered orally was shown to have potent antitumor activity against a series of seven different human tumors xenografts (71). Unfortunately, antitumor doses of MS-275 in mice were also myelosuppressive, causing decreases in red and white blood cells as well as platelets (111). A similar pattern of toxicity was observed with CI-994 (112); and thrombocytopenia was a major dose-limiting toxicity seen in Phase I and II clinical testing with CI-994 as well as with the cyclic peptide depsipeptide (113, 114).

Clinical Toxicity and Antitumor Activity

Dose-limiting clinical toxicities and reported antitumor responses have been noted in Phase I and II clinical trials for the limited number of structurally varied HDAC inhibitors that have entered clinical testing to date. The carboxylate phenylbutyrate given by prolonged intravenous infusion has a dose-limiting toxicity (DLT) of somnolence and confusion, which has not been reported for the benzamide or hydroxamate HDAC inhibitors (115) or for the carboxylate prodrug AN-9 (109). The carboxylate valproic acid has been in clinical use for more than two decades as an anticonvulsant and thus has well-described pharmacologic properties and a well-tolerated side effect profile; clinical trials are in progress evaluating the antitumor potential of valproic acid as an HDAC inhibitor. Despite thrombocytopenia being a DLT for both CI-994 and depsipeptide, evidence for antitumor clinical activity upon oral daily dosing of CI-994 has been noted in patients with several epithelial types of advanced solid malignancies [including nonsmall cell lung cancer (NSCLC), renal cell carcinoma, and bladder cancer]. Likewise, two Phase I trials of depsipeptide have suggested that patients with T cell leukemia or lymphoma, as well as other occasional cases of refractory malignancies, may achieve clinical benefit from this HDAC inhibitor (113, 116). Curiously, depsipeptide is the only clinically tested HDAC inhibitor reported to date that is associated with a significant incidence of cardiac dysrhythmias and nonspecific EKG abnormalities (113). Among the hydroxamates, daily infusions of pyroxamide and LAQ824 are currently under Phase I clinical evaluation, whereas a trial of infusional SAHA was recently completed (115, 117). When given by 2-h infusions daily five times, SAHA had a MTD of 300 mg/m^2/day. Among treated patients with advanced hematologic malignancies, myelosuppression (thrombocytopenia) was the DLT. In those with advanced solid tumors, myelosuppression was observed but was not a DLT; as well, nonspecific EKG changes without clinical signs or symptoms were common. Fatigue was commonly observed with SAHA treatment but was not dose-limiting and was similar to that previously reported for depsipeptide. Importantly, patients with renal cell carcinoma, head and neck squamous

carcinoma, papillary thyroid carcinoma, mesothelioma, B and T cell lymphomas, and Hodgkin's disease all showed some degree of clinical improvement (115).

Pharmacokinetic Considerations

Although receiving less reported attention than studies elucidating the pharmacodynamics of various HDAC inhibitors, the pharmacokinetic characteristics and limitations of different HDAC inhibitors are of critical interest and will likely prove to be an important determinant of their ultimate clinical utility. Inhibition of intracellular HDAC activity commonly requires continuous systemic circulation and drug exposure to achieve maximal tumor cytostasis or apoptosis and clinical response. Rapid clearance, a high degree of protein binding, rapid metabolism, or rapid inactivation of reactive functional groups (i.e., epoxy groups) are factors that can adversely affect HDAC inhibitor bioavailability and antitumor activity. Although occasionally used in the clinic, prolonged or daily infusions of any drug are generally undesirable. The requirement of constant systemic exposure by parenteral administration to achieve an active antitumor drug concentration will most likely limit the clinical development of any HDAC inhibitor that is not orally bioavailable. For this reason, the clinical development of SAHA shifted from Phase I evaluation of daily intravenous infusions to a more recently designed oral formulation (115).

Novel drug delivery systems that allow for controlled drug release may help circumvent the clinical inconvenience of daily infusions as well as generally enhance the therapeutic index of HDAC inhibitors. We have recently evaluated the potential of liposomes for delivering the HDAC inhibitor LAQ824. When formulated properly, liposomes can entrap and concentrate amphipathic drugs (achieving $>10{,}000$ drug molecules per liposomal nanoparticle), releasing them slowly over time in the plasma or delivering them specifically to solid tumors where they deposit their drug in close proximity to the tumor, allowing for increased tumor accumulation and drug exposure (118). In a pilot study administering liposomal LAQ824 on a once-weekly schedule for three weeks, we observed significant growth arrest of rapidly growing human breast tumor xenografts (Figure 5*B*). As shown in other studies involving various tumor model systems, free LAQ824 requires daily injections of generally higher doses to slow tumor growth (Figure 5*A*). More recent studies with optimized formulations and targeted liposomal constructs have shown even greater efficacy (119).

HDAC INHIBITORS IN COMBINATION WITH OTHER AGENTS

The greatest potential of HDAC inhibitors may lie in their ability to modulate the activity of other therapeutic agents. A variety of different drug combinations have demonstrated considerable promise in treating cancer. These are reviewed

more extensively in a separate review of the field (65), and are summarized in Table 3. The pretreatment or coadministration of HDAC inhibitors with a wide range of agents has repeatedly been shown to additively or synergistically enhance apoptosis of cancer cells in culture (68, 82, 85, 120–125) as well as antitumor efficacy in vivo (126–128). Notably, enhancements in activity have been observed when HDAC inhibitors are combined with a number of different commonly used chemotherapeutics (82, 120, 121). Nuclear receptor ligands (123, 127, 129, 130), Hsp90 antagonists (85), proteasome inhibitors (68, 84, 131), signal transduction inhibitors (80, 82, 124, 125, 132–136), and DNA demethylating agents (72, 73, 122, 128, 137) represent some of the more promising classes of agents. Demethylating agents such as 5-aza-2′-deoxycytidine (DAC) are particularly interesting owing to the interaction of DNA methylation with histone deacetylation in gene silencing of tumor suppressor genes, as mentioned above. Combinations of DAC with TSA or depsipeptide were shown to reactivate silenced tumor suppressor genes including *MLH1*, *TIMP3*, *CDKN2B*, *CDKN2A*, *ARHI*, gelsolin, and maspin (72, 73, 137), synergistically increasing the level of tumor cell apoptosis (122). Combinations of nuclear receptor ligands, such as all-*trans* retinoic acid (ATRA), or vitamin D analogs, such as 1,25-dihydroxyvitamin D, with HDAC inhibitors have been shown to increase differentiation and apoptosis in cancer cells (123, 127, 130) and also inhibit tumor growth in vivo (127, 130, 138). Small-molecule kinase inhibitors may also be rationally combined with HDAC inhibitors. Imatinib (Gleevec®) is a specific inhibitor of Bcr/Abl with impressive clinical activity in the treatment of chronic myeloid leukemia and selected other malignancies. Because LAQ824 has been shown to downregulate the expression of Bcr/Abl and also promote its degradation through acetylation of Hsp90 (80), combinations of imatinib with LAQ824 as well as other HDAC inhibitors, such as SAHA and apicidin, have been tested and shown to dramatically increase the apoptosis of Bcr/Abl positive leukemic cells (80, 83, 132, 133). A similar effect is seen when malignant cells known to be transformed by oncogenic tyrosine kinases (ErbB2/HER2, Src/Abl, PI3 kinase) are treated with HDAC inhibitors in combination with appropriate kinase inhibitors like Herceptin®, PD180970, or LY294002 (80, 82, 124).

As noted above, expression of the CDK inhibitor $p21^{WAF1/CIP1}$ is regulated by HDACs and plays a critical role in determining whether cells undergo differentiation or apoptosis in response to treatment with HDAC inhibitors (66, 70, 139). Flavopiridol is a CDK inhibitor that results in a disruption of $p21^{WAF1/CIP1}$ induction and induces apoptosis. Its combination with HDAC inhibitors (SAHA, depsipetpide, sodium butyrate) has been shown to result in a disruption of p21 induction and an additive or synergistic increase in tumor cell apoptosis (125, 135, 139, 140).

Proteasome inhibitors and Hsp90 antagonists represent two other groups of interesting agents that may be rationally combined with HDAC inhibitors. Hsp90 is a molecular chaperone that stabilizes and controls the intracellular trafficking of important client proteins, including ErbB2/HER2, Bcr/Abl, EGF, cyclin D1, c-Raf, and steroid receptors. The inhibition of Hsp90 with amsacrine antagonists, such as

TABLE 3 Therapeutic agents used in combination with HDAC inhibitors in preclinical and clinical studies

Combined therapeutic agent	HDAC inhibitor	Combined antitumor effects	Reference(s)
Standard chemotherapy			
Gemcitabine	CI-994	Phase II trial—increased toxicity	(176)
VP-16 (etoposide)	TSA, SAHA	Synergistic	(120)
cytarabine, etoposide, and topotecan	PB	Synergistic	(121)
Doxorubicin, melphalan, chloroambucil, cisplatin, carboplatin, fludarabine	PB	Additive	(121)
Taxotere, gemcitabine, epothilone B	LAQ824	Additive	(82)
Etoposide	TSA	Antagonistic	(177)
Fludarabine	MS-275	Synergistic	(178)
IMID-1, dexamethasone	SAHA	Synergistic	(68)
Demethylating agents			
DAC	TSA, depsipeptide	Enhanced	(122)
	Depsipeptide	Synergistic	(73)
	PB	Synergistic	(128)
	PB	Enhanced	(179)
Nuclear receptor ligands			
ATRA	CBHA	Synergistic	(127)
1α,25-Dihydroxyvitamin D_3	TSA	Synergistic	(123)
	SB		
Signal transduction inhibitors			
Imatinib mesylate (Gleevec)	Apicidin	Synergistic	(83)
	SAHA	Synergistic	(132)
Imatinib mesylate or PD180970	LAQ824	Synergistic	(80)
Herceptin	LAQ824	Synergistic	(82)
LY-29, 4002	SAHA, SB, MS-275	Synergistic	(124)
Flavopiridol	SAHA	Synergistic	(125)
	SB	Synergistic	(135)
	Depsipeptide	Synergistic	(136, 140)
TRAIL	SB	Enhanced	(180)
	SB, SAHA	Synergistic	(181)
	LAQ824	Enhanced	(182)
Hsp90 antagonists and proteasome inhibitors			
17-AAG	SAHA	Synergistic	(85)
Bortezomib (PS-341)	SAHA	Synergistic	(68)
	SAHA, SB	Synergistic	(84)
	SB	Synergistic	(141)
MG132	SB	Synergistic	(131)

ATRA, all-*trans* retinoic acid; CBHA, m-carboxylcinnamic acid bis-hydroxamide; DAC; 5-aza-2′-deoxycytidine; IMID-1, immunomodulatory thalidomide derivative 1; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

17-AAG, results in proteasomal degradation of client proteins. Owing to the regulation of Hsp90 function by acetylation, the combination of HDAC inhibitors and Hsp90 antagonists is a reasonable therapeutic strategy. Experimentally, 17-AAG in combination with SAHA or sodium butyrate inhibited induction of $p21^{WAF1/CIP1}$, inducing Bcl-2 cleavage, and synergistically enhanced tumor cell apoptosis (85). Proteasome inhibitors slow the degradation of many important and diverse cellular proteins, and their combination with HDAC inhibitors results in more complete inhibition of proteasome activity, which may synergistically enhance tumor cell apoptosis (68, 84, 131, 141).

There is additional evidence that HDAC inhibitors may improve the efficacy of radiation therapy (142). In this study, pretreatment with depsipeptide greatly increased radiation-induced apoptosis. In another study, the HDAC inhibitors phenylbutyrate, TSA, and valproic acid were able to reduce cutaneous radiation toxicity following radiotherapy (143). This poorly understood interaction whereby HDAC inhibitors potentially increase radiation-induced tumor cell death while decreasing normal host cell toxicity deserves further study, as it may lead to more novel clinical indications for HDAC inhibitors.

Although these provocative combination regimens based on cell culture studies have rational appeal, they must be explored more fully in vivo to assure that they do not also lead to enhanced host toxicity. One recent Phase II study of the combination of gemcitabine and CI-994 in patients with NSCLC demonstrated no improvement in efficacy over gemcitabine alone, primarily because of increased toxicity that limited dose intensity and reduced the net therapeutic index of the two-drug combination (176).

CONCLUSIONS

The complexities of the histone code and the various other nuclear as well as cytoplasmic nonhistone proteins whose functions are modulated by acetylation underscore why HDAC inhibition was an empirically discovered, as well as novel, form of cancer therapy. The biology of the various HDAC isoforms and their relationship to tumorigenesis is just beginning to be elucidated and is largely driven by the perceived clinical potential of HDAC inhibitors. It remains to be seen if a more detailed understanding of the specific roles played by various HDAC isoforms during human tumorigenesis leads not only to development of isoform-specific inhibitors but also to more effective or less toxic antitumor therapeutics, as compared to the multiclass HDAC inhibitors that are currently undergoing clinical evaluation. Rationally designed combinations of HDAC inhibitors with various other types of approved or investigational anticancer agents are showing promise in tumor cell culture systems but must yet be proven in clinical trials. Of great interest to many cancer investigators is the potential ability to derepress the expression of epigenetically silenced tumor suppressor genes by administering HDAC inhibitors in combination with inhibitors of DNA methyltransferases. There is a

similar level of preclinical interest in combining inhibitors of oncogenic kinases with HDAC inhibitors to strategically downregulate critical oncogenic pathways at transcriptional and posttranslational levels. The potential ability of HDAC inhibitors to overcome various drug-resistance phenotypes is yet another preclinical strategy warranting clinical evaluation. Finally, little is presently published on the pharmacokinetics and biodistribution of various HDAC inhibitors now under clinical development. Owing to the preclinically determined need for constant drug exposure to achieve in vivo tumor mass reduction by net inhibitory effects on tumor cell proliferation and survival mechanisms, a more detailed study and comparison of the pharmacokinetic profiles for various HDAC inhibitors is needed. Present evidence suggests that more novel formulations and drug delivery strategies may be able to significantly enhance the therapeutic index of even the most potent and biologically active of currently available HDAC inhibitors. Although a clinical role for HDAC inhibitors as novel cancer therapeutics seems almost inevitable at present, their general clinical utility will likely depend greatly on the future development of molecular or cellular predictors of their antitumor activity.

ACKNOWLEDGMENTS

We are grateful to the following for supplying their respective HDAC inhibitors: Dr. Peter Atadja and Novartis Oncology for NVP-LAQ824, Dr. Alan Kraker and Pfizer Oncology for CI-994, Dr. Victoria Richon and Aton Pharma for SAHA, and Dr. Keith Glaser and Abbott for A-16,1906. We thank Crystal Berger and Cliff Amend at the Buck Institute for their excellent technical assistance. Daryl Drummond was supported in part by a New Investigator Award from the California Breast Cancer Research Program of the University of California, Grant Number 7KB-0066A. This work was supported in part by NIH grant R01-CA36773 (CCB) and a development project award from the National Cancer Institute Specialized Programs of Research Excellence (SPORE) in Breast Cancer (P50-CA 58207-01; CCB).

The *Annual Review of Pharmacology and Toxicology* is online at http://pharmtox.annualreviews.org

LITERATURE CITED

1. Cress WD, Seto E. 2000. Histone deacetylases, transcriptional control, and cancer. *J. Cell Physiol.* 184:1–16
2. Glaser KB, Staver MJ, Waring JF, Stender J, Ulrich RG, Davidsen SK. 2003. Gene expression profiling of multiple histone deacetylase (HDAC) inhibitors: defining a common gene set produced by HDAC inhibition in T24 and MDA carcinoma cell lines. *Mol. Cancer Ther.* 2:151–63
3. Van Lint C, Emiliani S, Verdin E. 1996. The expression of a small fraction of cellular genes is changed in response to histone hyperacetylation. *Gene Expr.* 5:245–53
4. Turner BM. 2002. Cellular memory and the histone code. *Cell* 111:285–91
5. Strahl BD, Allis CD. 2000. The language of covalent histone modifications. *Nature* 403:41–45

6. Sterner DE, Berger SL. 2000. Acetylation of histones and transcription-related factors. *Microbiol. Mol. Biol. Rev.* 64:435–59
7. Jenuwein T. 2001. Re-SET-ting heterochromatin by histone methyltransferases. *Trends Cell Biol.* 11:266–73
8. Lachner M, Jenuwein T. 2002. The many faces of histone lysine methylation. *Curr. Opin. Cell Biol.* 14:286–98
9. Cheung WL, Ajiro K, Samejima K, Kloc M, Cheung P, et al. 2003. Apoptotic phosphorylation of histone H2B is mediated by mammalian sterile twenty kinase. *Cell* 113:507–17
10. Jason LJ, Moore SC, Lewis JD, Lindsey G, Ausio J. 2002. Histone ubiquitination: a tagging tail unfolds? *Bioessays* 24:166–74
11. Nathan D, Sterner DE, Berger SL. 2003. Histone modifications: now summoning sumoylation. *Proc. Natl. Acad. Sci. USA* 100:13118–20
12. Geiman TM, Robertson KD. 2002. Chromatin remodeling, histone modifications, and DNA methylation—how does it all fit together? *J. Cell Biochem.* 87:117–25
13. Nephew KP, Huang TH. 2003. Epigenetic gene silencing in cancer initiation and progression. *Cancer Lett.* 190:125–33
14. Claus R, Lubbert M. 2003. Epigenetic targets in hematopoietic malignancies. *Oncogene* 22:6489–96
15. Czermin B, Imhof A. 2003. The sounds of silence—histone deacetylation meets histone methylation. *Genetica* 117:159–64
16. Daujat S, Bauer UM, Shah V, Turner B, Berger S, Kouzarides T. 2002. Crosstalk between CARM1 methylation and CBP acetylation on histone H3. *Curr. Biol.* 12: 2090–97
17. Jenuwein T, Allis CD. 2001. Translating the histone code. *Science* 293:1074–80
18. Beisel C, Imhof A, Greene J, Kremmer E, Sauer F. 2002. Histone methylation by the Drosophila epigenetic transcriptional regulator Ash1. *Nature* 419:857–62
19. Imhof A, Becker PB. 2001. Modifications of the histone N-terminal domains. Evidence for an "epigenetic code"? *Mol. Biotechnol.* 17:1–13
20. Jacobs SA, Taverna SD, Zhang Y, Briggs SD, Li J, et al. 2001. Specificity of the HP1 chromo domain for the methylated N-terminus of histone H3. *EMBO J.* 20:5232–41
21. Hassan AH, Prochasson P, Neely KE, Galasinski SC, Chandy M, et al. 2002. Function and selectivity of bromodomains in anchoring chromatin-modifying complexes to promoter nucleosomes. *Cell* 111:369–79
22. Agalioti T, Chen G, Thanos D. 2002. Deciphering the transcriptional histone acetylation code for a human gene. *Cell* 111:381–92
23. Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, et al. 2002. Active genes are tri-methylated at K4 of histone H3. *Nature* 419:407–11
24. Zhang X, Yang Z, Khan SI, Horton JR, Tamaru H, et al. 2003. Structural basis for the product specificity of histone lysine methyltransferases. *Mol. Cell.* 12:177–85
25. Imhof A, Yang XJ, Ogryzko VV, Nakatani Y, Wolffe AP, Ge H. 1997. Acetylation of general transcription factors by histone acetyltransferases. *Curr. Biol.* 7:689–92
26. Juan LJ, Shia WJ, Chen MH, Yang WM, Seto E, et al. 2000. Histone deacetylases specifically down-regulate p53-dependent gene activation. *J. Biol. Chem.* 275:20436–43
27. Fu M, Rao M, Wang C, Sakamaki T, Wang J, et al. 2003. Acetylation of androgen receptor enhances coactivator binding and promotes prostate cancer cell growth. *Mol. Cell Biol.* 23:8563–75
28. Wang C, Fu M, Angeletti RH, Siconolfi-Baez L, Reutens AT, et al. 2001. Direct acetylation of the estrogen receptor alpha hinge region by p300 regulates transactivation and hormone sensitivity. *J. Biol. Chem.* 276:18375–83
29. Yamagata T, Mitani K, Oda H, Suzuki T, Honda H, et al. 2000. Acetylation of GATA-3 affects T-cell survival and

homing to secondary lymphoid organs. *EMBO J.* 19:4676–87
30. Boyes J, Byfield P, Nakatani Y, Ogryzko V. 1998. Regulation of activity of the transcription factor GATA-1 by acetylation. *Nature* 396:594–98
31. Herrera JE, Sakaguchi K, Bergel M, Trieschmann L, Nakatani Y, Bustin M. 1999. Specific acetylation of chromosomal protein HMG-17 by PCAF alters its interaction with nucleosomes. *Mol. Cell Biol.* 19:3466–73
32. Imai S, Armstrong CM, Kaeberlein M, Guarente L. 2000. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 403:795–800
33. Finnin MS, Donigian JR, Pavletich NP. 2001. Structure of the histone deacetylase SIRT2. *Nat. Struct. Biol.* 8:621–25
34. Kao HY, Verdel A, Tsai CC, Simon C, Juguilon H, Khochbin S. 2001. Mechanism for nucleocytoplasmic shuttling of histone deacetylase 7. *J. Biol. Chem.* 276:47496–507
35. Wang AH, Yang XJ. 2001. Histone deacetylase 4 possesses intrinsic nuclear import and export signals. *Mol. Cell Biol.* 21:5992–6005
36. Gray SG, Ekstrom TJ. 2001. The human histone deacetylase family. *Exp. Cell Res.* 262:75–83
37. Guardiola AR, Yao TP. 2002. Molecular cloning and characterization of a novel histone deacetylase HDAC10. *J. Biol. Chem.* 277:3350–56
38. Verdel A, Curtet S, Brocard MP, Rousseaux S, Lemercier C, et al. 2000. Active maintenance of mHDA2/mHDAC6 histone-deacetylase in the cytoplasm. *Curr. Biol.* 10:747–49
39. de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. 2003. Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem. J.* 370:737–49
40. Grozinger CM, Hassig CA, Schreiber SL. 1999. Three proteins define a class of human histone deacetylases related to yeast Hda1p. *Proc. Natl. Acad. Sci. USA* 96:4868–73
41. Petrie K, Guidez F, Howell L, Healy L, Waxman S, et al. 2003. The histone deacetylase 9 gene encodes multiple protein isoforms. *J. Biol. Chem.* 278:16059–72
42. Hu E, Dul E, Sung CM, Chen Z, Kirkpatrick R, et al. 2003. Identification of novel isoform-selective inhibitors within class I histone deacetylases. *J. Pharmacol. Exp. Ther.* 307:720–28
43. Jung M. 2001. Inhibitors of histone deacetylase as new anticancer agents. *Curr. Med. Chem.* 8:1505–11
44. Miller TA, Witter DJ, Belvedere S. 2003. Histone deacetylase inhibitors. *J. Med. Chem.* 46:5097–116
45. Meinke PT, Liberator P. 2001. Histone deacetylase: a target for antiproliferative and antiprotozoal agents. *Curr. Med. Chem.* 8:211–35
46. Finnin MS, Donigian JR, Cohen A, Richon VM, Rifkind RA, et al. 1999. Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. *Nature* 401:188–93
47. Breslow R, Belvedere S, Gershell L. 2000. Development of cytodifferentiating agents for cancer chemotherapy. *Helv. Chim. Acta* 83:1685–92
48. Woo SH, Frechette S, Abou Khalil E, Bouchain G, Vaisburg A, et al. 2002. Structurally simple trichostatin A-like straight chain hydroxamates as potent histone deacetylase inhibitors. *J. Med. Chem.* 45:2877–85
49. Yoshida M, Horinouchi S, Beppu T. 1995. Trichostatin A and trapoxin: novel chemical probes for the role of histone acetylation in chromatin structure and function. *Bioessays* 17:423–30
50. Shute RE, Dunlap B, Rich DH. 1987. Analogues of the cytostatic and antimitogenic agents chlamydocin and HC-toxin: synthesis and biological activity of chloromethyl ketone and diazomethyl

ketone functionalized cyclic tetrapeptides. *J. Med. Chem.* 30:71–78

51. Gore SD, Carducci MA. 2000. Modifying histones to tame cancer: clinical development of sodium phenylbutyrate and other histone deacetylase inhibitors. *Expert Opin. Invest. Drugs* 9:2923–34
52. Furumai R, Matsuyama A, Kobashi N, Lee KH, Nishiyama M, et al. 2002. FK228 (depsipeptide) as a natural prodrug that inhibits class I histone deacetylases. *Cancer Res.* 62:4916–21
53. Komatsu Y, Tomizaki KY, Tsukamoto M, Kato T, Nishino N, et al. 2001. Cyclic hydroxamic-acid-containing peptide 31, a potent synthetic histone deacetylase inhibitor with antitumor activity. *Cancer Res.* 61:4459–66
54. Furumai R, Komatsu Y, Nishino N, Khochbin S, Yoshida M, Horinouchi S. 2001. Potent histone deacetylase inhibitors built from trichostatin A and cyclic tetrapeptide antibiotics including trapoxin. *Proc. Natl. Acad. Sci. USA* 98: 87–92
55. el-Beltagi HM, Martens AC, Lelieveld P, Haroun EA, Hagenbeek A. 1993. Acetyldinaline: a new oral cytostatic drug with impressive differential activity against leukemic cells and normal stem cells—preclinical studies in a relevant rat model for human acute myelocytic leukemia. *Cancer Res.* 53:3008–14
56. Suzuki T, Ando T, Tsuchiya K, Fukazawa N, Saito A, et al. 1999. Synthesis and histone deacetylase inhibitory activity of new benzamide derivatives. *J. Med. Chem.* 42: 3001–3
57. Mai A, Massa S, Ragno R, Esposito M, Sbardella G, et al. 2002. Binding mode analysis of 3-(4-benzoyl-1-methyl-1H-2-pyrrolyl)-N-hydroxy-2-propenamide: a new synthetic histone deacetylase inhibitor inducing histone hyperacetylation, growth inhibition, and terminal cell differentiation. *J. Med. Chem.* 45:1778–84
58. Ragno R, Mai A, Massa S, Cerbara I, Valente S, et al. 2004. 3-(4-Aroyl-1-methyl-1H-pyrrol-2-yl)-N-hydroxy-2-propenamides as a new class of synthetic histone deacetylase inhibitors. 3. Discovery of novel lead compounds through structure-based drug design and docking studies. *J. Med. Chem.* 47:1351–59
59. Pina IC, Gautschi JT, Wang GY, Sanders ML, Schmitz FJ, et al. 2003. Psammaplins from the sponge *Pseudoceratina purpurea*: inhibition of both histone deacetylase and DNA methyltransferase. *J. Org. Chem.* 68:3866–73
60. Haggarty SJ, Koeller KM, Wong JC, Butcher RA, Schreiber SL. 2003. Multidimensional chemical genetic analysis of diversity-oriented synthesis-derived deacetylase inhibitors using cell-based assays. *Chem. Biol.* 10:383–96
61. Haggarty SJ, Koeller KM, Wong JC, Grozinger CM, Schreiber SL. 2003. Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation. *Proc. Natl. Acad. Sci. USA* 100:4389–94
62. Pandolfi PP. 2001. Transcription therapy for cancer. *Oncogene* 20:3116–27
63. Timmermann S, Lehrmann H, Polesskaya A, Harel-Bellan A. 2001. Histone acetylation and disease. *Cell Mol. Life Sci.* 58: 728–36
64. Marks P, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. 2001. Histone deacetylases and cancer: causes and therapies. *Nat. Rev. Cancer* 1:194–202
65. Rosato RR, Grant S. 2004. Histone deacetylase inhibitors in clinical development. *Expert Opin. Invest. Drugs* 13: 21–38
66. Richon VM, Sandhoff TW, Rifkind RA, Marks PA. 2000. Histone deacetylase inhibitor selectively induces p21WAF1 expression and gene-associated histone acetylation. *Proc. Natl. Acad. Sci. USA* 97:10014–19
67. Vigushin DM, Coombes RC. 2002. Histone deacetylase inhibitors in cancer treatment. *Anticancer Drugs* 13:1–13

68. Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Shringarpure R, et al. 2004. Transcriptional signature of histone deacetylase inhibition in multiple myeloma: biological and clinical implications. *Proc. Natl. Acad. Sci. USA* 101:540–45
69. Petti MC, Fazi F, Gentile M, Diverio D, De Fabritiis P, et al. 2002. Complete remission through blast cell differentiation in PLZF/RARalpha-positive acute promyelocytic leukemia: in vitro and in vivo studies. *Blood* 100:1065–67
70. Han JW, Ahn SH, Park SH, Wang SY, Bae GU, et al. 2000. Apicidin, a histone deacetylase inhibitor, inhibits proliferation of tumor cells via induction of p21WAF1/Cip1 and gelsolin. *Cancer Res.* 60:6068–74
71. Saito A, Yamashita T, Mariko Y, Nosaka Y, Tsuchiya K, et al. 1999. A synthetic inhibitor of histone deacetylase, MS-27-275, with marked in vivo antitumor activity against human tumors. *Proc. Natl. Acad. Sci. USA* 96:4592–97
72. Cameron EE, Bachman KE, Myohanen S, Herman JG, Baylin SB. 1999. Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. *Nat. Genet.* 21:103–7
73. Primeau M, Gagnon J, Momparler RL. 2003. Synergistic antineoplastic action of DNA methylation inhibitor 5-AZA-2′-deoxycytidine and histone deacetylase inhibitor depsipeptide on human breast carcinoma cells. *Int. J. Cancer* 103:177–84
74. Henderson C, Brancolini C. 2003. Apoptotic pathways activated by histone deacetylase inhibitors: implications for the drug-resistant phenotype. *Drug Resist. Update* 6:247–56
75. Johnstone RW, Licht JD. 2003. Histone deacetylase inhibitors in cancer therapy: is transcription the primary target? *Cancer Cell.* 4:13–18
76. Kwon SH, Ahn SH, Kim YK, Bae GU, Yoon JW, et al. 2002. Apicidin, a histone deacetylase inhibitor, induces apoptosis and Fas/Fas ligand expression in human acute promyelocytic leukemia cells. *J. Biol. Chem.* 277:2073–80
77. Cao XX, Mohuiddin I, Ece F, McConkey DJ, Smythe WR. 2001. Histone deacetylase inhibitor downregulation of bcl-xl gene expression leads to apoptotic cell death in mesothelioma. *Am. J. Respir. Cell Mol. Biol.* 25:562–68
78. Sawa H, Murakami H, Ohshima Y, Sugino T, Nakajyo T, et al. 2001. Histone deacetylase inhibitors such as sodium butyrate and trichostatin A induce apoptosis through an increase of the bcl-2-related protein Bad. *Brain Tumor. Pathol.* 18:109–14
79. Slack JL. 1999. The biology and treatment of acute progranulocytic leukemia. *Curr. Opin. Oncol.* 11:9–13
80. Nimmanapalli R, Fuino L, Bali P, Gasparetto M, Glozak M, et al. 2003. Histone deacetylase inhibitor LAQ824 both lowers expression and promotes proteasomal degradation of Bcr-Abl and induces apoptosis of imatinib mesylate-sensitive or -refractory chronic myelogenous leukemia-blast crisis cells. *Cancer Res.* 63:5126–35
81. Scott GK, Marden C, Xu F, Kirk L, Benz CC. 2002. Transcriptional repression of ErbB2 by histone deacetylase inhibitors detected by a genomically integrated ErbB2 promoter-reporting cell screen. *Mol. Cancer Ther.* 1:385–92
82. Fuino L, Bali P, Wittmann S, Donapaty S, Guo F, et al. 2003. Histone deacetylase inhibitor LAQ824 down-regulates Her-2 and sensitizes human breast cancer cells to trastuzumab, taxotere, gemcitabine, and epothilone B. *Mol. Cancer Ther.* 2:971–84
83. Kim JS, Jeung HK, Cheong JW, Maeng H, Lee ST, et al. 2004. Apicidin potentiates the imatinib-induced apoptosis of Bcr-Abl-positive human leukaemia cells by enhancing the activation of mitochondria-dependent caspase cascades. *Br. J. Haematol.* 124:166–78

84. Yu C, Rahmani M, Conrad D, Subler M, Dent P, Grant S. 2003. The proteasome inhibitor bortezomib interacts synergistically with histone deacetylase inhibitors to induce apoptosis in Bcr/Abl+ cells sensitive and resistant to STI571. *Blood* 102:3765–74
85. Rahmani M, Yu C, Dai Y, Reese E, Ahmed W, et al. 2003. Coadministration of the heat shock protein 90 antagonist 17-allylamino-17-demethoxygeldanamycin with suberoylanilide hydroxamic acid or sodium butyrate synergistically induces apoptosis in human leukemia cells. *Cancer Res.* 63:8420–27
86. Liu LT, Chang HC, Chiang LC, Hung WC. 2003. Histone deacetylase inhibitor upregulates RECK to inhibit MMP-2 activation and cancer cell invasion. *Cancer Res.* 63:3069–72
87. Sasakawa Y, Naoe Y, Noto T, Inoue T, Sasakawa T, et al. 2003. Antitumor efficacy of FK228, a novel histone deacetylase inhibitor, depends on the effect on expression of angiogenesis factors. *Biochem. Pharmacol.* 66:897–906
88. Michaelis M, Michaelis UR, Fleming I, Suhan T, Cinatl J, et al. 2004. Valproic acid inhibits angiogenesis in vitro and in vivo. *Mol. Pharmacol.* 65:520–27
89. Ruefli AA, Bernhard D, Tainton KM, Kofler R, Smyth MJ, Johnstone RW. 2002. Suberoylanilide hydroxamic acid (SAHA) overcomes multidrug resistance and induces cell death in P-glycoprotein-expressing cells. *Int. J. Cancer* 99:292–98
90. Tsurutani J, Soda H, Oka M, Suenaga M, Doi S, et al. 2003. Antiproliferative effects of the histone deacetylase inhibitor FR901228 on small-cell lung cancer lines and drug-resistant sublines. *Int. J. Cancer* 104:238–42
91. Jing Y, Xia L, Waxman S. 2002. Targeted removal of PML-RARalpha protein is required prior to inhibition of histone deacetylase for overcoming all-trans retinoic acid differentiation resistance in acute promyelocytic leukemia. *Blood* 100:1008–13
92. Cote S, Zhou D, Bianchini A, Nervi C, Gallagher RE, Miller WH Jr. 2000. Altered ligand binding and transcriptional regulation by mutations in the PML/RARalpha ligand-binding domain arising in retinoic acid-resistant patients with acute promyelocytic leukemia. *Blood* 96:3200–8
93. Castro-Galache MD, Ferragut JA, Barbera VM, Martin-Orozco E, Gonzalez-Ros JM, et al. 2003. Susceptibility of multidrug resistance tumor cells to apoptosis induction by histone deacetylase inhibitors. *Int. J. Cancer* 104:579–86
94. Peart MJ, Tainton KM, Ruefli AA, Dear AE, Sedelies KA, et al. 2003. Novel mechanisms of apoptosis induced by histone deacetylase inhibitors. *Cancer Res.* 63:4460–71
95. Batova A, Shao LE, Diccianni MB, Yu AL, Tanaka T, et al. 2002. The histone deacetylase inhibitor AN-9 has selective toxicity to acute leukemia and drug-resistant primary leukemia and cancer cell lines. *Blood* 100:3319–24
96. Takakura M, Kyo S, Sowa Y, Wang Z, Yatabe N, et al. 2001. Telomerase activation by histone deacetylase inhibitor in normal cells. *Nucleic Acids Res.* 29:3006–11
97. Nakamura M, Saito H, Ebinuma H, Wakabayashi K, Saito Y, et al. 2001. Reduction of telomerase activity in human liver cancer cells by a histone deacetylase inhibitor. *J. Cell Physiol.* 187:392–401
98. Vigushin DM, Ali S, Pace PE, Mirsaidi N, Ito K, et al. 2001. Trichostatin A is a histone deacetylase inhibitor with potent antitumor activity against breast cancer in vivo. *Clin. Cancer Res.* 7:971–76
99. Svechnikova I, Gray SG, Kundrotiene J, Ponthan F, Kogner P, Ekstrom TJ. 2003.

Apoptosis and tumor remission in liver tumor xenografts by 4-phenylbutyrate. *Int. J. Oncol.* 22:579–88

100. Sasakawa Y, Naoe Y, Inoue T, Sasakawa T, Matsuo M, et al. 2003. Effects of FK228, a novel histone deacetylase inhibitor, on tumor growth and expression of p21 and c-myc genes in vivo. *Cancer Lett.* 195:161–88
101. Remiszewski SW, Sambucetti LC, Bair KW, Bontempo J, Cesarz D, et al. 2003. N-hydroxy-3-phenyl-2-propenamides as novel inhibitors of human histone deacetylase with in vivo antitumor activity: discovery of (2E)-N-hydroxy-3-[4-[[(2-hydroxyethyl)[2-(1H-indol-3-yl)ethyl]amino]methyl]phenyl]-2-propenamide (NVP-LAQ824). *J. Med. Chem.* 46:4609–24
102. Kuefer R, Hofer MD, Altug V, Zorn C, Genze F, et al. 2004. Sodium butyrate and tributyrin induce in vivo growth inhibition and apoptosis in human prostate cancer. *Br. J. Cancer* 90:535–41
103. Kosugi H, Ito M, Yamamoto Y, Towatari M, Ueda R, et al. 2001. In vivo effects of a histone deacetylase inhibitor, FK228, on human acute promyelocytic leukemia in NOD/Shi-scid/scid mice. *Jpn. J. Cancer Res.* 92:529–36
104. Butler LM, Webb Y, Agus DB, Higgins B, Tolentino TR, et al. 2001. Inhibition of transformed cell growth and induction of cellular differentiation by pyroxamide, an inhibitor of histone deacetylase. *Clin. Cancer Res.* 7:962–70
105. Butler LM, Agus DB, Scher HI, Higgins B, Rose A, et al. 2000. Suberoylanilide hydroxamic acid, an inhibitor of histone deacetylase, suppresses the growth of prostate cancer cells in vitro and in vivo. *Cancer Res.* 60:5165–70
106. Arts J, Van Emelen K, Angibaud P, Van Brandt S, Poncelet V, et al. 2003. *Small molecule inhibitors of histone deacetylases* (*HDACs*): *identification of JNJ16241199, a potent oral antitumoral agent*. Presented at AACR-NCI-EORTC Int. Conf. Mol. Targets Cancer Ther., Boston
107. Piekarz RL, Robey R, Sandor V, Bakke S, Wilson WH, et al. 2001. Inhibitor of histone deacetylation, depsipeptide (FR901228), in the treatment of peripheral and cutaneous T-cell lymphoma: a case report. *Blood* 98:2865–68
108. Siu LL, Von Hoff DD, Rephaeli A, Izbicka E, Cerna C, et al. 1998. Activity of pivaloyloxymethyl butyrate, a novel anticancer agent, on primary human tumor colony-forming units. *Invest. New Drugs* 16:113–19
109. Patnaik A, Rowinsky EK, Villalona MA, Hammond LA, Britten CD, et al. 2002. A phase I study of pivaloyloxymethyl butyrate, a prodrug of the differentiating agent butyric acid, in patients with advanced solid malignancies. *Clin. Cancer Res.* 8:2142–48
110. Jaboin J, Wild J, Hamidi H, Khanna C, Kim CJ, et al. 2002. MS-27-275, an inhibitor of histone deacetylase, has marked in vitro and in vivo antitumor activity against pediatric solid tumors. *Cancer Res.* 62:6108–15
111. Fournel M, Trachy-Bourget MC, Yan PT, Kalita A, Bonfils C, et al. 2002. Sulfonamide anilides, a novel class of histone deacetylase inhibitors, are antiproliferative against human tumors. *Cancer Res.* 62:4325–30
112. Volpe DA, LoRusso PM, Foster BJ, Parchment RE. 2004. In vitro and in vivo effects of acetyldinaline on murine megakaryocytopoiesis. *Cancer Chemother. Pharmacol.* 54(1):89–94
113. Sandor V, Bakke S, Robey RW, Kang MH, Blagosklonny MV, et al. 2002. Phase I trial of the histone deacetylase inhibitor, depsipeptide (FR901228, NSC 630176), in patients with refractory neoplasms. *Clin. Cancer Res.* 8:718–28
114. Prakash S, Foster BJ, Meyer M, Wozniak A, Heilbrun LK, et al. 2001. Chronic oral administration of CI-994: a phase 1 study. *Invest. New Drugs* 19:1–11

115. Kelly WK, Richon VM, O'Connor O, Curley T, MacGregor-Curtelli B, et al. 2003. Phase I clinical trial of histone deacetylase inhibitor: suberoylanilide hydroxamic acid administered intravenously. *Clin. Cancer Res.* 9:3578–88
116. Piekarz RL, Robey R, Sandor V, Bakke S, Wilson WH, et al. 2001. Inhibitor of histone deacetylation, depsipeptide (FR901228), in the treatment of peripheral and cutaneous T-cell lymphoma: a case report. *Blood* 98:2865–68
117. Atadja P, Gao L, Kwon P, Trogani N, Walker H, et al. 2004. Selective growth inhibition of tumor cells by a novel histone deacetylase inhibitor, NVP-LAQ824. *Cancer Res.* 64:689–95
118. Drummond DC, Meyer OM, Hong K, Kirpotin DB, Papahadjopoulos D. 1999. Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. *Pharmacol. Rev.* 51:691–743
119. Benz C, Scott G, Berger C, Amend C, Guo Z, et al. 2003. Liposome encapsulation of histone deacetylase inhibitor enhances in vivo activity against ErbB2-positive breast cancers. *Clin. Cancer Res.* 9:6259s
120. Kim MS, Blake M, Baek JH, Kohlhagen G, Pommier Y, Carrier F. 2003. Inhibition of histone deacetylase increases cytotoxicity to anticancer drugs targeting DNA. *Cancer Res.* 63:7291–300
121. Witzig TE, Timm M, Stenson M, Svingen PA, Kaufmann SH. 2000. Induction of apoptosis in malignant B cells by phenylbutyrate or phenylacetate in combination with chemotherapeutic agents. *Clin. Cancer Res.* 6:681–92
122. Zhu WG, Lakshmanan RR, Beal MD, Otterson GA. 2001. DNA methyltransferase inhibition enhances apoptosis induced by histone deacetylase inhibitors. *Cancer Res.* 61:1327–33
123. Rashid SF, Moore JS, Walker E, Driver PM, Engel J, et al. 2001. Synergistic growth inhibition of prostate cancer cells by 1 alpha,25 dihydroxyvitamin D(3) and its 19-nor-hexafluoride analogs in combination with either sodium butyrate or trichostatin A. *Oncogene* 20:1860–72
124. Rahmani M, Yu C, Reese E, Ahmed W, Hirsch K, et al. 2003. Inhibition of PI-3 kinase sensitizes human leukemic cells to histone deacetylase inhibitor-mediated apoptosis through p44/42 MAP kinase inactivation and abrogation of p21(CIP1/WAF1) induction rather than AKT inhibition. *Oncogene* 22:6231–42
125. Almenara J, Rosato R, Grant S. 2002. Synergistic induction of mitochondrial damage and apoptosis in human leukemia cells by flavopiridol and the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA). *Leukemia* 16:1331–43
126. Keen JC, Yan L, Mack KM, Pettit C, Smith D, et al. 2003. A novel histone deacetylase inhibitor, scriptaid, enhances expression of functional estrogen receptor alpha (ER) in ER negative human breast cancer cells in combination with 5-aza 2′-deoxycytidine. *Breast Cancer Res. Treat.* 81:177–86
127. Coffey DC, Kutko MC, Glick RD, Butler LM, Heller G, et al. 2001. The histone deacetylase inhibitor, CBHA, inhibits growth of human neuroblastoma xenografts in vivo, alone and synergistically with all-trans retinoic acid. *Cancer Res.* 61:3591–94
128. Belinsky SA, Klinge DM, Stidley CA, Issa JP, Herman JG, et al. 2003. Inhibition of DNA methylation and histone deacetylation prevents murine lung cancer. *Cancer Res.* 63:7089–93
129. Kosugi H, Towatari M, Hatano S, Kitamura K, Kiyoi H, et al. 1999. Histone deacetylase inhibitors are the potent inducer/enhancer of differentiation in acute myeloid leukemia: a new approach to anti-leukemia therapy. *Leukemia* 13:1316–24
130. Zhou DC, Kim SH, Ding W, Schultz C, Warrell RP Jr, Gallagher RE. 2002. Frequent mutations in the ligand-binding domain of PML-RARalpha after multiple relapses of acute promyelocytic leukemia:

analysis for functional relationship to response to all-trans retinoic acid and histone deacetylase inhibitors in vitro and in vivo. *Blood* 99:1356–63

131. Giuliano M, Lauricella M, Calvaruso G, Carabillo M, Emanuele S, et al. 1999. The apoptotic effects and synergistic interaction of sodium butyrate and MG132 in human retinoblastoma Y79 cells. *Cancer Res.* 59:5586–95
132. Yu C, Rahmani M, Almenara J, Subler M, Krystal G, et al. 2003. Histone deacetylase inhibitors promote STI571-mediated apoptosis in STI571-sensitive and -resistant Bcr/Abl+ human myeloid leukemia cells. *Cancer Res.* 63:2118–26
133. Nimmanapalli R, Fuino L, Stobaugh C, Richon V, Bhalla K. 2003. Cotreatment with the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) enhances imatinib-induced apoptosis of Bcr-Abl-positive human acute leukemia cells. *Blood* 101:3236–39
134. Rosato RR, Almenara JA, Yu C, Grant S. 2004. Evidence of a functional role for p21WAF1/CIP1 down-regulation in synergistic antileukemic interactions between the histone deacetylase inhibitor sodium butyrate and flavopiridol. *Mol. Pharmacol.* 65:571–81
135. Rosato RR, Almenara JA, Cartee L, Betts V, Chellappan SP, Grant S. 2002. The cyclin-dependent kinase inhibitor flavopiridol disrupts sodium butyrate-induced p21WAF1/CIP1 expression and maturation while reciprocally potentiating apoptosis in human leukemia cells. *Mol. Cancer Ther.* 1:253–66
136. Nguyen DM, Schrump WD, Tsai WS, Chen A, Stewart JHt, et al. 2003. Enhancement of depsipeptide-mediated apoptosis of lung or esophageal cancer cells by flavopiridol: activation of the mitochondria-dependent death-signaling pathway. *J. Thorac. Cardiovasc. Surg.* 125:1132–42
137. Fujii S, Luo RZ, Yuan J, Kadota M, Oshimura M, et al. 2003. Reactivation of the silenced and imprinted alleles of ARHI is associated with increased histone H3 acetylation and decreased histone H3 lysine 9 methylation. *Hum. Mol. Genet.* 12:1791–800
138. Pili R, Kruszewski MP, Hager BW, Lantz J, Carducci MA. 2001. Combination of phenylbutyrate and 13-cis retinoic acid inhibits prostate tumor growth and angiogenesis. *Cancer Res.* 61:1477–85
139. Rosato RR, Wang Z, Gopalkrishnan RV, Fisher PB, Grant S. 2001. Evidence of a functional role for the cyclin-dependent kinase-inhibitor p21WAF1/CIP1/MDA6 in promoting differentiation and preventing mitochondrial dysfunction and apoptosis induced by sodium butyrate in human myelomonocytic leukemia cells (U937). *Int. J. Oncol.* 19:181–91
140. Nguyen DM, Schrump WD, Chen GA, Tsai W, Nguyen P, et al. 2004. Abrogation of p21 expression by flavopiridol enhances depsipeptide-mediated apoptosis in malignant pleural mesothelioma cells. *Clin. Cancer Res.* 10:1813–25
141. Denlinger CE, Keller MD, Mayo MW, Broad RM, Jones DR. 2004. Combined proteasome and histone deacetylase inhibition in non-small cell lung cancer. *J. Thorac. Cardiovasc. Surg.* 127:1078–86
142. Zhang Y, Adachi M, Zhao X, Kawamura R, Imai K. 2004. Histone deacetylase inhibitors FK228, N-(2-aminophenyl)-4-[N-(pyridin-3-yl-methoxycarbonyl) amino-methyl]benzamide and m-carboxycinnamic acid bis-hydroxamide augment radiation-induced cell death in gastrointestinal adenocarcinoma cells. *Int. J. Cancer* 110:301–8
143. Chung YL, Wang AJ, Yao LF. 2004. Antitumor histone deacetylase inhibitors suppress cutaneous radiation syndrome: implications for increasing therapeutic gain in cancer radiotherapy. *Mol. Cancer Ther.* 3:317–25
144. Spotswood HT, Turner BM. 2002. An increasingly complex code. *J. Clin. Invest.* 110:577–82

145. Gu W, Roeder RG. 1997. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell* 90:595–606
146. Sakaguchi K, Herrera JE, Saito S, Miki T, Bustin M, et al. 1998. DNA damage activates p53 through a phosphorylation-acetylation cascade. *Genes Dev.* 12:2831–41
147. Tomita A, Towatari M, Tsuzuki S, Hayakawa F, Kosugi H, et al. 2000. c-Myb acetylation at the carboxyl-terminal conserved domain by transcriptional co-activator p300. *Oncogene* 19: 444–51
148. Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, et al. 2002. HDAC6 is a microtubule-associated deacetylase. *Nature* 417:455–58
149. Munshi N, Merika M, Yie J, Senger K, Chen G, Thanos D. 1998. Acetylation of HMG I(Y) by CBP turns off IFN beta expression by disrupting the enhanceosome. *Mol. Cell.* 2:457–67
150. Waltzer L, Bienz M. 1998. Drosophila CBP represses the transcription factor TCF to antagonize Wingless signalling. *Nature* 395:521–25
151. Naryzhny SN, Lee H. 2004. The post-translational modifications of proliferating cell nuclear antigen (PCNA): acetylation, not phosphorylation, plays an important role in the regulation of its function. *J. Biol. Chem.* 279:20194–99
152. Zhang W, Bieker JJ. 1998. Acetylation and modulation of erythroid Kruppel-like factor (EKLF) activity by interaction with histone acetyltransferases. *Proc. Natl. Acad. Sci. USA* 95:9855–60
153. Chen H, Lin RJ, Xie W, Wilpitz D, Evans RM. 1999. Regulation of hormone-induced histone hyperacetylation and gene activation via acetylation of an acetylase. *Cell* 98:675–86
154. Soutoglou E, Katrakili N, Talianidis I. 2000. Acetylation regulates transcription factor activity at multiple levels. *Mol. Cell.* 5:745–51
155. Bannister AJ, Miska EA, Gorlich D, Kouzarides T. 2000. Acetylation of importin-alpha nuclear import factors by CBP/p300. *Curr. Biol.* 10:467–70
156. Chen LF, Greene WC. 2003. Regulation of distinct biological activities of the NF-kappaB transcription factor complex by acetylation. *J. Mol. Med.* 81:549–57
157. Goel A, Janknecht R. 2003. Acetylation-mediated transcriptional activation of the ETS protein ER81 by p300, P/CAF, and HER2/Neu. *Mol. Cell Biol.* 23:6243–54
158. Jacob AL, Lund J, Martinez P, Hedin L. 2001. Acetylation of steroidogenic factor 1 protein regulates its transcriptional activity and recruits the coactivator GCN5. *J. Biol. Chem.* 276:37659–64
159. Cohen HY, Lavu S, Bitterman KJ, Hekking B, Imahiyerobo TA, et al. 2004. Acetylation of the C terminus of Ku70 by CBP and PCAF controls Bax-mediated apoptosis. *Mol. Cell* 13:627–38
160. Pelletier G, Stefanovsky VY, Faubladier M, Hirschler-Laszkiewicz I, Savard J, et al. 2000. Competitive recruitment of CBP and Rb-HDAC regulates UBF acetylation and ribosomal transcription. *Mol. Cell.* 6:1059–66
161. Ammanamanchi S, Freeman JW, Brattain MG. 2003. Acetylated sp3 is a transcriptional activator. *J. Biol. Chem.* 278:35775–80
162. Huang S, Qiu Y, Shi Y, Xu Z, Brandt SJ. 2000. P/CAF-mediated acetylation regulates the function of the basic helix-loop-helix transcription factor TAL1/SCL. *EMBO J.* 19:6792–803
163. Yao YL, Yang WM, Seto E. 2001. Regulation of transcription factor YY1 by acetylation and deacetylation. *Mol. Cell Biol.* 21:5979–91
164. Martinez-Balbas MA, Bauer UM, Nielsen SJ, Brehm A, Kouzarides T. 2000. Regulation of E2F1 activity by acetylation. *EMBO J.* 19:662–71
165. Sartorelli V, Puri PL, Hamamori Y, Ogryzko V, Chung G, et al. 1999.

Acetylation of MyoD directed by PCAF is necessary for the execution of the muscle program. *Mol. Cell.* 4:725–34

166. Park SH, Lee SR, Kim BC, Cho EA, Patel SP, et al. 2002. Transcriptional regulation of the transforming growth factor beta type II receptor gene by histone acetyltransferase and deacetylase is mediated by NF-Y in human breast cancer cells. *J. Biol. Chem.* 277:5168–74
167. Lee BI, Park SH, Kim JW, Sausville EA, Kim HT, et al. 2001. MS-275, a histone deacetylase inhibitor, selectively induces transforming growth factor beta type II receptor expression in human breast cancer cells. *Cancer Res.* 61:931–34
168. Butler LM, Zhou X, Xu WS, Scher HI, Rifkind RA, et al. 2002. The histone deacetylase inhibitor SAHA arrests cancer cell growth, up-regulates thioredoxin-binding protein-2, and down-regulates thioredoxin. *Proc. Natl. Acad. Sci. USA* 99:11700–5
169. Kim MS, Kwon HJ, Lee YM, Baek JH, Jang JE, et al. 2001. Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. *Nat. Med.* 7:437–43
170. Andoh A, Shimada M, Araki Y, Fujiyama Y, Bamba T. 2002. Sodium butyrate enhances complement-mediated cell injury via down-regulation of decay-accelerating factor expression in colonic cancer cells. *Cancer Immunol. Immunother.* 50:663–72
171. Zhang XD, Gillespie SK, Borrow JM, Hersey P. 2004. The histone deacetylase inhibitor suberic bishydroxamate regulates the expression of multiple apoptotic mediators and induces mitochondria-dependent apoptosis of melanoma cells. *Mol. Cancer Ther.* 3:425–35
172. Bernhard D, Skvortsov S, Tinhofer I, Hubl H, Greil R, et al. 2001. Inhibition of histone deacetylase activity enhances Fas receptor-mediated apoptosis in leukemic lymphoblasts. *Cell Death Differ.* 8:1014–21
173. Huang H, Reed CP, Zhang JS, Shridhar V, Wang L, Smith DI. 1999. Carboxypeptidase A3 (CPA3): a novel gene highly induced by histone deacetylase inhibitors during differentiation of prostate epithelial cancer cells. *Cancer Res.* 59:2981–88
174. Yang X, Ferguson AT, Nass SJ, Phillips DL, Butash KA, et al. 2000. Transcriptional activation of estrogen receptor alpha in human breast cancer cells by histone deacetylase inhibition. *Cancer Res.* 60:6890–94
175. Walker GE, Wilson EM, Powell D, Oh Y. 2001. Butyrate, a histone deacetylase inhibitor, activates the human IGF binding protein-3 promoter in breast cancer cells: molecular mechanism involves an Sp1/Sp3 multiprotein complex. *Endocrinology* 142:3817–27
176. Pawel JV, Shepherd F, Gatzmeier U, Natale RB, O'Brien ME, et al. 2002. Randomized phase 2 study of the oral histone deacetylase inhibitor CI-994 plus gemcitabine (Gem) vs placebo (PBO) plus GEM in second-line nonsmall cell lung cancer (NSCLC). *Am. Soc. Clin. Oncol. Annu. Meet.* A1239, Orlando, Florida
177. Johnson CA, Padget K, Austin CA, Turner BM. 2001. Deacetylase activity associates with topoisomerase II and is necessary for etoposide-induced apoptosis. *J. Biol. Chem.* 276:4539–42
178. Maggio SC, Rosato RR, Kramer LB, Dai Y, Rahmani M, et al. 2004. The histone deacetylase inhibitor MS-275 interacts synergistically with fludarabine to induce apoptosis in human leukemia cells. *Cancer Res.* 64:2590–600
179. Lemaire M, Momparler LF, Farinha NJ, Bernstein M, Momparler RL. 2004. Enhancement of antineoplastic action of 5-aza-2′-deoxycytidine by phenylbutyrate on L1210 leukemic cells. *Leuk. Lymphoma* 45:147–54
180. Hernandez A, Thomas R, Smith F, Sandberg J, Kim S, et al. 2001. Butyrate sensitizes human colon cancer cells to TRAIL-mediated apoptosis. *Surgery* 130:265–72

181. Rosato RR, Almenara JA, Dai Y, Grant S. 2003. Simultaneous activation of the intrinsic and extrinsic pathways by histone deacetylase (HDAC) inhibitors and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) synergistically induces mitochondrial damage and apoptosis in human leukemia cells. *Mol. Cancer Ther.* 2:1273–84

182. Guo F, Sigua C, Tao J, Bali P, George P, et al. 2004. Cotreatment with histone deacetylase inhibitor LAQ824 enhances Apo-2L/tumor necrosis factor-related apoptosis inducing ligand-induced death inducing signaling complex activity and apoptosis of human acute leukemia cells. *Cancer Res.* 64:2580–89

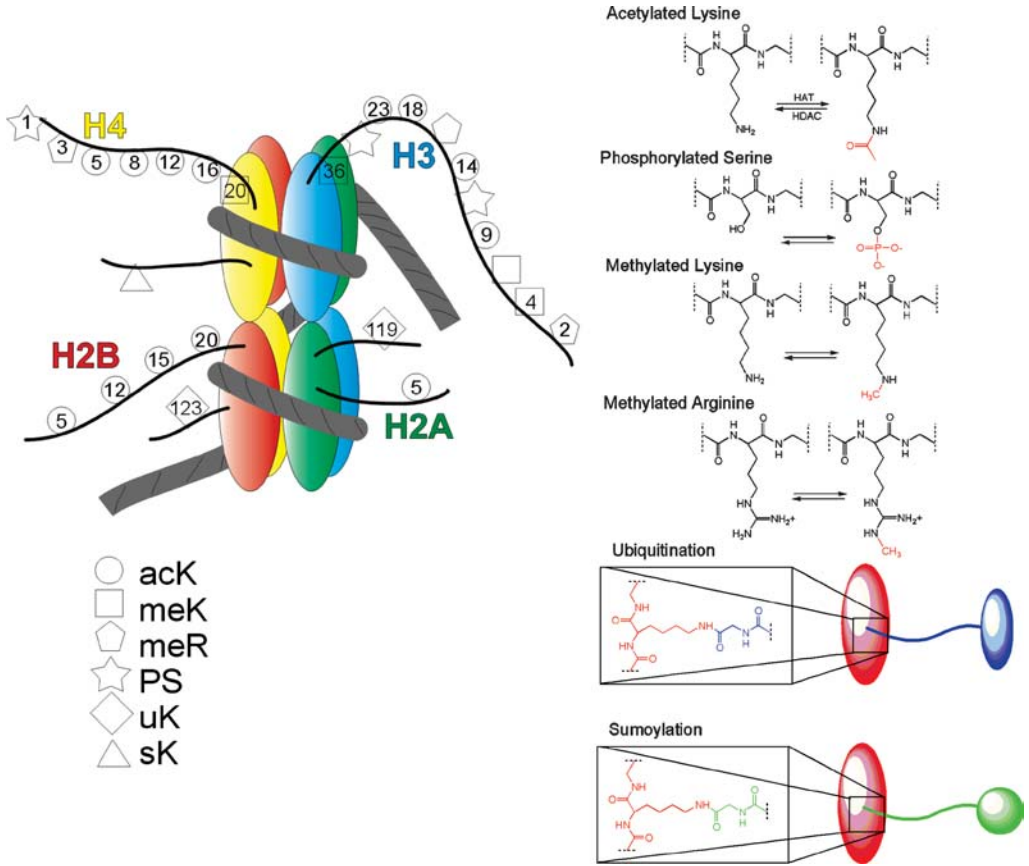


Figure 1 See legend on next page

Figure 1 Histones can be modified in a variety of ways, primarily in the tails of core histones in a process that is referred to as the histone code. Acetylated lysine residues, methylated arginines, methylated lysines, phosphorylated serines, sumoylated lysines, and ubquitinated lysine residues all contribute to the histone code. The relative positions for each modification on the various histone tails are depicted by symbols that are defined in the key. The actual chemical modification of the various amino acids in the histone tails is shown with the colored bonds indicating the modification and the amino acid residue shown in black. This figure was adapted from Turner (4) and Spotswood & Turner (144) with permission.

Annual Review of Pharmacology and Toxicology
Volume 45, 2005

CONTENTS