Cellular Oncology 28 (2006) 191–222
IOS Press

Review

Acetylation and histone deacetylase inhibitors in cancer

Madeleine S.Q. Kortenhorst [a], Michael A. Carducci [b,*] and Shabana Shabbeer [b]
[a] *Utrecht University, The Netherlands*
[b] *Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD, USA*

1. The diverse field of epigenetics

In recent years, the role of epigenetics in the development and treatment of cancer has gained interest and the effects of internal and external factors on the epigenetic profile are under investigation. The term "epigenetics" refers to modifications that influence phenotype without altering genotype. Epigenetic changes are potentially reversible but generally stably maintained during the cell cycle. Since Feinberg et al. described differences in DNA methylation in human cancer in 1983 [66], several mechanisms of epigenetic control have been identified such as DNA methylation and histone modifications including acetylation, methylation and phosphorylation. Recently Seligson et al. reported that specific histone modification patterns are predictive of the risk of prostate cancer recurrence [183] and Fraga et al. showed that a profound disruption in histone modification patterns is a common feature of cancer [70]. Epigenetic abnormalities can be acquired during fetal development and during the course of a life contributing to common cancer risk in adults [65]. Recently Fraga et al. reported that, although monozygotic twins are epigenetically identical during the first years of life, the patterns of global and locus specific epigenetic modifications and gene expression patterns in monozygotic twin pairs diverge as they become older [70]. These differences could be explained by the influence of external factors such as smoking, physical activity and diet [17,61] as well as by accumulation of small defects in epigenetic information, that could be considered an "epigenetic drift" associated with the aging process [13].

In 1974, Kornberg suggested that the structural organization of chromatin is based on a repeating unit of about 200 base pairs and eight histone molecules [116]. A year later Baldwin proposed that this histone octamer serves as a protein spool around which DNA is wrapped [7]. In the same year Oudet et al. provided the first electron microscopic images of eukaryotic genome proving the existence of the repeating uniformly sized particles in DNA [151]. Referring to their nuclear origin and their resemblance to "ν" (nu bodies) [148], Oudet et al. named these spherical particles nucleosomes. Since then several others have tried to elucidate the structure of the histone complex, but it took until 1991 to solve the definitive X-ray structure of the octameric histone core of the nucleosome [2]. A chronologic review of the discoveries that led to the establishment of the nucleosome as a repeating unit in DNA was published in 2003 by Olins and Olins [149].

The nucleosome core consists of 147 base pairs [57] of double-stranded DNA wrapped 1.65 times around an octamer of two copies each of histone 2A (H2A), 2B (H2B), 3 (H3) and 4 (H4) protein (Fig. 1). The atomic structures of the dimerized H2A [106] and H3 and H4 [117] are shown in Fig. 2. There are >120 direct atomic interactions of the nucleosome core with the DNA backbone at 14 super helix locations [126]. The repeating nucleosome core particles assemble into higher order helices, which are stabilized by a linker histone H1 to further condense the chromatin and together make up the nucleosome. About 25% of nucleosome core histone is comprised of amino-terminal tails that protrude the enveloping DNA double helix [206].

*Corresponding author: M.A. Carducci, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, 1650 Orleans Street, Room 1M59, Baltimore, MD 21231, USA. Tel.: +1 410 614 3977; Fax: +1 410 614 8160; E-mail: carducci@jhmi.edu.

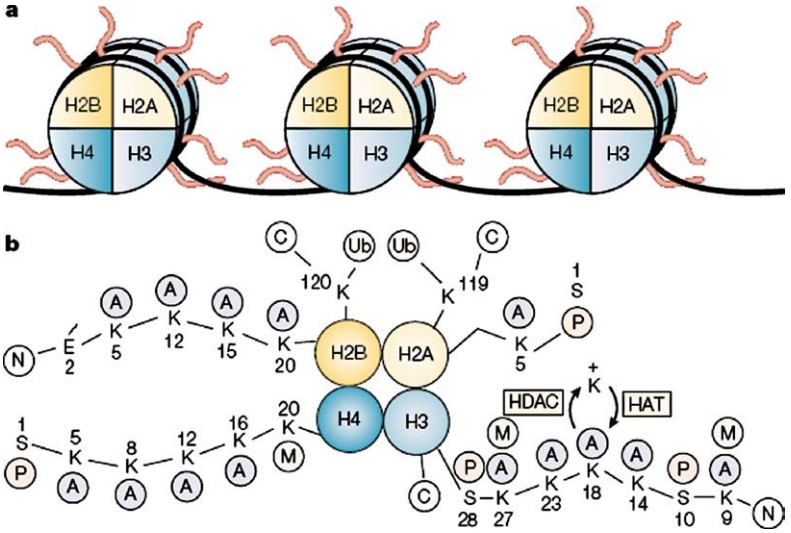


Fig. 1. Schematic structure of histones in nucleosomes. (a) The core proteins of nucleosomes are designated H2A (Histone 2A), H2B (Histone 2B), H3 (histone 3) and H4 (histone 4). Each histone is present in two copies, so the DNA (black) wraps around an octamer of histones called the core nucleosome. (b) The amino terminal tails of core histones, lysines (K) in the amino-terminal tails of histones H2A, H2B, H3 and H4 are potential acetylation/deacetylation sites for histone acetyltransferases (HATs) and histone deacetylases (HDACs). Acetylation neutralizes the charge on lysines. A, acetyl; C, carboxyl terminus; E, glutamic acid; M, methyl; N, amino terminus; P, phosphate; S, serine; Ub, ubiquitin. *(Reproduced with permission from Nature Reviews Cancer (Nat. Rev. Cancer **1**(3) (2001), 194–202), copyright (2001) Macmillan Magazines Ltd.)*

Although they are unstructured on a single nucleosome level, these N-terminal tails are thought to mediate interactions with other nucleosomes and chromatin proteins affecting higher order chromatin structure. The highly efficient way of DNA packaging by nucleosome formation compresses the DNA ∼10,000 times forcing chromatin in a repressive state and inaccessible for nuclear processes like transcription. When Oudet et al. provided the first images of repeating uniformly sized particles in DNA in 1975, they hypothesized that their observations might have a function in genomic expression. Consistent with these predictions Boeger et al. showed in 2003 that nucleosomes can unfold completely at transcriptionally active promoters [18]. An overview on the dynamic structure and function of chromatin has been published by Hansen in 2002 [89].

2. The histone code

In 1993, Turner presented the first evidence that post-translational modification of histone tails was functionally significant [191]. In the years that followed, increasing experimental data provided support for the hypothesis that distinct patterns of covalent histone marks make up a histone ‘language’. Encoded on histone tail domains and read by other proteins or proteins modules, these modifications are thought to determine the transcriptional state of genes. Post-translational modifications important in the development and progression of cancer include acetylation, methylation, phosphorylation and ubiquitination. Figure 1 gives a schematic overview of the core nucleosome and important modification sites on amino terminal tails.

In 2000, Strahl and Allis referred for the first time to this language as the ‘histone code’ defining it as “multiple histone modifications acting in a combinatorial or sequential fashion on one or multiple histone tails, specifying unique downstream functions” [187]. An important concept in this hypothesis is that the histone code uses combinations of modifications on each histone and that modifications on different histone tails may be interdependent. It is therefore essential that histone modifications are site-specific and that these modifications cause site specific chromatin modification [187]. Recently two other hypotheses have been added to explain the important function histone modifications serve. The ‘modification cassettes model’ proposes that numerous residues in linear strings of densely modifiable sites can have a large array of different biological readouts by forming cassettes. In the second model neighboring modifications act together as ‘binary switches’ [68].

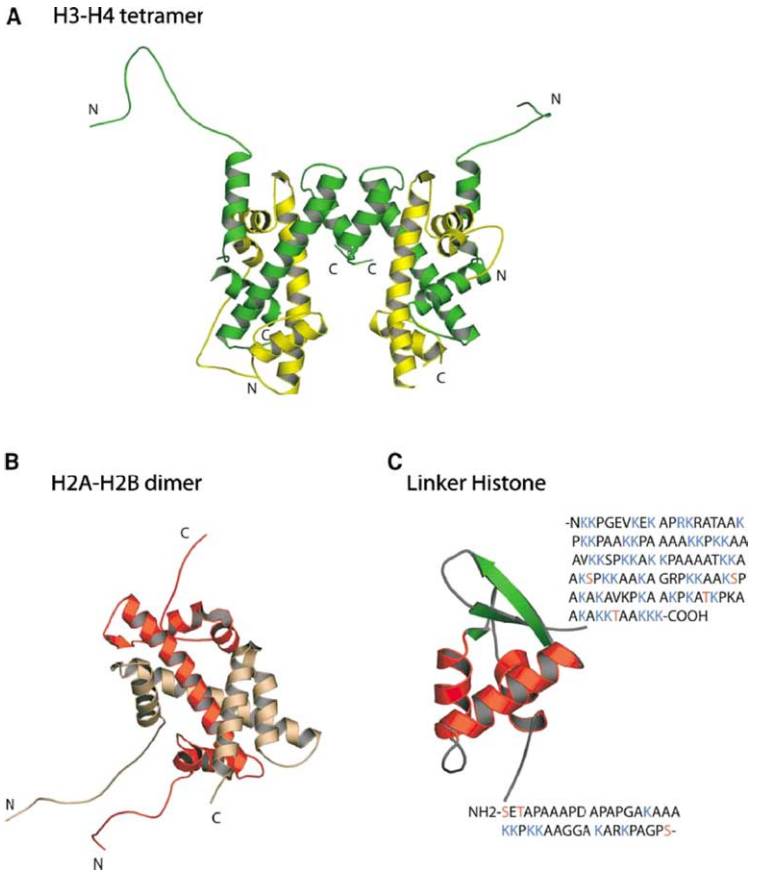


Fig. 2. Atomic structure of the core (A and B) and linker (C) histones. (a) A tetramer of H3 (green) and H4 (yellow). (b) A dimer of H2A (red) and H2B (pink). The linker histone has a conserved wing helix fold; the avian erythrocyte variant H5 is shown [166] (*Reprinted from Cell, 116, Khorasanizadeh, The Nucleosome: from genomic organization to genomic regulation, 259–272, Copyright (2004), with permission from Elsevier).*

3. Nucleosome dynamics and epigenetic modifications

Although DNA packaging is necessary to store the ∼2 meters of DNA that make up the human genome in the nucleus, the complex structure of nucleosomes and many interactions with the DNA backbone impairs accessibility to chromatin. Access to chromatin is essential for proteins that regulate biological processes like transcription, DNA repair and replication to exert their function. To counterbalance the repressive nature of chromatin, a sophisticated mechanism has evolved that regulates chromatin accessibility.

3.1. Chromatin remodeling complexes and chromatin modification complexes

Unlike assumed previously, nucleosomes have emerged to be highly specialized, serving many functions in the regulation of individual genes and chromosome regions. Although all nucleosomes contain a histone octamer around which DNA is wrapped, chromatin remodeling and chromatin modifying complexes allow nucleosomes to have a specialized and dynamic composition [31].

N-terminal histone tails, which protrude from the surface of the chromatin polymer, possess many post-translational modification sites. These modification sites can be recognized by ATP-dependent nucleosome-remodeling complexes that possess chromatin-binding domains that recognize specific modifications patterns (see below). ATP-dependent chromatin-remodeling complexes regulate chromatin accessibility using ATP hydrolysis to weaken histone-DNA contacts. At least three ways of creating access to nucleosomal DNA by chromatin remodeling complexes have been identified [110]: octamer sliding, DNA looping and histone substitution [64,102,136]. By weakening DNA con-

tacts these complexes are able to expose DNA to proteins that for instance regulate transcription to reach their target sites.

An overview of recent literature on chromatin remodeling complexes is provided by Cosgrove et al. [55] and Cairns [31].

The ε-amino groups evolutionarily conserved on lysine residues of histones tails can be post-translationally modified by chromatin-modifying enzymes causing acetylation, methylation and ubiquitination. Such seemingly small modifications determine not only the structural organization of chromatin, by virtue of their ionic charges, but also attract other proteins that possess modification-specific chromatin-binding domains (see below) to sites in the chromatin where transcription may be regulated [205].

3.2. Chromatin-binding domains

As described, both chromatin remodeling and chromatin modification make use of chromatin-binding domains, called protein motifs, which recognize specific modifications caused by chromatin modifying complexes on histone tails and non-histone proteins. Several chromatin-binding domains can be identified. Below three domains important in the context of the histone code hypothesis will be discussed: bromodomains, chromodomains and SANT domains.

3.2.1. Bromodomains

Bromodomains form an extensive family of small protein domains that preferentially bind acetylated peptides irrespective of the protein to which it belongs [58]. Bromodomains are widely distributed among different enzymes such as chromatin remodeling enzymes that utilize ATP to modify chromatin structure, but also in subunits of the chromatin remodeling complexes that do not have a catalytic function. In that case bromodomains mainly help to recognize previously modified chromatin and stabilize the complex [103]. Bromodomains have also been found in enzymes that cause methylation and acetylation. Interestingly, since histone acetyl transferases (HATs) acetylate a wide variety of target proteins (see below), the presence of a bromodomain on most HATs suggests self perpetuation through a positive feedback loop [58].

3.2.2. Chromodomains

As with bromodomains, chromodomains bind to their target protein independent of the protein to which they belong. Although their distribution among enzymes is more restricted than with bromodomains, chromodomains have been found in ATP-dependent chromatin remodeling factors, histone acetyl transferases and methyl transferases too. Chromodomains have been shown to recognize methyl-lysines [10], DNA and RNA and point to an involvement in protein–protein interactions [21]. However their exact function in the context of gene expression is not yet fully understood.

3.2.3. SANT domains

Unlike bromodomains and chromodomains, SANT domains primarily mediate interactions between remodeling complexes and unmodified chromatin substrates through the recruitment of chromatin modifying enzymes and by mediating interactions between histones and enzymes. SANT domains have been shown to be present in several components of complexes containing Histone Deacetylase (HDAC) or HAT activity; although no HAT or HDAC enzyme itself has been found to possess a SANT domain to date [58]. In ATP-dependent chromatin remodeling complexes, SANT domains are broadly present. By direct binding, ATP-dependent chromatin remodeling complexes cause conformational change of histone tails thereby promoting binding of modifying enzymes and subsequent catalytic processes. As is suggested by Yu et al., the interaction of SANT domains with unacetylated histone tails could block the binding of HATs, thereby maintaining a deacetylated state [214]. A review on the unique function of SANT-domains has been written by Boyer et al. in 2004 [22].

A more extensive review on the diversity of proteins containing protein motifs and their function has been published by de la Cruz et al. in 2005 [58].

4. Acetylation

As briefly discussed above, modification of histone tails is an important feature in the regulation of gene expression. Limited by the 20 encoded amino acids available, modification of proteins extends the range of molecular structures and functions possible. Many proteins are post- or co-translationally acetylated. Out of the more than 200 covalent modifications that have been reported, acetylation is the most common modification of eukaryotic proteins affecting many protein functions such as transcription, nuclear import, microtubule function, hormone responses, peptide-receptor recognition, DNA binding and protein–protein interactions. Modification of proteins not only changes its

molecular structure, but also provides binding sites for modification recognizing protein motifs such as bromodomains. The acetylation status of histones is regulated by the antagonistic actions of HATs and HDACs. Both HATs and HDACs function as part of multiprotein complexes with other proteins [81,177], or self-associate with each other [84,197].

4.1. HDACs

HDACs may be classified into four subfamilies, i.e., class I, class II, class III and the HDAC11-related enzymes (Table 1) [82]. The class I HDACs possess a molecular weight of 22 to 55 kDa and share homologous catalytic sites. The class II HDACs range from 120 to 135 kDa and, unlike the class I HDACs, exhibit greater diversity within the class. HDAC6 and −10 for example are characterized by duplicated HDAC catalytic domains while the other members of the same class display only one catalytic domain [197]. Class I HDACs are located exclusively in the nucleus with the exception of HDAC8 which has recently been shown to be predominantly expressed in the cytosol [201]. Class II HDACs shuttle between the nucleus and cytoplasm depending upon perceived cellular signals. Class III HDACs are known as the Sir2-like HDACs due to their homology to yeast Sir2 proteins. While class I and II are Zn-dependent HDACs, the class III HDACs are Zn-independent and NAD-dependent. In addition, the HDAC11-related enzymes, which share the features of class I and II HDACs but may have a distinct physiological role, could potentially constitute a fourth class of HDACs.

Despite the existence of several HDAC subfamilies, the different HDACs are by no means redundant. Robyr et al. generated 'acetylation maps' by inactivating six different HDACs, demonstrating that only a small degree of functional overlap was present among the different HDACs [171]. HDAC5 and 9 are for example involved in stress response of the heart [41], HDAC2 in apoptosis [220], HDAC1 in modulating the cell cycle and HDAC8 in smooth muscle contractility [200]. HDAC activity also differs between different types of tissues [48,109]. Screening of HDAC expression in human prostate cancer for instance revealed distinct class I HDAC profiles between stromal and epithelial cells [201] and *in vitro* experiments with prostate cancer cell lines showed a marked increase in HDAC levels for most HDACs compared to normal prostate tissue [99]. Furthermore, different isoforms of HDACs may have distinct localization and functions [152]. Even during embryonic development the levels of HDACs are continuously changing [129]. Targeted disruption of both HDAC1 alleles results in severe proliferation defects and retardation in development, leading to embryonic lethality [121]. Certain disease states can be characterized by loss or gain of specific or generalized HDAC activity, for example, reduced protein expression of HDAC1 and −2 proteins and decreased enzymatic HDAC activity is observed in asthma patients [97], while class II HDACs suppress cardiac hypertrophy [130] and higher expression of HDAC2 and −9 has been reported in many colon cancer cell lines compared with the primary cell from corresponding normal tissue [152,220].

Table 1
Classification of HDAC subfamilies

Class I	Class II	Class III	Class IV
HDAC1	HDAC4	SIRT1	HDAC11
HDAC2	HDAC5	SIRT2	
HDAC3	HDAC6	SIRT3	
HDAC8	HDAC7	SIRT4	
	HDAC9	SIRT5	
	HDAC10	SIRT6	
		SIRT7	

The classification is based on the homology of human HDACs to yeast HDACs, their sub-cellular expression and enzymatic activity. HDAC – histone deacetylase, SIRT – silent information regulator.

Malignant diseases are exemplified by aberrant transcriptional regulation which may be triggered by increased recruitment of HDACs to the site of transcriptional initiation. In acute promyelocytic leukemia (APL) for example, it has been shown that the PLZF/RARα fusion protein causes oligomerization of RAR, imposing an altered interaction with transcriptional co-regulators that recruit HDACs [133]. The resulting continuous activity of the HDAC-complex at the promoters of target genes of the PLZF/RARα fusion protein leads to a repression of these genes and is the determinant of resistance to retinoic acid (RA) treatment [83]. Co-treatment with HDACIs relieves this transcriptional repression and leads to differentiation of myeloid cells *in vitro* and induced a clinical response in an RA-resistant APL patient [203]. Oligomerization and altered recruitment of HDACs are also responsible for malignant transformation by the non-APL Acute Myeloid Leukemia (AML) fusion protein AML1-ETO [127,202], which blocks RA signaling too [134], suggesting that interference with the RA pathway by HDAC recruitment may be a common theme in AMLs [134]. Thus, as malignant cells express

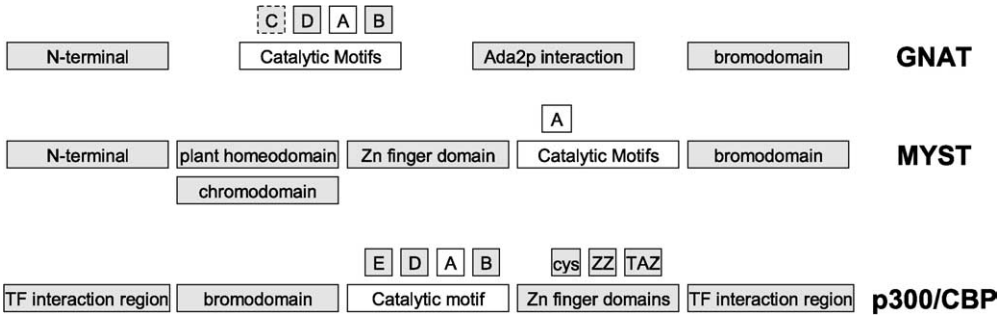


Fig. 3. Functional domains of HATs. *For the function of the different domains see text (source:* [177]).

increased HDAC activity, irrespective of the trigger of such activity, it is thought that inhibitors of HDACs return this transient aberrant transcription in malignant cells to the transcription status of their normal cell counterpart, while leaving the surrounding normal cells unaltered. With this premise, HDAC inhibitors (HDACIs) were investigated for activity and found to be relatively non-toxic to normal cells *in vitro* and *in vivo* despite the accumulation of acetylation in normal cells as well as tumor cells [96]. Successful preclinical studies and development of animal models has resulted in a number of HDACIs being evaluated in human clinical trials (see below).

4.2. HATs

Although hyperacetylation has been mainly associated with transcriptional activation, the effects of acetylation depend on the setting and the genes involved. As is shown for instance by Guidez et al. [86] acetylation of lysine residues can also lead to transcriptional repression. Acetylation of internal lysine residues is facilitated by the action of HATs. By definition HATs acetylate histone tails by transferring acetyl groups from acetyl coenzyme A (acetyl-CoA) onto the ε-amino group of conserved lysine residues. However, HATs are also able to acetylate non-histone proteins and are therefore, sometimes referred to as FATs (factor acetyltransferases). Post-translational acetylation of lysine residues occurs in histones, high mobility group (HMG) proteins, transcription factors, nuclear receptors and α-tubulin [159]. Important in the context of cancer is that post-translational acetylation of the ε-amino group of lysine residues is known to be reversible, making it an attractive therapeutic target. HATs are composed of several domains making them substrate- and site-specific under distinct physiological situations. Three super families of HATs can be identified based on their composition: GNAT (Gcn5-related N-acetyl transferases) [142], MYST (named after its founding members MOZ, Ybf2/Sas3, Sas2 and Tip60) [20] and p300/CBP (CREB-binding protein) [8,147].

4.2.1. GNAT super family

The GNAT super family acetylates a distinct subset of genes. All GNAT enzymes contain four functional domains (Fig. 3): an amino terminal, a catalytic motif domain, Ada2p interaction domain and a carboxy-terminal bromodomain. The amino-terminal is variable in length and is thought to facilitate recognition of nucleosomal substrates [207]. The catalytic motif domain actually contains up to four conserved motifs labeled A–D of which motif A, the highly conserved acetyl-CoA binding site, is common to HATs from all super families and essential for HAT activity [35] (Fig. 3). The Ada2p interaction domain enables GNATs to acetylate physiologically relevant nucleosomal substrates *in vivo*. Ada2 proteins interact with DNA-bound transcriptional activators [35]. The C-terminal bromodomain binds to acetylated lysine residues facilitating protein–protein interactions. Secondly, the bromodomain is able to affect HAT activity by autoacetylation causing the HAT to fold into an inactive state [182].

4.2.2. MYST super family

Besides an amino terminal tail, an acetyltransferase domain A and a C-terminal domain, MYST super family HATs sometimes contain zinc-finger domains and a chromodomain. The function of the chromodomain in the context of HATs is unclear. Likely, chromodomains serve as chromatin targeting modules for the MYST family similar to the bromodomains of the GNAT family (Fig. 3).

4.2.3. p300/CBP super family

Contrary to the GNAT super family, the p300/CBP super family generally acts as a global transcriptional

activator. p300 and CBP are two of the most widely studied HATs in the context of transcriptional regulation. They are largely interchangeable in function and are therefore, often referred to as p300/CBP. As co-activators for transcription, p300/CBP is particularly recruited to promoters by DNA-bound transcription factors that need p300/CBP to function in transcriptional activation. p300/CBP contain at least two independent regions for interaction with multiple transcription factors such as c-Jun [9] and nuclear hormone receptors [101]. Furthermore p300/CBP contains a bromodomain, three Zinc finger regions (cys, ZZ and TAZ domains) and a HAT domain containing a p300/CBP specific E motif (Fig. 3). The distribution of p300/CBP among the different target proteins might provide a mechanism for integrating several signaling and transcription response pathways since p300/CBP is present in limited amounts in the cell [101]. Research on HATs to date is basic and preclinical with limited, if any, clinical candidates. We preferred to include HATs because of their potential as therapeutic targets. A more detailed review on histone acetyltransferases is provided by Roth et al. [177].

5. Histone deacetylase inhibitors

The important role of acetylation and deacetylation in transcriptional regulation makes HATs and HDACs a promising target for anticancer therapies. Although the field of epigenetics is in its infancy, discoveries and translation to the clinic have moved at a remarkable speed. Drugs targeting epigenetic changes are in phase I trials and are moving to phase II trials for several types of histone deacetylase inhibitors (HDACIs). DNA methyl transferase inhibitors were recently approved by the US FDA for their clinical benefit in patients with myelodysplasia.

The microbial toxin Trichostatin A (TSA) was an early compound that was identified to possess HDAC inhibitory activity. After establishing its efficacy as an HDACI, TSA was used to model other HDACI such as vorinostat (also known as suberoylanilide hydroxamic acid, SAHA) and m-carboxycinnamic acid bishydroxamide (CBHA; Memorial Sloan-Kettering Cancer Center) [170], which in turn served as a template for PXD-101 (Prolifix Ltd/CuraGen Corp) and LAQ-824 (Novartis AG). Another natural compound to be discovered as a non-competitive inhibitor of HDAC was sodium butyrate (SB) [36]. Like other HDACIs of the same structural type, the clinical relevance of

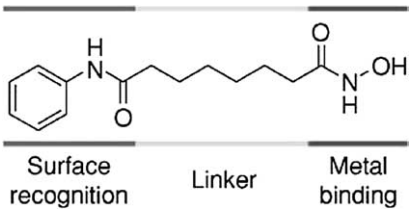


Fig. 4. Structural features of hydroxamic acid-based HDACIs. *Reprinted from Advances in Cancer Research, 91, PA Marks, VM Richon, T Miller, WK Kelly, Histone Deacetylase Inhibitors, 137–168, Copyright 2004, with permission from Elsevier.*

SB was limited by its short half-life and low potency. However, several improved derivatives of SB were later characterized and screened, including the drugs phenyl butyrate, phenyl acetate (which is the β-oxidation product of phenyl butyrate *in vivo*), valproic acid (VPA), and AN-9 (pivaloyloxymethyl butyrate, Titan Pharmaceuticals Inc), the prodrug form of butanoic acid [146].

The rate of development of newer HDACIs has greatly increased following the elucidation of the HDACI–HDAC interaction [67]. The ability of HDACIs to chelate zinc bound to the active site of the HDAC appeared to be crucial to maximum inhibitory activity, as exemplified by the general structure illustrated in Fig. 4, which is based on vorinostat. This explained why HDACIs such as the aliphatic acids, which lack a zinc binding moiety, required higher concentrations (mM) of drug for activity, compared with the nanomolar quantities of drugs of the hydroxamic acid type. As a result of this research, a novel class of HDACIs with a Zn^{2+}-chelating motif-tethered to short-chain fatty acids has been synthesized [125]. Cyclic peptides, such as FK-228 (National Cancer Institute/Gloucester Pharmaceuticals Inc), which do not possess the characteristic structure outlined above, are also active at HDAC inhibition at nanomolar concentrations, and research conducted by Furumai et al. helped to decipher the mechanism of action of such compounds [73]. Cellular reducing activity converts FK-228 to its active, reduced form (redFK), which possesses a functional sulfhydryl group capable of interacting with the zinc in the active-site pocket of HDACs. Because FK-228 is more stable than redFK in medium and serum, FK-228 may be the natural prodrug which is activated after incorporation in to the cells. More recent developments of HDACIs belong to the class of electrophilic ketones and include various trifluoromethyl ketones and α-ketoamides which are active at micromolar concentrations [71,195]. Special attention should also be given to the emergence of

HDAC-specific HDACIs such as Tubacin, an HDAC6-specific HDACI [88] and MGCD0103 an HDAC1-3 specific HDACI [137].

5.1. Mechanisms of action

By changing the acetylation status of histone tails HDACIs modify the histone code leading to changes in gene expression [187] (see above). Furthermore, by accumulating acetylated histones, HDACIs relax chromatin structure by virtue of their ionic charges thereby enhancing the accessibility of the transcription machinery to DNA leading to increased transcription [205]. The accumulation of acetylated histones may also affect cell cycle progression. By inhibition of chromatin separation, the ability of tumor cells to undergo mitosis is altered [50]. Additionally, HDACIs specifically affect the cell cycle of tumor cells by targeting cells with deficient cell cycle checkpoint controls causing apoptosis [204]. Generation of reactive oxygen species also plays a role in HDACI-induced cell death. In a study published in 2005, Ruefli et al. showed that vorinostat-induced Bid cleavage and disruption of the mitochondrial membrane lead to cell death in the absence of activation of key caspases [179]. Recent work by Xu et al., suggests that anti-tumor effects of vorinostat in treated cancer cells is achieved in part by induction of polyploidy followed by senescence [208].

6. The cellular and biological effects of HDAC inhibitors

HDACIs demonstrate pleiotropic effects on cells in culture and *in vivo*. Their effect is dependent on several factors including cell type. Recently reported frameshift mutations in HDAC2 in colon, gastric and endometrial tumors have shown to diminish the effect of treatment with some, but not all, HDACIs using colorectal cell lines *in vivo* [172]. Other factors that are important for the response to HDACI include dose and duration of treatment and whether HDACIs are given in combination with other agents.

6.1. Activity in vitro of HDACIs

TSA was evaluated for its biological activity in a variety of mammalian cell lines and was demonstrated to prolong the half-life of acetylated histones in mouse mammary gland tumor cells [211]. Pulse-chase experiments revealed that histone hyperacetylation induced by TSA was not due to increased acetylation but was a result of decreased deacetylation of histones. Many mammalian cell lines have since been investigated for their sensitivity to various HDACIs. Examples include differentiation by TSA in breast cancer cell lines [198], apoptosis in endometrial cancer cell lines [188] and hepatoma cells [45], and apoptosis of neuroblastoma cell lines by CBHA either administered alone or in combination with retinoic acid [52]. Without testing its ability to act as an HDAC inhibitor, Carducci et al., demonstrated the pro-apoptotic effect of PB on prostate cancer cell lines [40]. Later, Butler et al., demonstrated the anti-proliferative effect of vorinostat on prostate cancer cell lines and simultaneously demonstrated the HDAC inhibitory activity by detecting presence of acetylated histones [28].

6.1.1. Cell cycle effects

HDACIs arrest growth in different phases of the cell cycle in different cell lines [163]. Most HDACIs, with the notable exception of tubacin, exert a dose-dependent effect on growth arrest in G_1 [88]. The arrest is mediated by the p53-independent induction of $p21^{CIP1/WAF1}$ [169,199], loss of activity of cyclin-dependent kinase (Cdk) [181] and transcriptional inactivation of the enzymes CTP synthase and thymidylate synthetase, which are responsible for DNA synthesis in the S phase [220]. G_2 arrest has been detected in comparatively fewer cell lines and requires a higher dose of HDACIs compared with G_1 arrest [27,181]. However, higher doses of HDACIs also induce apoptosis of cancer cells (as discussed below). Research by Qiu et al. and Warrener et al. has emphasized this dilemma by highlighting the role of checkpoints in cell cycle progression [163,204]. Cells exhibiting an intact G_2 checkpoint were growth arrested on treatment with HDACIs while cells possessing a defective G_2 checkpoint undergo apoptosis within hours of mitotic exit [204] (Table 2).

6.1.2. Induction of apoptosis

Treatment of cells with HDACIs can induce or repress genes to cause cell cycle arrest in different cancer cell types. HDACIs can also increase expression of pro-apoptotic genes such as Bak, Bax, CD95, CD95 ligand, gelsolin, p53, GADD45 β, DRAK1, Apaf-1, DFF45 α, caspase-9, caspase-8, caspase-3, Bim, Bid and Bad, and decrease expression of anti-apoptotic genes including Bcl-2, Bcl-x_L, c-FLIP, survivin, XIAP, Mcl-1 and NFκB [3,44,62,91,92,98,135,217]. Apoptosis, mediated by varied pathways, was induced in many cell lines treated with HDACIs as a function

Table 2
Effects of HDAC inhibitors

Effects	Process	Pathway	References
Cell cycle arrest	G1-arrest	p53-independent p21 induction	[169,199]
		Loss of Cdk activity	[181]
Apoptosis	Defective G2/M checkpoint	Loss of the G2/M checkpoint results in catastrophic mitosis	[163,204]
	Mitochondrial injury	Induction of expression of pro-apoptotic genes	[199]
	p21-deficiency	Induction of p21 inhibits apoptosis by inducing G1 cell cycle arrest. Conversely p21-deficient cell lines do not arrest cells in G1. p21-deficiency can therefore cause catastrophic mitosis in cells with a defective G2/M checkpoint.	[27]
	Generation of ROS	Activation of caspase cascade	[174,179]

HDAC – histone deacetylase, Cdk – cyclin-dependent kinase, ROS – reactive oxygen species.

of dose and duration of incubation. In U937 human leukemia cells, vorinostat induced differentiation at low doses (sub-micromolar) but at higher doses the drug caused apoptosis by triggering mitochondrial injury [199]. The apoptotic effect of HDACIs is also cell line-specific, as demonstrated by the analysis of a large panel of HDACI-sensitive cell lines, in which selected cell lines required up to 10-fold lower concentrations of HDACIs in order to achieve apoptosis [27]. Cancer cells were revealed to be 10-fold more sensitive to hydroxamates than normal fibroblasts, possibly due to the loss of the G_2 checkpoint, a feature exploited by HDACIs to selectively kill proliferating and non-proliferating tumor cells but not normal cells [26] (Table 2).

Recently, apoptosis has been reported to be induced by generation of reactive oxygen species (ROS) by various HDACIs (reviewed in reference [164]). ROS production leads to activation of the caspase cascade and degradation of critical proteins such as $p21^{CIP1/WAF1}$, $p27^{KIP}$, Bcl-2 and pRb. Proof of this was established by administration of the free radical scavenger L-N-acetylcysteine, which blocked MS-275-mediated mitochondrial injury and apoptosis [174]. In acute T-cell leukemic cell lines, vorinostat induced a cell-death pathway acting via cleavage of Bid and production of ROS [179]. Conversely, SB, which induces apoptosis independently of ROS generation or activation of the extrinsic pathway, prompted degradation of anti-apoptotic proteins Bcl-2 and $p21^{CIP1/WAF1}$ in human leukemia cell lines [176]. Induction of $p21^{CIP1/WAF1}$ was also observed in breast cancer cell lines co-treated with SB and tumor necrosis factor (TNF)-α, TNF-related apoptosis inducing ligand (TRAIL) or anti-FAS agonist antibody [49] (Table 2).

6.1.3. Gene expression affected by HDAC inhibition

Gene expression profiles of T24 bladder and MDA breast carcinoma cells treated with TSA or other HDACIs were studied to define a common set of genes that are induced or repressed by HDAC inhibition [78]. Examples of those genes induced and repressed are provided in Table 3.

6.1.4. Acetylation of non-histone proteins

Phylogenetic studies have proven that the evolution of HDACs predates the evolution of histones, indicating that there are non-histone substrates of HDACs [82]. p53 represents one example of a non-histone protein that is maintained in the acetylated state by HDACIs [192,216]. Other examples include the transcriptional repressor Bcl-6 [14], the 90-kDa heat shock protein (Hsp90) [215] and tubulin [88]. Hyperacetylation of Ku70 protein on treatment with HDACIs results in release of Bax, which may provide one of the underlying mechanisms behind apoptosis [53].

HDACIs demonstrate pleiotropic effects by altering transcriptional status and preventing/inhibiting proliferation of tumor cells. Studies have exhibited synergism in gene re-expression and anti-proliferative effects when HDACIs are combined with other agents. The pleiotropy arises from the variety of histone deacetylase substrates, many of which are still unknown. The name 'histone' deacetylase inhibitors may be misleading, as many 'non-histone' substrates have been identified for HDACs.

6.2. Activity in vivo

The antitumor activity of HDACIs was demonstrated in several *in vivo* models of cancer including human xenografts. Significant reduction in tumor volume of breast cancer xenografts and lung metastasis was observed in animals treated with valproic acid [80]. MS-275, pyroxamide (Aton Phama/National Cancer Institute (NCI)) and vorinostat have also exhibited antitumor activity in many cancer cell lines *in*

Table 3
Genes induced or repressed by HDACIs

Induced genes	Repressed genes
$p21^{CIP1/WAF1}$ [169], and $p27^{KIP1}$ [94]	Thymidylate synthetase [78]
Retinoic acid receptor β (RAR β) [156]	CTP synthetase [78]
Estrogen receptor [209]	Genes controlled by signal transducer and activator of transcription-5 (STAT5) [167]
TRAIL cell surface death receptors: TRAIL-R1/DR4 and TRAIL-R2/DR5 [217]	Bcr-Abl fusion gene [144,145]
Semaphorin III, a vascular endothelial cell growth factor (VEGF) competitor [60]	VEGF receptors VEGFR-1 and VEGFR-2, and neuropilin-1 [60,162]
α-Fucosidase [78]	Inactivation of the heat shock protein-90 (Hsp90) molecular chaperone leading to mutant and wild-type androgen receptor depletion. As well as other Hsp90 client proteins HER-2 (ErbB2), Akt/PKB, and Raf-1 [43]
Histone H2B [78]	Lipopolysaccharide (LPS) induced inflammatory cytokines TNF-α, interleukin (IL)-1β, IL-6 and interferon (IFN)-γ [124]
α-Tubulin [78]	Androgen receptor (AR) and prostate-specific antigen (PSA) [43]
Glutaredoxin [78]	
Metallothionein 1L [78]	

vivo [28,54]. In addition to their role in direct growth arrest, death and differentiation of tumor cells *in vivo*, HDACIs act as chemopreventive agents to inhibit tumor growth by preventing neovascularization of the tumor and thus exerting an anti-angiogenesis function. TSA, vorinostat, FK-228 and LAQ-824 have all demonstrated an anti-angiogenic effect *in vivo* [60,120, 162], and recently, the chemopreventive sulforaphane has also indicated HDACI activity [141].

6.2.1. Histone acetylation

With the exception of the small molecule tubacin, all known HDACIs maintain histones in the acetylated state [88]. For example, hyperacetylated histone H4 was detected in peripheral blood mononuclear cells (PBMCs) at 1 h and 2 h after a single injection of PXD-101 in tumor-bearing mice [158]. In another example, xenografts from mice treated with pyroxamide displayed increased levels of histone acetylation and increased expression of the cell cycle regulator $p21^{CIP1/WAF1}$ compared with tumors from vehicle-treated control animals [29].

7. SIRT inhibitors

The above discussed HDAC inhibitors affect the activity of class I and II HDACs. Translational research on these compounds has resulted in phase 1 and 2 clinical trials testing its efficacy in cancer patients. Although it is known that class III HDACs such as Silent Information Regulator (SIRT) 1 have cancer relevance, SIRT1 regulates gene silencing [85], p53 function [122,196] and plays a critical role in stress signaling [25], SIRT has been primarily appreciated for its role in the biology of aging. A few recent findings might shift the focus a little more in the direction of neoplasms. Ford et al. [69] reported in 2005 that inhibition of SIRT1 by siRNA in cancer cells causes growth arrest and/or apoptosis in absence of stress. Non-cancerous cells are shown to be refractory to SIRT1 silencing and the effect of SIRT1 inhibition is therefore highly cancer-specific. A paper published in March 2006 by Pruitt et al. [161] showed furthermore that pharmacologic, dominant negative and siRNA-mediated inhibition of SIRT1 in colon and breast cancer cell lines reactivates epigenetically silenced tumor suppressor genes (TSG) such as mismatch-repair gene MLH1 and cell-cell adhesion associated protein E-cadherin. Previously described synergistic reactivation of epigenetically silenced TSG using HDACIs have been shown to depend on pre-treatment with methyltransferase inhibitors to (partially) demethylate the promoter regions [32]. Surprisingly, the process of reactivation using SIRT-inhibitors takes place without the loss of promoter DNA hypermethylation. The recent findings suggest new directions for targeting reversal of gene silencing by epigenetic pathways and possible therapeutic approaches.

8. HAT regulation: The other side of acetylation

Over the past years substantial progress has been made in the field of HDACIs, but less research has

been done on the young field of HATIs. Several papers however, have established a direct relationship between HAT activity and the development or progression of disease [154]. As mentioned earlier, since CBP and p300 are available in limited concentrations in the cell, competition for them between different transcription factors can facilitate integration of several signaling and transcription response pathways.

8.1. Gain of p300/CBP and HAT inhibitors

As a result of fusion to other proteins HATs can become oncogenic. These gain-of-function mutations presumably increase proliferation by inappropriately enhancing activation of certain transcription pathways. MOZ (monocytic leukemia zinc finger), a putative HAT, has been described in acute myeloid leukemia (AML), to be fused with at least two different gene products: CBP (CREB binding protein) [20] and TIF2, a nuclear receptor coactivator [37], causing gain-of-function. Furthermore, the fusion of MLL (a homeotic regulator, mixed lineage leukemia) with CBP in patients with therapy-related AML, myelodysplastic syndrome and CML and the fusion of MLL with p300 in patients with AML, suggests an important (oncogenic) role for these gain of function mutations. Initially HAT inhibitors were synthesized as mechanistic tool for research focused on the identification of functional effects of protein acetylation in specific pathways [219].

Lys–CoA, a conjugate of the amino acid lysine and coenzyme A, specifically blocks the HAT activity of p300. Although Lys–CoA has been intensively used for transcription studies during *in vitro* and *in vivo* studies, the use of microinjection or cell permeabilizing agents is necessary since Lys–CoA is not easily taken up by cells in cell culture conditions. The same holds true for H3-CoA-20, a p300/CREB binding protein-associated factor (PCAF)-specific inhibitor of the same class [123], and anacardic acid (AA) the first naturally occurring HAT inhibitor. AA was isolated from cashew nut shell liquid which inhibits HAT activity from both p300 and PCAF very effectively [6]. Their inability to penetrate the cell membrane makes them unsuitable for future use in animal models and humans. Recognizing the importance of HAT inhibitors in a clinical setting the first cell permeable HAT inhibitor garcinol was reported by Balasubramanyam et al. in 2004. Garcinol is a polyisoprenylated benzophenone derived from *Garcinia indica* fruit rind and shows to be a potent inhibitor of both p300 and PCAF HATs *in vitro* and *in vivo*. Treatment of HeLa cells with garcinol was shown to inhibit activated histone acetylation, induce apoptosis and down-regulate gene expression of proto-oncogenes [5]. However, the effect of garcinol in normal (untransformed) cells and other cancer types remains to be elucidated.

8.2. Loss of p300/CBP and HAT activators

In addition to functioning as oncogenes p300 as well as CBP have been shown to be potent tumor suppressor genes. Mice heterozygous for loss of CBP have been shown to develop tumors. Consistent with these data Rubenstein–Taybi syndrome patients lacking one functional allele of CBP show a predisposition to cancer [131]. Interestingly mice heterozygous for loss of p300 have not been shown to develop tumors. Despite this lack of direct evidence for p300 acting as a tumor suppressor gene, heterozygosity studies show p300 involvement in a number of different cancer types in humans. Analysis of colorectal, gastric, and epithelial cancer samples for instance show missense mutations as well as deletion mutations in the p300 gene [140] and it has been found that colorectal tumors as well as 80% of glioblastoma is associated with a loss of heterozygosity of the p300 gene. Gayther et al. identified in 10/193 tumor samples and cancer cell lines (breast, colorectal, ovarian, lung, pancreatic cancer and glioma) truncation mutations, insertions and missense mutations of p300 with or without inactivation or deletion of the second allele. Although their study indicates that p300 mutations are relatively rare, they do support the idea that loss of p300 activity contributes to tumor development since the nature of the p300 mutations suggests that most of the mutations would clearly lead to a loss of function [75]. In that perspective, it would be an interesting idea to use a HAT activator to study its effect on p300 function. Little has been published however on HAT activators, especially in the context of cancer. By using HAT inhibitor AA, Balasubramanyam et al. synthesized the first small molecule HAT activator called CTPB (N-(4-chloro-3-trifluoromethylphenyl)-2-ethoxy-6-pentadecyl-benzamide). Just like AA however, cells are poorly permeable to CTPB [5]. It remains to be seen in *in vitro* and *in vivo* studies whether HAT activators can serve as potential anticancer agents. A review on the implications of small molecule activators and inhibitors of HATs in chromatin therapy has been written in 2004 by Varier et al. [193].

9. HDACIs in clinical trials

Over the last five years more than 20 HDACIs have been investigated in cancer clinical trials, either alone or in combination with other agents. A brief summary of some selected clinical trials is presented in Table 4. This includes the early clinical trials which paved the way for more HDACIs to be evaluated, as well as the most recent trials showing promise.

9.1. 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 HDACIs that have entered clinical testing. Short chain fatty acids such as phenylbutyrate show a dose-limiting toxicity (DLT) of somnolence and confusion when administered using prolonged intravenous infusion. This neurotoxicity has not been reported for the benzamide or hydroxamate HDACIs or for the carboxylate prodrug AN-9. 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 non-small 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 HDACI. Unfortunately, depsipeptide has been reported to be associated with a significant incidence of cardiac dysrhythmias and nonspecific electrocardiogram (EKG) abnormalities. In some patients, the hydroxamates, LAQ824 and LBH-589 too have demonstrated some EKG changes. Fatigue was commonly observed with vorinostat treatment but was not dose-limiting and was similar to that previously reported for depsipeptide. Importantly, many patients with solid cancers showed some degree of clinical improvement.

9.2. Pharmacokinetics and pharmacodynamics

Owing to the reversible nature of epigenetic modifications, and assuming these to be the key determinants of tumor growth, inhibition of intracellular HDAC activity or demethylation commonly will require continuous 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 HDACI bioavailability and antitumor activity. Most HDACIs are rapidly metabolized in rodents and dogs. With a few exceptions, for example, LBH-589A (Novartis AG; $t_{1/2} = 15$ to 20 h) [11] and MS-275 ($t_{1/2} = 100$ h) [79,180], reported half-lives for HDACIs are a maximum of 1 h in humans. This short half-life poses a significant limitation to the design of both *in vivo* studies and clinical trials with HDACIs. In particular, butyrate and phenylbutyrate degraded rapidly after intravenous administration, requiring doses $\geqslant$400 mg/kg/day to be administered as a continuous i.v. infusion for 120 h (repeated every 21 days) in certain clinical trials [39,203].

Therefore, most phase I trials continue to focus on the pharmacokinetics of different HDACIs. In case of drugs with a shorter half-life, continuous dosing may be required. However, continuous dosing is not always desirable. Therefore, drugs available as oral formulations like valproic acid and vorinostat, are more potentially attractive candidates. In the case of valproic acid, the already well-investigated neuropsychiatric drug, indicated availability of a pharmacokinetic profile with a $T_{1/2}$ of $\sim$14 h [194] and at therapeutically tolerated doses, effective plasma concentrations are in the achievable 0.5–0.75 mM range. The drug is conveniently bioavailable in an oral formulation. Trials on prostate cancer and other malignancies with this drug have been guided by this pre-existing knowledge.

9.3. Biomarkers of evaluation

Because of the differentiating properties of epigenetic modifiers, the conventional marker of evaluation, PSA, is often upregulated even when tumor burden is reduced [39]. Hence, PSA cannot be used as a marker of disease prognosis when using differentiating agents. Till date, acetylation of histones in PBMCs is relied upon as a marker of exposure to HDACI, in both blood and solid cancers. As markers of effect in blood cancers, acetylation of the p21 gene and upregulation of the protein is a vital endpoint of drug activity, but not of antitumor response. In order to avoid biopsies, and in the absence of validated biomarkers of effect in surrogate tissue, the inability to evaluate acetylated histones in solid tumors, has led to difficulty in trial design. However, many novel designs of evaluating patient and tumor response to HDACI are currently underway. These designs include treating patient populations

Table 4
Clinical trials with HDACIs

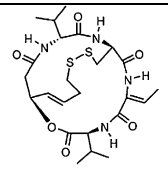
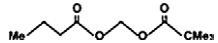
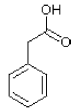
Class	HDACI	Structure	Cancer (trial phase)	Schedule	Outcome	Adverse events	Reference
Cyclic peptides	Depsipeptide		hematological malignancies (Phase I)	13 mg/m^2 i.v. on days 1, 8, and 15 every 4 weeks	CR, PR: none Evidence of antitumor activity and histone acetylation increases of at least 100%. Increase in p21 promoter H4 acetylation, p21 protein and 1D10 antigen expression.	Progressive fatigue, nausea, and other constitutional symptoms prevented repeated dosing. Neither life-threatening toxicities nor cardiac toxicities were noted.	[30]
			CTCL + PTCL (Phase II)		CR: 21% (3/14 CTCL) PR: 29% (4/14 CTCL) PR: 24% (4/17 PTCL)	Fatigue, N/V, granulocytopenia, hypocalcemia, neutropenia, thrombocytopenia. Cardiac: non-specific ST-T wave changes, but no change in cardiac function.	[155]
Butyric acid	AN-9		NSCLC (Phase II)	2.34 g/m^2/day i.v. in 6 h infusion on 3 consecutive days every 3 weeks	PR: 6.43% (3/47) SD $>$ 12 weeks: 30% (14/47)	Fatigue, nausea, dysgeusia, dyspnea (G1-2: 9%, G4: 4%), chest pain (G1-2: 6%, G3-4: 4%)	[105,168]
Aliphatic acid	Phenylacetate		solid tumors (Phase I)	Single i.v. bolus followed by 14-day continuous i.v. infusion (maintaining blood concentration at 200–300 μg/ml)	SD $>$ 9 months: 17% (1/6 glioblastoma) SD $>$ 2 months: 33% (3/9 HRPC) Nonlinear pharmacokinetics, induction of drug clearance	Confusion, lethargy, emesis DLT: reversible CNS depression	[189]
			malignant glioblastoma (Phase II)	400 mg/kg/d continuous i.v. infusion 2 weeks/4 weeks	CR: none PR: 7.5% (3/40) SD: 17.5% (7/40) $>$50% reduction of tumor: 7.5% (3/40) treatment failure $<$ 2 months: 75% (30/40)	Infection, malaise, fatigue, lethargy, reversible disorientation, somnolence, weakness, N/V, edema, granulocytopenia.	[42]

Table 4
(Continued)

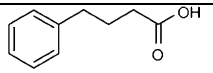
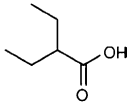
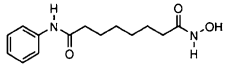
Class	HDACI	Structure	Cancer (trial phase)	Schedule	Outcome	Adverse events	Reference
	Phenyl butyrate		solid tumors (Phase I)	150–515 mg/kg/day as 120 h i.v. infusion every 21 days	CR: none SD: 8% (2/24, 19 PCa) Significant bone pain reduction in HRPC Recommended phase II dose: 410 mg/kg/day for 120 h	DLT: neurocortical (excessive somnolence, confusion), hypokalemia, hyponatremia, hyperuricemia (2 patients, 515 and 345 mg/kg/day) MTD: 410 mg/kg/day for 5 days Other toxicities: fatigue, nausea	[39]
			solid tumors (Phase I)	9–45 g/day p.o. in 3 doses/day	PR, CR: none SD $>$ 6 months: 25% (7/28, 12 PCa) Recommended phase II dose: 27 g/day.	dyspepsia (G1-2), edema (G1-4), fatigue, neurocortical toxicity, N/V DLT: N/V, hypocalcemia at 36 g/day (2/7) MTD: 27 g/day	[77]
	Valproic acid		advanced cancer (Phase I)	30–120 mg/kg/day as 2 $\times$ 1!h i.v. infusion for 5 days every 2 weeks	PBC: hyperacetylation observed in majority of patients Recommended phase II dose: 60 mg/kg	neurological toxicity (G3-4, 9/26, dose 75-, 90- and 120-mg/kg) no hemalogical toxicity $>$G3-4 MTD	[4,153]
Hydroxamate	vorinostat		solid tumor + hematological malignancies (Phase I)	200 mg qd, 400 mg qd, 600 mg qd, or 400 mg bid per day p.o.	CR: 1/73 PR: 4% (3/73 + 2 unconfirmed) linear pharmacokinetics from 200 to 600 mg, Mean $T_{1/2}$: 91–127 min dose-dependent accumulation of acetylated histones from 200–600 mg	MTD: 400 mg qd and 200 mg bid for continuous daily dosing and 300 mg bid for 3 consecutive days per week dosing. DLT: anorexia, dehydration, diarrhea, and fatigue	[107]

Table 4
(Continued)

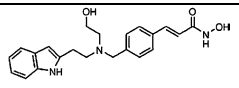
Class	HDACI	Structure	Cancer (trial phase)	Schedule	Outcome	Adverse events	Reference
			Solid tumors + hematological malignancies + refractory lymphomas (Phase I)	75–900 mg/m^2/day in 2 h i.v. infusion 3–5 days/week for 1–3 weeks	Objective tumor regression with clinical improvement in tumor related symptoms: 4 patients Mean $T_{1/2}$: 21–58 min	Fatigue (G1-3), anorexia (G1-2), vomiting (G1-2), diarrhea (G1-3), constipation (G1-4), hypokalemia, non-specific EKG changes, increased creatinine, dysgeusia MTD: 300 mg/m^2/day × 5 days for 3 weeks (2/5 hematological), 900 mg/m^2/day × 5 days for 3 weeks (1/6) DLT: leucopenia (G3-4), thrombocytopenia (G3-4), acute respiratory distress, hypotension (G3)	[108]
			advanced CTCL (Phase II)	400 mg daily until disease progression or intolerable toxicity	Objective Response Rate was 29.5% (18 PR including 1 with later CR)	AE $\geqslant$ Grade 3 included fatigue (5%), pulmonary embolism (5%), nausea (4%) and thrombocytopenia (4%). Twenty-five pts discontinued due to progressive disease. Causes of the 3 deaths on study were: unknown (d 2), ischemic stroke (d 227) and disease progression (d 52)	[150]
	LAQ-824		advanced solid tumors (Phase I)	Dose-escalating i.v. infusion on days 1–3 for 21 days	Increase and maintenance of acetylation for 24 h, inhibition of Hsp90 activity with increased expression of H sp70 and decreased downstream target c-Raf	Not reported	[118]

Table 4
(Continued)

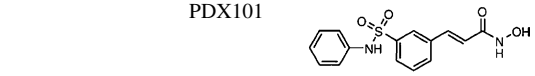
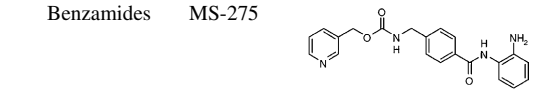
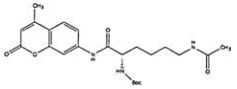
Class	HDACI	Structure	Cancer (trial phase)	Schedule	Outcome	Adverse events	Reference
			advanced solid tumors + hematological malignancies (Phase I)	6–200 mg/m^2/day as 3 h i.v. infusion on days 1–3 for 21 days	PK analysis showed dose proportionality, $T_{1/2}$: 6–26 h PBL: Sustained histone acetylation 24 h post-dosing of $\geqslant$ 36 mg/m^2/day	QTcF day 1: no change QTcF day 3: dose-related increases of $<$20 msec QTcF $>$ 60 msec: 10% (8/77) at 36–200 mg/m^2. QTc $>$ 500 msec: 1/77 (200 mg/m^2) Frequent non-specific T wave flattening Small increase in troponin without increase in CK MB (2/77)	[178]
	PDX101		advanced solid tumors (Phase I)	150, 300, 600, 900 and 1200 mg/m^2 as 30 min. i.v. infusion 5 days every 3 weeks Ongoing Schedule: 1000 mg/m^2	SD $>$ 2 cycles: 7/21 linear kinetics displayed $T_{1/2}$: 0.5–1 h PMN: Dose dependent histone H4 hyperacetylation, increases in p21, p19, and Apaf-1 expression	DLT: 14% (3/21, all 1200 mg/m^2), fatigue (G3), reversible atrial fibrillation (G3), diarrhea (G3) and lethargy. All other AE were $\leqslant$ G2. No hematological toxicity was observed	[186]
	LBH-589	not published	Solid tumor (Phase I)	1.2–7.2 mg/m^2/day as 30 min. i.v. infusion day 1–3 and 8–10 every 3 weeks (arm 1) or day 1–3 and 15–17 every 4 weeks (arm 2)	SD: 46% (6/13) Rapid onset (1 h) of prolonged acetylation (up to 7 days) in some patients. $T_{1/2}$: 15 to 20 h.	Neutropenia (G3, 1/13), hypoglycemia (G3, 1/13), thrombocytopenia (G2, 2/13), anemia (G2, 2/13) DLT: prolonged thrombocytopenia (G2, 7.2 mg/m^2/day in arm 1)	[11]
Benzamides	MS-275		solid tumors + lymphomas (Phase I)	$>$2 mg/m^2/day (dose escalation) p.o. 4/10 weeks or 4/6 weeks	$T_{1/2}$: 39–80 h Linear PK suggested PBMC: increased histone H3 acetylation was apparent at all dose levels	MTD: 10 mg/m^2 DLT: N/V, anorexia, and fatigue	[25]

Table 4
(Continued)

Class	HDACI	Structure	Cancer (trial phase)	Schedule	Outcome	Adverse events	Reference
			solid tumors + lymphomas (Phase I)	2–6 mg/m^2/2 weeks p.o. or 2 mg/m^2 p.o. twice weekly for 2/3 weeks or 4 mg/m^2/week p.o. for 3 weeks	$T_{1/2}$: 100 h T_{max}: 0.5–2.0 h PBMC: increased histone H3 acetylation. HDACI observed. PR: 6% (1/17) SD: 17% (3/17)	Hypophosphatemia, asthenia, nausea, anorexia. MS-275 p.o. on the daily schedule was intolerable at a dose and schedule explored. AE: hypophosphatemia, asthenia, nausea and anorexia (all G1–3)	[79,180]
			Metastatic melanoma (Phase II)	3 mg on day 1 + 15 or 7 mg on day 1 + 8 + 15 of a 4 week cycle	SD for 8–48 weeks: 29% of 3 mg group and 21% of 7 mg group Stabilization of certain metastatic lesions No tumor reponse was noted	Nausea (32%, G1–G2), hypophosphatemia (29%, G1–G3), diarrhea (18%, G1–G2)	[90]
	MGCD0103		advanced solid tumors (Phase I)	12.5, 20, 27, 36 and 45 mg/m^2/day 3 days/week, 2 out of 3 weeks	SD (>2 cycles) seen in renal cell cancer (2 patients, 4 and 6 cycles) and colorectal cancer (1 patient, 4 cycles) Dose-dependent $T_{1/2}$: 7.7–11.3 h (±0.6–1.6) Maximal HDAC inhibition in patients co-administered an acidic beverage which reduces previous PK variability.	AEs: grade 1–3 fatigue (91%), grade 1–2 nausea (70%) and vomiting (48%), anorexia (26%), constipation (39%) and dehydration (14%). MTD has not yet been reached	[38,184]
			advanced solid tumors + NHL (Phase I)	12.5–27 mg/m^2/day p.o.	SD > 6 cycles: 1/27 to date (thymic carcinoma) HDACI correlates positively with histone acetylation in PBC lasting >24 h post-dosing. $T_{1/2}$: 8.8 h	Fatigue (20/27, 5/20 G3), nausea 13 pts (13/27, 1/13 G3), anorexia (8/27), vomiting (6/27) and diarrhea (5/27)	[76,100]

AML – acute myeloid leukemia, CNS – central nerve system, CRi – complete remission, CTCL – cutaneous T-cell lymphoma, CR – complete response, DAC – 5-Aza-2′-deoxycytidine, DNMT – DNA methyl transferase, DLT – dose-limiting toxicity, ECG – electrocardiogram, ER – estrogen receptor, GSTPi – glutathione-S-transferase Pi, HDACI – histone deacetylase inhibition, HRPC – hormone refractory prostate cancer, HGB F – fetal hemoglobin, i.v. – intravenous, MDS – Myelodaysplastic Syndrome, N.A. – non available, NHL – non-Hodgkin's lymphoma, NSCLC – non-small-cell lung cancer, N/V – nausea/vomiting, PCa – prostate cancer, p.o. – per oral, PMN – peripheral mononuclear cells, POD – post-operative death, PR – partial response, PTCL – peripheral T-cell lymphoma, RR – relative risk, SD – stable disease.

Table 5
Preclinical combination of HDACIs with other agents

HDAC inhibitor(s)	Combination agent(s)	Rationale/mechanism underlying synergism	Reference
TSA FK-228 Phenylbutyrate vorinostat CBHA	Drugs or hormones that act on retinoic acid, estrogen receptors or other nuclear receptors	Aberrant fusion proteins bind to RARE (or other nuclear receptors) where HDAC-containing repressor complexes are recruited to silence expression of genes from these promoters. Ligands and HDACIs reactivate such silenced and repressed chromatin to cause expression of hormone-inducible genes to overcome retinoid resistance.	[51,52,56,59,132,156,190]
TSA vorinostat FK-228 Phenylbutyrate FR901228	DNA methyl transferase inhibitors (e.g. DAC, decitabine)	Eliminated the dominant effect of hypermethylation of promoters.	[12,19,32,74,104,114,139,160,221]
3n-Butyrate	5-Fluorouracil (5-FU)	Enhanced apoptosis.	[24]
FK-228 Phenylbutyrate vorinostat	Flavopiridol (NCI)	Flavopiridol, a synthetic Cdk inhibitor, interfered with expression of the cellular Cdk inhibitor $p21^{CIP1/WAF1}$, to cause apoptosis as opposed to cell cycle arrest and differentiation induced by HDACIs.	[1,143,175]
vorinostat Phenylbutyrate Apicidin LAQ-824	STI-571	HDACIs caused apoptosis in Imantinib-resistant cells and enhanced apoptosis in Bcr-Abl expressing cells.	[112,144,145,212,213]
Sodium butyrate	Topoisomerase II inhibitors (e.g., etoposide, epirubicin)	HDACIs upregulated topoisomerase II expression, which in turn rendered cells sensitive to topoisomerase II inhibitors.	[119]
LAQ-824 LBH-589	17-AAG (Hsp90 antagonist)	Inhibition of chaperone association of Hsp90 with Flt-3 and Bcr-Abl, resulting in polyubiquitination and proteosomal degradation of Flt-3 and Bcr-Abl. Levels of Flt-3 and Bcr-Abl were greatly attenuated to result in enhanced apoptosis.	[15,165]
vorinostat FK-228	Standard chemotherapy agents (e.g., VP-16, ellipticine, doxorubicin, and cisplatin, oxaliplatin)	Enhanced apoptosis.	[113]
TSA vorinostat MS-275 FK-228	γ-Irradiation	γ-H2AX foci expression was prolonged and histones were inhibited from participating in DNA repair. vorinostat also caused differential expression of several oncoproteins and DNA damage repair proteins (epidermal growth factor receptor, AKT, DNA-PK, and Rad51) that affect susceptibility of cells to radiation induced damage response	[16,33,34,46,111,218]
vorinostat Sodium butyrate	Bortezomib	Enhanced apoptosis.	[213]

Table 5
(Continued)

HDAC inhibitor(s)	Combination agent(s)	Rationale/mechanism underlying synergism	Reference
Sodium butyrate TSA vorinostat	Activators of extrinsic, receptor-mediated apoptotic pathway (TRAIL, TNF-α)	HDACIs sensitized cells to TRAIL by decreasing FLIP protein expression to cause cell death.	[93,95,173]
LAQ824	Apo-2L/tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)	Exposure to LAQ824 increased the mRNA and protein expressions of the death receptors DR5 and/or DR4, but reduced the mRNA and protein levels of cellular FLICE-inhibitory protein (c-FLIP). As compared with treatment with Apo-2L/tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) or LAQ824 alone, pretreatment with LAQ824 increased the assembly of Fas-associated death domain and caspase-8, but not of c-FLIP, into the Apo-2L/TRAIL-induced death-inducing signaling complex. This increased the processing of caspase-8 and Bcl-2 interacting domain (BID), augmented cytosolic accumulation of the prodeath molecules cytochrome-c, Smac and Omi, as well as led to increased activity of caspase-3 and apoptosis. Treatment with LAQ824 also down-regulated the levels of Bcl-2, Bcl-x(L), XIAP, and survivin. Partial inhibition of apoptosis due to LAQ824 or Apo-2L/TRAIL exerted by Bcl-2 overexpression was reversed by cotreatment with LAQ824 and Apo-2L/TRAIL.	[87]
vorinostat	Signal transduction modulators (e.g., STI-571)	Effectively induced apoptosis in Bcr/Abl^{+} cells that were STI-571-resistant and non-resistant.	[144,145,212]
vorinostat	ErbB signaling inhibitors (e.g., CI-1033 (Pfizer Inc)	Dual targeted therapy abrogated EGFR and Akt signaling.	[47]
LAQ-824	VEGFR tyrosine kinase inhibitor (PTK-787/ZK-222584)	Increased anti-angiogenic factors in tumor and surrounding endothelial cells, and inhibited tumor growth.	[162]
AQ-824	Herceptin and/or Taxotere or Epothilone B against human breast cancer	Causes down regulation of the mRNA and protein expression of Her-2, which correlated with the attenuation of pAKT. Promoting the proteasomal degradation of Her-2 and sensitizes human breast cancer cells (with Her-2 amplification) to Herceptin and apoptosis due to tubulin polymerizing agents, Taxotere and Epothilone B	[72]

5AC – 5-Aza-cytidine, DAC – 5-Aza-2′-deoxycytidine, CNS – central nerve system, CTCL – cutaneous T-cell lymphoma, CR – complete response, MTD – maximum tolerated dose, DLT – dose-limiting toxicity, ECG – electrocardiogram, GSTPi – glutathione-S-transferase Pi, HDACI – histone deacetylase inhibition, HRPC – hormone refractory prostate cancer, HGB F – fetal hemoglobin, N.A. – non-available, ND – not determined, NHL – non-Hodgkin's lymphoma, NSCLC – non-small-cell lung cancer, N/V – nausea/vomiting, p.o. – per oral, PMN – peripheral mononuclear cells, POD – post-operative death, PR – partial response, PTCL – peripheral T-cell lymphoma, RR – relative risk, SD – stable disease.

Table 6
Clinical Trials of HDACIs with other agents

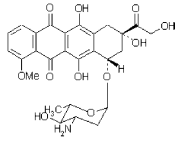
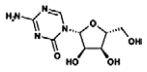
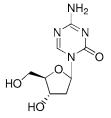
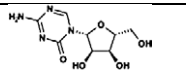
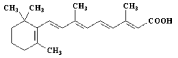
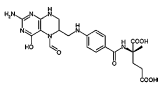
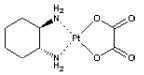
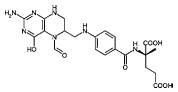
HDACI	Combining agent	Structure	Cancer (trial phase)	Schedule	Outcome	Adverse events	Reference
Valproic acid	Epirubicin		Advanced solid tumors (Phase I)	VPA loading dose followed by 6 oral doses (q12h) prior to epirubicin in 3-week cycles Arm 1: i.v. VPA (15–75 mg/kg/d) followed by epirubicin (75–100 mg/m^2/d) Arm 2: p.o. VPA (75–160 mg/kg/d) followed by epirubicin (100 mg/m^2/d)	PR:19% (7/37) SD/minor response: 43% (16/37) H3 and H4 acetylation and topo II expression correlated to VPA dose, plasma concentration and response	DLT: somnolence (1/42) and neutropenia (1/42) MTD: 160 mg/kg/d VPA combined with 100 mg/m^2/d epirubicin	[138]
	DAC		Advanced cancer (Phase I)	DAC: 20–47 mg/m^2/day for 10 days every 28 days VPA:11.9–25.7 mg/kg/day p.o.	SD for 6 months: 5% (1/22) SD for 4 months: 15% (3/22) Median LINE methylation decreased from 65% to 61% after 10 days treatment, but had returned back to baseline at the start of the next cycle	Drowsiness (5/22) Tremor (4/22) Hypomagnesemia (2/22) Anemia G2 (2/22) Neutropenia (2/22) DLT: neutropenic fever (1/22 at 37.5 mg/m^2)	[23]
	decitabine		relapsed/refractory AML + untreated AML with age >60 (Phase I)	15–20 mg/m^2/day decitabine as 1 h i.v. infusion for 10 days every 28 days ± 15 mg/kg/day p.o. VPA on days 5–21 every 28 d	Decrease DNA methylation: 64% (9/14) Depletion of DNMT: 67% (4/6) >100% increase of p15 or ER expression: 77% (10/13) of which all had a clinical response <100% increase of p15 or ER expression: 23% (3/13) of which none had a clinical response decitabine C_{max}: 93 ng/ml ($n = 7$) CR: 13% (2/15) CRi: 27% (4/15) 4/15 patients had clinical improvement	Not reported	[115]

Table 6
(Continued)

HDACI	Combining agent	Structure	Cancer (trial phase)	Schedule	Outcome	Adverse events	Reference
	DAC and ATRA	 and 	High risk MDS + relapsed/refractory AML + untreated AML with age >60 (Phase I/II)	DAC: 75 mg/m^2/day for 7 days ATRA: 45 mg/m^2/day p.o. on days 3–7 VPA: 50, 62.5 or 75 mg/kg/day p.o. for 7 days	All patients received fixed doses of DAC and ATRA. The dose VPA varied between the patients. CR: 6% (1/16, 75 mg/kg VPA) Marrow blasts <5%: 13% (2/16) CRP (CR minus bone marrow response): 6% (1/16) Median LINE methylation decreased during treatment from 62% to 58%, but returned to baseline by day 0 of the next cycle	Confusion (G3): 1/6 in 50 mg/kg VPA treated patients, 0/6 in 62.5 mg/kg VPA treated patients and 2/6 in 75 mg/kg VPA treated patients.	[185]
Vorinostat	5-fluorouracil and leucovorin and Oxaliplatin (FOLFOX)	 and  and 	Advanced colorectal cancer (Phase I)	vorinostat: 100–300 mg p.o. twice daily on day 1–7 every 2 weeks FOLFOX: 400 mg/m^2 leukovorin + 85 mg/m^2 oxiplatin over 2 h followed by 2500 mg/m^2 5-FU over 46 h on days 4–5 every 2 weeks	SD for 5 months: 1/6 SD for 2 months: 3/6 2 liver patient biopsies showed major downregulation of thymidilate synthase after 3 days treatment with vorinostat	Neutropenia (1/8, G2), mucositis (1/8, G2), N/V (2/8, G2)	[63]
MS-275	13-*cis* retinoic acid		Advanced solid tumors (Phase I)	MS-275: 4–5 mg/m^2 p.o. once per week CRA: 1 mg/kg/day p.o. twice daily for 3 weeks every 4 weeks	$T_{1/2}$ MS-275: 108.2 ± 47.9 h Clearance MS-275: 9.4 ± 6.0 L/h/m^2 Median CRA levels: 182.4 ng/ml 38% (5/13) remained on treatment for >4 months Histones isolated from PBMCs post-therapy showed transient but consistent acetylation Post-therapy liver lesion biopsies showed increased histone acetylation and decreased phosphorylated ERK and STAT3 protein expression	MTD: 5 mg/m^2 MS-275 + 1 mg/kg CRA (G3 hyponatremia, neutropenia, anemia) Fatigue (G1–2)	[157]

AML – acute myeloid leukemia, ATRA – all-trans retinoic acid, CRA – 13-*cis* retinoic acid, CR – complete response, DAC – 5-Aza-2′-deoxycytidine, DNMT – DNA methyl transferase, DLT – dose-limiting toxicity, ER – estrogen receptor, HDACI – histone deacetylase inhibitor, i.v. – intravenous, MDS – myelodaysplastic syndrome, MTD – maximum tolerated dose, N/V – nausea/vomiting, p.o. – per orale, PBMC – peripheral mononuclear cells, PR – partial response, PTCL – SD stable disease, VPA – valproic acid.

with HDACIs in the window period before surgery (pre-prostatectomy for example) and evaluating biomarkers of drug activity in post-surgery samples. Similarly, in case of DNA Methyl Transferase Inhibitors (DNMTIs), the frequency of methylation markers, like GSTPi, RASSF1A, CDH1, EDNRB1, which are frequently methylated in prostate cancer [128,210], etc. may be evaluated. However, it is still not known if decreases in methylation of these genes or others have independent prognostic significance.

10. Combination studies with HDACIs

In vitro, HDACIs have been combined with an array of chemically and structurally diverse compounds. In some cases, the combination was supported by a rationale, and in others the mechanism underlying the synergistic activity of the combination was subsequently analyzed. A summary of selected preclinical combination studies is provided in Table 5. Many of these preclinical observations have now forwarded to phase I/II clinical trials. Table 6 provides an overview of clinical trials using HDACIs in combination with other agents.

11. Conclusion

In the clinic, HDACIs are competing with conventional chemotherapeutic drugs, and are favorable because of their low toxicities. Further analysis into the common pathways between conventional drugs and HDACIs, significance of individual HDACs and their isoforms, and other substrates of HDACs will reveal the mechanism behind the success of HDACIs in the clinic. HDACIs with a more favorable PK such as MS-275 and to a lower extent LBH-589 and LAQ-824 may be preferred than the aliphatic HDACIs, many of which have very short half lives. Ease of administration and type of toxicity are additional considerations that will influence the development of next generation HDACIs. Currently, studies on mechanism of action of HDACIs are ongoing and the next few years should see a continued increase in the number of HDACIs under investigation in clinical trials.

Glossary of abbreviations

AA	anacardic acid
Acetyl-CoA	acetyl coenzyme A
AML	acute myeloid leukemia
AN-9	pivaloyloxymethyl butyrate
APL	acute promyelocytic leukemia
ATP	adenosine triphosphate
ATRA	all-trans retinoic acid
CBHA	m-carboxycinnamic acid bis-hydroxamide
CBP	CREB-binding protein
Cdk	cyclin-dependent kinase
CML	chronic myeloid leukemia
CNS	central nerve system
CR	complete response
CRA	13-*cis* retinoic acid
CTCL	cutaneous T-cell lymphoma
CTPB	N-(4-chloro-3-trifluoromethyl-phenyl)-2-ethoxy-6-pentadecyl-benzamide
DAC	5-Aza-2′-deoxycytidine
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
DNMT	DNA methyl transferase
DNMTI	DNA methyl transferase inhibitor
ER	estrogen receptor
ECG/EKG	electrocardiogram
FAT	factor acetyl transferases
GNAT	Gcn5-related N-acetyl transferases
GSTPi	glutathione-S-transferase Pi
H2A	histone 2A
H2B	histone 2B
H3	histone 3
H4	histone 4
HAT	histone acetyl transferase
HATI	histone acetyl transferase inhibitor
HDAC	histone deacetylase
HDACI	histone deacetylase inhibitor
MDS	myelodaysplastic syndrome
HGB F	fetal hemoglobin
HMG	high mobility group
HRPC	hormone refractory prostate cancer
Hsp90	90-kDa heat shock protein
i.v.	intravenous
Lys–CoA	conjugate of amino acid lysine and coenzyme A
MLL	mixed lineage leukemia
MOZ	monocytic leukemia zink finger
MTD	maximum tolerated dose
MYST	super family of HATs named after its founding members MOZ, Ybf2/Sas3, Sas2 and Tip60
N.A.	not available
NCI	National Cancer Institute
NHL	non-Hodgkin's lymphoma
NSCLC	non-small cell lung carcinoma
N/V	nausea/vomiting

PCa	prostate cancer
PCAF	p300/CREB binding protein (CBP)-associated factor
PBMC	peripheral blood mononuclear cells
PK	pharmacokinetic
PMN	peripheral mononuclear cells
p.o.	per oral
POD	post operative death
PR	partial response
PTCL	peripheral T-cell lymphoma
RA	retinoic acid
RNA	ribonucleic acid
ROS	reactive oxygen species
RR	relative risk
SAHA	suberoylanilide hydroxamic acid
SB	sodium butyrate
SD	stable disease
SIRT	silent information regulator
TNF α	tumor necrosis factor α
TRAIL	TNF-related apoptosis inducing ligand
TSA	trichostatin A
TSG	tumor suppressor gene
US	FDA United States Federal Drug Administration
VPA	valproic acid

Acknowledgements

Supported by Catharine van Tussenbroek Fund, NCI U0-1 CA 70095 and SPORE P50CA58236.

References

[1] J. Almenara, R. Rosato and S. Grant, Synergistic induction of mitochondrial damage and apoptosis in human leukemia cells by flavopiridol and the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA), *Leukemia* **16** (2002), 1331–1343.

[2] G. Arents, R.W. Burlingame, B.C. Wang, W.E. Love and E. N. Moudrianakis, The nucleosomal core histone octamer at 3.1 A resolution: a tripartite protein assembly and a left-handed superhelix, *Proc. Natl. Acad. Sci. USA* **88** (1991), 10148–10152.

[3] J.L. Aron, M.R. Parthun, G. Marcucci, S. Kitada, A.P. Mone, M.E. Davis, T. Shen, T. Murphy, J. Wickham, C. Kanakry, D.M. Lucas, J.C. Reed, M.R. Grever and J.C. Byrd, Depsipeptide (FR901228) induces histone acetylation and inhibition of histone deacetylase in chronic lymphocytic leukemia cells concurrent with activation of caspase 8-mediated apoptosis and down-regulation of c-FLIP protein, *Blood* **102** (2003), 652–658.

[4] A. Atmaca, A. Maurer, T. Heinzel, M. Gottlicher, A. Neumann, S.E. Al-Batran, E. Martin, I. Bartsch, A. Knuth and E. Jaeger, A dose-escalating phase I study with valproic acid in patinets with advanced cancer, in: *American Society of Clinical Oncology Annual Meeting*, USA, 2004.

[5] K. Balasubramanyam, M. Altaf, R.A. Varier, V. Swaminathan, A. Ravindran, P.P. Sadhale and T.K. Kundu, Polyisoprenylated benzophenone, garcinol, a natural histone acetyltransferase inhibitor, represses chromatin transcription and alters global gene expression, *J. Biol. Chem.* **279** (2004), 33716–33726.

[6] K. Balasubramanyam, V. Swaminathan, A. Ranganathan and T.K. Kundu, Small molecule modulators of histone acetyltransferase p300, *J. Biol. Chem.* **278** (2003), 19134–19140.

[7] J.P. Baldwin, P.G. Boseley, E.M. Bradbury and K. Ibel, The subunit structure of the eukaryotic chromosome, *Nature* **253** (1975), 245–249.

[8] A.J. Bannister and T. Kouzarides, The CBP co-activator is a histone acetyltransferase, *Nature* **384** (1996), 641–643.

[9] A.J. Bannister, T. Oehler, D. Wilhelm, P. Angel and T. Kouzarides, Stimulation of c-Jun activity by CBP: c-Jun residues Ser63/73 are required for CBP induced stimulation in vivo and CBP binding in vitro, *Oncogene* **11** (1995), 2509–2514.

[10] A.J. Bannister, P. Zegerman, J.F. Partridge, E.A. Miska, J.O. Thomas, R.C. Allshire and T. Kouzarides, Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain, *Nature* **410** (2001), 120–124.

[11] J. Beck, T. Fischer, E. Rowinsky, C. Huber, M. Mita, P. Atadja, B. Peng, C. Kwong, M. Dugan and A. Patnaik, Phase I pharmacokinetic and pharmacodynamic study of LBH589A: a novel histone deacetylase inhibitor, in: *American Society of Clinical Oncology Annual Meeting*, 2004.

[12] S.A. Belinsky, D.M. Klinge, C.A. Stidley, J.P. Issa, J.G. Herman, T.H. March and S.B. Baylin, Inhibition of DNA methylation and histone deacetylation prevents murine lung cancer, *Cancer Res.* **63** (2003), 7089–7093.

[13] P.E. Bennett-Baker, J. Wilkowski and D.T. Burke, Age-associated activation of epigenetically repressed genes in the mouse, *Genetics* **165** (2003), 2055–2062.

[14] O.R. Bereshchenko, W. Gu and R. Dalla-Favera, Acetylation inactivates the transcriptional repressor BCL6, *Nat. Genet.* **32** (2002), 606–613.

[15] K. Bhalla, P. George, R. Gutti, P. Bali, J. Tao, F. Guo, C. Sigua, Y. Li, P. Cohen, P. Atadja and H. Lee, A combination of histone deacetylase inhibitor LBH589 and the hsp90 inhibitor 17-AAG is highly active against human CML-BC and AML cells with constitutively active mutant FLT-3 tyrosine kinase, in: *American Society of Clinical Oncology Annual Meeting,* USA, 2004.

[16] S. Biade, C.C. Stobbe, J.T. Boyd and J.D. Chapman, Chemical agents that promote chromatin compaction radiosensitize tumour cells, *Int. J. Radiat. Biol.* **77** (2001), 1033–1042.

[17] H.T. Bjornsson, M.D. Fallin and A.P. Feinberg, An integrated epigenetic and genetic approach to common human disease, *Trends Genet.* **20** (2004), 350–358.

[18] H. Boeger, J. Griesenbeck, J.S. Strattan and R.D. Kornberg, Nucleosomes unfold completely at a transcriptionally active promoter, *Mol. Cell* **11** (2003), 1587–1598.

[19] A.J. Boivin, L.F. Momparler, A. Hurtubise and R.L. Momparler, Antineoplastic action of 5-aza-2′-deoxycytidine and phenylbutyrate on human lung carcinoma cells, *Anticancer Drugs* **13** (2002), 869–874.

[20] J. Borrow, V.P. Stanton, Jr., J.M. Andresen, R. Becher, F.G. Behm, R.S. Chaganti, C.I. Civin, C. Disteche, I. Dube, A.M. Frischauf, D. Horsman, F. Mitelman, S. Volinia, A.E. Watmore and D.E. Housman, The translocation t(8;16)(p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein, *Nat. Genet.* **14** (1996), 33–41.

[21] K. Bouazoune, A. Mitterweger, G. Langst, A. Imhof, A. Akhtar, P.B. Becker and A. Brehm, The dMi-2 chromodomains are DNA binding modules important for ATP-dependent nucleosome mobilization, *Embo J.* **21** (2002), 2430–2440.

[22] L.A. Boyer, R.R. Latek and C.L. Peterson, The SANT domain: a unique histone-tail-binding module?, *Nat. Rev. Mol. Cell Biol.* **5** (2004), 158–163.

[23] F. Braiteh, A. Soriano, C.H. Luis, H.S. David, C. Ng, G. Garcia-Manero and R. Kurzrock, Phase I study of low-dose hypomethylating agent azacitidine (5-AC) combined with the histone deacetylase inhibitor valproic acid (VPA) in patients with advanced cancers, in: *American Society of Clinical Oncology Annual Meeting*, 2006.

[24] R.A. Bras-Goncalves, M. Pocard, J.L. Formento, F. Poirson-Bichat, G. De Pinieux, I. Pandrea, F. Arvelo, G. Ronco, P. Villa, A. Coquelle, G. Milano, T. Lesuffleur, B. Dutrillaux and M.F. Poupon, Synergistic efficacy of 3n-butyrate and 5-fluorouracil in human colorectal cancer xenografts via modulation of DNA synthesis, *Gastroenterology* **120** (2001), 874–888.

[25] A. Brunet, L.B. Sweeney, J.F. Sturgill, K.F. Chua, P.L. Greer, Y. Lin, H. Tran, S.E. Ross, R. Mostoslavsky, H.Y. Cohen, L.S. Hu, H.L. Cheng, M.P. Jedrychowski, S.P. Gygi, D.A. Sinclair, F.W. Alt and M.E. Greenberg, Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase, *Science* **303** (2004), 2011–2015.

[26] A. Burgess, A. Ruefli, H. Beamish, R. Warrener, N. Saunders, R. Johnstone and B. Gabrielli, Histone deacetylase inhibitors specifically kill nonproliferating tumour cells, *Oncogene* **23** (2004), 6693–6701.

[27] A.J. Burgess, S. Pavey, R. Warrener, L.J. Hunter, T.J. Piva, E.A. Musgrove, N. Saunders, P.G. Parsons and B.G. Gabrielli, Up-regulation of p21(WAF1/CIP1) by histone deacetylase inhibitors reduces their cytotoxicity, *Mol. Pharmacol.* **60** (2001), 828–837.

[28] L.M. Butler, D.B. Agus, H.I. Scher, B. Higgins, A. Rose, C. Cordon-Cardo, H.T. Thaler, R.A. Rifkind, P.A. Marks and V.M. Richon, Suberoylanilide hydroxamic acid, an inhibitor of histone deacetylase, suppresses the growth of prostate cancer cells in vitro and in vivo, *Cancer Res.* **60** (2000), 5165–5170.

[29] L.M. Butler, Y. Webb, D.B. Agus, B. Higgins, T.R. Tolentino, M.C. Kutko, M.P. LaQuaglia, M. Drobnjak, C. Cordon-Cardo, H.I. Scher, R. Breslow, V.M. Richon, R.A. Rifkind and P.A. Marks, Inhibition of transformed cell growth and induction of cellular differentiation by pyroxamide, an inhibitor of histone deacetylase, *Clin. Cancer Res.* **7** (2001), 962–970.

[30] J.C. Byrd, G. Marcucci, M.R. Parthun, J.J. Xiao, R.B. Klisovic, M. Moran, T.S. Lin, S. Liu, A.R. Sklenar, M.E. Davis, D.M. Lucas, B. Fischer, R. Shank, S.L. Tejaswi, P. Binkley, J. Wright, K.K. Chan and M.R. Grever, A phase 1 and pharmacodynamic study of depsipeptide (FK228) in chronic lymphocytic leukemia and acute myeloid leukemia, *Blood* **105** (2005), 959–967.

[31] B.R. Cairns, Chromatin remodeling complexes: strength in diversity, precision through specialization, *Curr. Opin. Genet. Dev.* **15** (2005), 185–190.

[32] E.E. Cameron, K.E. Bachman, S. Myohanen, J.G. Herman and S.B. Baylin, Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer, *Nat. Genet.* **21** (1999), 103–107.

[33] K. Camphausen, W. Burgan, M. Cerra, K.A. Oswald, J.B. Trepel, M.J. Lee and P.J. Tofilon, Enhanced radiation-induced cell killing and prolongation of gammaH2AX foci expression by the histone deacetylase inhibitor MS-275, *Cancer Res.* **64** (2004), 316–321.

[34] K. Camphausen, T. Scott, M. Sproull and P.J. Tofilon, Enhancement of xenograft tumor radiosensitivity by the histone deacetylase inhibitor MS-275 and correlation with histone hyperacetylation, *Clin. Cancer Res.* **10** (2004), 6066–6071.

[35] R. Candau, J.X. Zhou, C.D. Allis and S.L. Berger, Histone acetyltransferase activity and interaction with ADA2 are critical for GCN5 function in vivo, *Embo J.* **16** (1997), 555–565.

[36] E.P. Candido, R. Reeves and J.R. Davie, Sodium butyrate inhibits histone deacetylation in cultured cells, *Cell* **14**, (1978), 105–113.

[37] M. Carapeti, R.C. Aguiar, J.M. Goldman and N.C. Cross, A novel fusion between MOZ and the nuclear receptor coactivator TIF2 in acute myeloid leukemia, *Blood* **91** (1998), 3127–3133.

[38] M. Carducci, L.L. Siu, R. Sullivan, M. Maclean, A. Kalita, E.X. Chen, R. Pili, R.E. Martell, J. Besterman and G.K. Reid, Phase I study of isotype-selective histone deacetylase (HDAC) inhibitor MGCD0103 given as three-times weekly oral dose in patients (pts) with advanced solid tumors, in: *American Society of Clinical Oncology Annual Meeting*, 2006.

[39] M.A. Carducci, J. Gilbert, M.K. Bowling, D. Noe, M.A. Eisenberger, V. Sinibaldi, Y. Zabelina, T.L. Chen, L.B. Grochow and R.C. Donehower, A Phase I clinical and pharmacological evaluation of sodium phenylbutyrate on an 120-h infusion schedule, *Clin. Cancer Res.* **7** (2001), 3047–3055.

[40] M.A. Carducci, J.B. Nelson, K.M. Chan-Tack, S.R. Ayyagari, W.H. Sweatt, P.A. Campbell, W.G. Nelson and J.W. Simons, Phenylbutyrate induces apoptosis in human prostate cancer and is more potent than phenylacetate, *Clin. Cancer Res.* **2** (1996), 379–387.

[41] S. Chang, T.A. McKinsey, C.L. Zhang, J.A. Richardson, J.A. Hill and E.N. Olson, Histone deacetylases 5 and 9 govern responsiveness of the heart to a subset of stress signals and play redundant roles in heart development, *Mol. Cell Biol.* **24** (2004), 8467–8476.

[42] S.M. Chang, J.G. Kuhn, H.I. Robins, S.C. Schold, A.M. Spence, M.S. Berger, M.P. Mehta, M.E. Bozik, I. Pollack, D. Schiff, M. Gilbert, C. Rankin and M.D. Prados, Phase II study of phenylacetate in patients with recurrent malignant glioma:

a North American Brain Tumor Consortium report, *J. Clin. Oncol.* **17** (1999), 984–990.

[43] L. Chen, S. Meng, H. Wang, P. Bali, W. Bai, B. Li, P. Atadja, K.N. Bhalla and J. Wu, Chemical ablation of androgen receptor in prostate cancer cells by the histone deacetylase inhibitor LAQ824, *Mol. Cancer Ther.* **4** (2005), 1311–1319.

[44] Z. Chen, S. Clark, M. Birkeland, C.M. Sung, A. Lago, R. Liu, R. Kirkpatrick, K. Johanson, J.D. Winkler and E. Hu, Induction and superinduction of growth arrest and DNA damage gene 45 (GADD45) alpha and beta messenger RNAs by histone deacetylase inhibitors trichostatin A (TSA) and butyrate in SW620 human colon carcinoma cells, *Cancer Lett.* **188** (2002), 127–140.

[45] T. Chiba, O. Yokosuka, M. Arai, M. Tada, K. Fukai, F. Imazeki, M. Kato, N. Seki and H. Saisho, Identification of genes up-regulated by histone deacetylase inhibition with cDNA microarray and exploration of epigenetic alterations on hepatoma cells, *J. Hepatol.* **41** (2004), 436–445.

[46] P. Chinnaiyan, G. Vallabhaneni, E. Armstrong, S.M. Huang and P.M. Harari, Modulation of radiation response by histone deacetylase inhibition, *Int. J. Radiat. Oncol. Biol. Phys.* **62** (2005), 223–229.

[47] P. Chinnaiyan, S. Varambally, S.A. Tomlins, S. Huang, A.M. Chinnaiyan and P.M. Harari, Enhancing the anti-tumor activity of ErbB blockade with histone deacetylase inhibition, in: *American Society of Clinical Oncology Annual Meetin*g, USA, 2004.

[48] J.H. Choi, H.J. Kwon, B.I. Yoon, J.H. Kim, S.U. Han, H.J. Joo and D.Y. Kim, Expression profile of histone deacetylase 1 in gastric cancer tissues, *Jpn. J. Cancer Res.* **92** (2001), 1300–1304.

[49] V. Chopin, C. Slomianny, H. Hondermarck and X. Le Bourhis, Synergistic induction of apoptosis in breast cancer cells by cotreatment with butyrate and TNF-alpha, TRAIL, or anti-Fas agonist antibody involves enhancement of death receptors' signaling and requires P21(waf1), *Exp. Cell Res.* **298** (2004), 560–573.

[50] D. Cimini, M. Mattiuzzo, L. Torosantucci and F. Degrassi, Histone hyperacetylation in mitosis prevents sister chromatid separation and produces chromosome segregation defects, *Mol. Biol. Cell* **14** (2003), 3821–3833.

[51] D.C. Coffey, M.C. Kutko, R.D. Glick, L.M. Butler, G. Heller, R.A. Rifkind, P.A. Marks, V.M. Richon and M.P. La Quaglia, The histone deacetylase inhibitor, CBHA, inhibits growth of human neuroblastoma xenografts in vivo, alone and synergistically with all-trans retinoic acid, *Cancer Res.* **61** (2001), 3591–3594.

[52] D.C. Coffey, M.C. Kutko, R.D. Glick, S.L. Swendeman, L. Butler, R. Rifkind, P.A. Marks, V.M. Richon and M.P. LaQuaglia, Histone deacetylase inhibitors and retinoic acids inhibit growth of human neuroblastoma in vitro, *Med. Pediatr. Oncol.* **35** (2000), 577–581.

[53] H.Y. Cohen, S. Lavu, K.J. Bitterman, B. Hekking, T.A. Imahiyerobo, C. Miller, R. Frye, H. Ploegh, B.M. Kessler and D.A. Sinclair, Acetylation of the C terminus of Ku70 by CBP and PCAF controls Bax-mediated apoptosis, *Mol. Cell* **13** (2004), 627–638.

[54] L.A. Cohen, P.A. Marks, R.A. Rifkind, S. Amin, D. Desai, B. Pittman and V.M. Richon, Suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor, suppresses the growth of carcinogen-induced mammary tumors, *Anticancer Res.* **22** (2002), 1497–1504.

[55] M.S. Cosgrove, J.D. Boeke and C. Wolberger, Regulated nucleosome mobility and the histone code, *Nat. Struct. Mol. Biol.* **11** (2004), 1037–1043.

[56] S. Cote, A. Rosenauer, A. Bianchini, K. Seiter, J. Vandewiele, C. Nervi and W.H. Miller, Jr., Response to histone deacetylase inhibition of novel PML/RARalpha mutants detected in retinoic acid-resistant APL cells, *Blood* **100** (2002), 2586–2596.

[57] C.A. Davey, D.F. Sargent, K. Luger, A.W. Maeder and T.J. Richmond, Solvent mediated interactions in the structure of the nucleosome core particle at 1.9 a resolution, *J. Mol. Biol.* **319** (2002), 1097–1113.

[58] X. de la Cruz, S. Lois, S. Sanchez-Molina and M.A. Martinez-Balbas, Do protein motifs read the histone code?, *Bioessays* **27** (2005), 164–175.

[59] K. Demary, L. Wong and R.A. Spanjaard, Effects of retinoic acid and sodium butyrate on gene expression, histone acetylation and inhibition of proliferation of melanoma cells, *Cancer Lett.* **163** (2001), 103–107.

[60] C.F. Deroanne, K. Bonjean, S. Servotte, L. Devy, A. Colige, N. Clausse, S. Blacher, E. Verdin, J.M. Foidart, B.V. Nusgens and V. Castronovo, Histone deacetylases inhibitors as anti-angiogenic agents altering vascular endothelial growth factor signaling, *Oncogene* **21** (2002), 427–436.

[61] A. Eden, F. Gaudet, A. Waghmare and R. Jaenisch, Chromosomal instability and tumors promoted by DNA hypomethylation, *Science* **300** (2003), 455.

[62] F. Facchetti, S. Previdi, M. Ballarini, S. Minucci, P. Perego, C.A. Porta, M. Gottlicher, M.S. Jansen, S.C. Nagel, P.J. Miranda, E.K. Lobenhofer, C.A. Afshari, D.P. McDonnell, L. Tou, Q. Liu, R.A. Shivdasani, D. Zgouras, U. Becker, S. Loitsch, J. Stein, Y.L. Chung, A.J. Wang, L.F. Yao, N. Gurvich, O.M. Tsygankova, J.L. Meinkoth, P.S. Klein, Q. Lu, Y.T. Yang, C.S. Chen, M. Davis, J.C. Byrd, M.R. Etherton, A. Umar, V.L. Nelson-DeGrave, J.K. Wickenheisser, J.E. Cockrell, J.R. Wood, R.S. Legro, J.F. Strauss, 3rd, J.M. McAllister, O.H. Kramer, P. Zhu, H.P. Ostendorff, M. Golebiewski, J. Tiefenbach, M.A. Peters, B. Brill, B. Groner, I. Bach, T. Heinzel, E. Yildirim, Z. Zhang, T. Uz, C.Q. Chen, R. Manev, H. Manev, D.H. Ovakim, J.J. Heikkila, W.G. Zhu, G.A. Otterson, R.A. Blaheta, J. Cinatl, Jr., R.S. Williams, L. Cheng, A.W. Mudge, A.J. Harwood, A. Schimpf, S. Giavara, J.P. Sleeman, F. Lo Coco, C. Nervi and P.G. Pelicci, Modulation of pro- and anti-apoptotic factors in human melanoma cells exposed to histone deacetylase inhibitors, *Apoptosis* **9** (2004), 573–582.

[63] M.G. Fakih, L. Pendyala, K. Toth, P. Creaven, N. Soehnlein, A. Litwin and D. Trump, A phase I study of vorinostat (suberoylanilide hydroxamic acid, SAHA) in combination with 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX) in patients with advanced colorectal cancer, in: *American Society of Clinical Oncology Annual Meeting*, 2006.

[64] H.Y. Fan, X. He, R.E. Kingston and G.J. Narlikar, Distinct strategies to make nucleosomal DNA accessible, *Mol. Cell* **11** (2003), 1311–1322.

[65] A.P. Feinberg and B. Tycko, The history of cancer epigenetics, *Nat. Rev. Cancer* **4** (2004), 143–153.

[66] A.P. Feinberg and B. Vogelstein, Hypomethylation distinguishes genes of some human cancers from their normal counterparts, *Nature* **301** (1983), 89–92.

[67] M.S. Finnin, J.R. Donigian, A. Cohen, V.M. Richon, R.A. Rifkind, P.A. Marks, R. Breslow and N.P. Pavletich, Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors, *Nature* **401** (1999), 188–193.

[68] W. Fischle, Y. Wang and C.D. Allis, Binary switches and modification cassettes in histone biology and beyond, *Nature* **425** (2003), 475–479.

[69] J. Ford, M. Jiang and J. Milner, Cancer-specific functions of SIRT1 enable human epithelial cancer cell growth and survival, *Cancer Res.* **65** (2005), 10457–10463.

[70] M.F. Fraga, E. Ballestar, M.F. Paz, S. Ropero, F. Setien, M.L. Ballestar, D. Heine-Suner, J.C. Cigudosa, M. Urioste, J. Benitez, M. Boix-Chornet, A. Sanchez-Aguilera, C. Ling, E. Carlsson, P. Poulsen, A. Vaag, Z. Stephan, T.D. Spector, Y.Z. Wu, C. Plass and M. Esteller, Epigenetic differences arise during the lifetime of monozygotic twins, *Proc. Natl. Acad. Sci. USA* **102** (2005), 10604–10609.

[71] R.R. Frey, C.K. Wada, R.B. Garland, M.L. Curtin, M.R. Michaelides, J. Li, L.J. Pease, K.B. Glaser, P.A. Marcotte, J.J. Bouska, S.S. Murphy and S.K. Davidsen, Trifluoromethyl ketones as inhibitors of histone deacetylase, *Bioorg. Med. Chem. Lett.* **12** (2002), 3443–3447.

[72] L. Fuino, P. Bali, S. Wittmann, S. Donapaty, F. Guo, H. Yamaguchi, H.G. Wang, P. Atadja and K. Bhalla, 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** (2003), 971–984.

[73] R. Furumai, A. Matsuyama, N. Kobashi, K.H. Lee, M. Nishiyama, H. Nakajima, A. Tanaka, Y. Komatsu, N. Nishino, M. Yoshida and S. Horinouchi, FK228 (depsipeptide) as a natural prodrug that inhibits class I histone deacetylases, *Cancer Res.* **62** (2002), 4916–4921.

[74] J. Gagnon, S. Shaker, M. Primeau, A. Hurtubise and R.L. Momparler, Interaction of 5-aza-2′-deoxycytidine and depsipeptide on antineoplastic activity and activation of 14-3-3sigma, E-cadherin and tissue inhibitor of metalloproteinase 3 expression in human breast carcinoma cells, *Anticancer Drugs* **14** (2003), 193–202.

[75] S.A. Gayther, S.J. Batley, L. Linger, A. Bannister, K. Thorpe, S.F. Chin, Y. Daigo, P. Russell, A. Wilson, H.M. Sowter, J.D. Delhanty, B.A. Ponder, T. Kouzarides and C. Caldas, Mutations truncating the EP300 acetylase in human cancers, *Nat. Genet.* **24** (2000), 300–303.

[76] K.T.A. Gelmon, M. Carducci, G.K. Reid, Z. Li, A. Kalita, V. Callejas, J. Longstreth, J.M. Besterman and L.L. Siu, Phase I trials of the oral histone deacetylase (HDAC) inhibitor MGCD0103 given either daily or 3x weekly for 14 days every 3 weeks in patients (pts) with advanced solid tumors, in: *American Society of Clinical Oncology Annual Meeting*, 2005.

[77] J. Gilbert, S.D. Baker, M.K. Bowling, L. Grochow, W.D. Figg, Y. Zabelina, R.C. Donehower and M.A. Carducci, A phase I dose escalation and bioavailability study of oral sodium phenylbutyrate in patients with refractory solid tumor malignancies, *Clin. Cancer Res.* **7** (2001), 2292–2300.

[78] K.B. Glaser, M.J. Staver, J.F. Waring, J. Stender, R.G. Ulrich and S.K. Davidsen, 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** (2003), 151–163.

[79] L. Gore, S.N. Holden, M. Basche, S.K.S. Raj, I. Arnold, C. O'Bryant, S. Witta, B. Rohde, C. McCoy and S.G. Eckhardt, Updated results from a phase I trial of the histone deacetylase inhibitor MS-275 in patients with refractory solid tumors, in: *American Society of Clinical Oncology Annual Meeting*, USA, 2004.

[80] M. Gottlicher, S. Minucci, P. Zhu, O.H. Kramer, A. Schimpf, S. Giavara, J.P. Sleeman, F. Lo Coco, C. Nervi, P.G. Pelicci and T. Heinzel, Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells, *Embo J.* **20** (2001), 6969–6978.

[81] S.G. Gray and T.J. Ekstrom, The human histone deacetylase family, *Exp. Cell Res.* **262** (2001), 75–83.

[82] I.V. Gregoretti, Y.M. Lee and H.V. Goodson, Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis, *J. Mol. Biol.* **338** (2004), 17–31.

[83] F. Grignani, S. De Matteis, C. Nervi, L. Tomassoni, V. Gelmetti, M. Cioce, M. Fanelli, M. Ruthardt, F.F. Ferrara, I. Zamir, C. Seiser, M.A. Lazar, S. Minucci and P.G. Pelicci, Fusion proteins of the retinoic acid receptor-alpha recruit histone deacetylase in promyelocytic leukaemia, *Nature* **391** (1998), 815–818.

[84] C.M. Grozinger, C.A. Hassig and S.L. Schreiber, Three proteins define a class of human histone deacetylases related to yeast Hda1p, *Proc. Natl. Acad. Sci. USA* **96** (1999), 4868–4873.

[85] L. Guarente, Sir2 links chromatin silencing, metabolism, and aging, *Genes Dev.* **14** (2000), 1021–1026.

[86] F. Guidez, L. Howell, M. Isalan, M. Cebrat, R.M. Alani, S. Ivins, I. Hormaeche, M.J. McConnell, S. Pierce, P.A. Cole, J. Licht and A. Zelent, Histone acetyltransferase activity of p300 is required for transcriptional repression by the promyelocytic leukemia zinc finger protein, *Mol. Cell Biol.* **25** (2005), 5552–5566.

[87] F. Guo, C. Sigua, J. Tao, P. Bali, P. George, Y. Li, S. Wittmann, L. Moscinski, P. Atadja and K. Bhalla, 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** (2004), 2580–2589.

[88] S.J. Haggarty, K.M. Koeller, J.C. Wong, C.M. Grozinger and S.L. Schreiber, Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation, *Proc. Natl. Acad. Sci. USA* **100** (2003), 4389–4394.

[89] J.C. Hansen, Conformational dynamics of the chromatin fiber in solution: determinants, mechanisms, and functions, *Annu. Rev. Biophys. Biomol. Struct.* **31** (2002), 361–392.

[90] A. Hauschild, U.G.C. Trefzer, K. Kaehler, S. Ugurel, F. Kiecker, T. Eigentler and H. Krissel, A phase II multicenter study on the histone deacetylase (HDAC) inhibitor MS-275,

comparing two dosage schedules in metastatic melanoma, in: *American Society of Clinical Oncology Annual Meeting*, 2006.

[91] C. Henderson and C. Brancolini, Apoptotic pathways activated by histone deacetylase inhibitors: implications for the drug-resistant phenotype, *Drug Resist. Updat.* **6** (2003), 247–256.

[92] C. Henderson, M. Mizzau, G. Paroni, R. Maestro, C. Schneider and C. Brancolini, Role of caspases, Bid, and p53 in the apoptotic response triggered by histone deacetylase inhibitors trichostatin-A (TSA) and suberoylanilide hydroxamic acid (SAHA), *J. Biol. Chem.* **278** (2003), 12579–12589.

[93] A. Hernandez, R. Thomas, F. Smith, J. Sandberg, S. Kim, D.H. Chung and B.M. Evers, Butyrate sensitizes human colon cancer cells to TRAIL-mediated apoptosis, *Surgery* **130** (2001), 265–272.

[94] L. Huang and A.B. Pardee, Suberoylanilide hydroxamic acid as a potential therapeutic agent for human breast cancer treatment, *Mol. Med.* **6** (2000), 849–866.

[95] H. Inoue, K. Shiraki, S. Ohmori, T. Sakai, M. Deguchi, T. Yamanaka, H. Okano and T. Nakano, Histone deacetylase inhibitors sensitize human colonic adenocarcinoma cell lines to TNF-related apoptosis inducing ligand-mediated apoptosis, *Int. J. Mol. Med.* **9** (2002), 521–525.

[96] A. Insinga, S. Minucci and P.G. Pelicci, Mechanisms of selective anticancer action of histone deacetylase inhibitors, *Cell Cycle* **4** (2005), 741–743.

[97] K. Ito, G. Caramori, S. Lim, T. Oates, K.F. Chung, P.J. Barnes and I.M. Adcock, Expression and activity of histone deacetylases in human asthmatic airways, *Am. J. Respir. Crit. Care Med.* **166** (2002), 392–396.

[98] R.W. Johnstone, Histone-deacetylase inhibitors: novel drugs for the treatment of cancer, *Nat. Rev. Drug Discov.* **1** (2002), 287–299.

[99] S.K. Kachhap, M.S.Q. Kortenhorst, S. Shabbeer, E. Washington and M.A. Carducci, Comparison of expression of Class I and Class II histone deacetylase in prostate cancer cell lines and normal immortalized prostate epithelial cells, *Proceedings of AACR Annual Meeting*, 2004.

[100] A.M.C. Kalita, C. Bonfils, K. Gelmon, L.L. Siu, A. Tolcher, M. Carducci, J.M. Besterman, G.K. Reid and Z. Li, Pharmacodynamic effect of MGCD0103, an oral isotype-selective histone deacetylase (HDAC) inhibitor, on HDAC enzyme inhibition and histone acetylation induction in Phase I clinical trials in patients (pts) with advanced solid tumors or non-Hodgkin's lymphoma (NHL), in: *Annual meeting American Society of Clinical Oncology*, FL, USA, 2005.

[101] Y. Kamei, L. Xu, T. Heinzel, J. Torchia, R. Kurokawa, B. Gloss, S.C. Lin, R.A. Heyman, D.W. Rose, C.K. Glass and M.G. Rosenfeld, A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors, *Cell* **85** (1996), 403–414.

[102] S.R. Kassabov, B. Zhang, J. Persinger and B. Bartholomew, SWI/SNF unwraps, slides, and rewraps the nucleosome, *Mol. Cell* **11** (2003), 391–403.

[103] M. Kasten, H. Szerlong, H. Erdjument-Bromage, P. Tempst, M. Werner and B.R. Cairns, Tandem bromodomains in the chromatin remodeler RSC recognize acetylated histone H3 Lys14, *Embo J.* **23** (2004), 1348–1359.

[104] J.C. Keen, L. Yan, K.M. Mack, C. Pettit, D. Smith, D. Sharma, and N.E. Davidson, 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** (2003), 177–186.

[105] H. Keer, T. Reid and S. Sreedharan, Pivanex activity in refractory non-small cell lung cancer, a phase II study, in: *American Society of Clinical Oncology Annual Meeting*, USA, 2002.

[106] R.I. Kelley, Isolation of a histone IIb1–IIb2 complex, *Biochem. Biophys. Res. Commun.* **54** (1973), 1588–1594.

[107] W.K. Kelly, O.A. O'Connor, L.M. Krug, J.H. Chiao, M. Heaney, T. Curley, B. MacGregore-Cortelli, W. Tong, J.P. Secrist, L. Schwartz, S. Richardson, E. Chu, S. Olgac, P.A. Marks, H. Scher and V.M. Richon, Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer, *J. Clin. Oncol.* **23** (2005), 3923–3931.

[108] W.K. Kelly, V.M. Richon, O. O'Connor, T. Curley, B. MacGregor-Curtelli, W. Tong, M. Klang, L. Schwartz, S. Richardson, E. Rosa, M. Drobnjak, C. Cordon-Cordo, J.H. Chiao, R. Rifkind, P.A. Marks and H. Scher, Phase I clinical trial of histone deacetylase inhibitor: suberoylanilide hydroxamic acid administered intravenously, *Clin. Cancer Res.* **9** (2003), 3578–3588.

[109] S. Khochbin, A. Verdel, C. Lemercier and D. Seigneurin-Berny, Functional significance of histone deacetylase diversity, *Curr. Opin. Genet. Dev.* **11** (2001), 162–166.

[110] S. Khorasanizadeh, The nucleosome: from genomic organization to genomic regulation, *Cell* **116** (2004), 259–272.

[111] J.H. Kim, J.H. Shin and I.H. Kim, Susceptibility and radiosensitization of human glioblastoma cells to trichostatin A, a histone deacetylase inhibitor, *Int. J. Radiat. Oncol. Biol. Phys.* **59** (2004), 1174–1180.

[112] J.S. Kim, H.K. Jeung, J.W. Cheong, H. Maeng, S.T. Lee, J.S. Hahn, Y.W. Ko and Y.H. Min, 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** (2004), 166–178.

[113] M.S. Kim, M. Blake, J.H. Baek, G. Kohlhagen, Y. Pommier and F. Carrier, Inhibition of histone deacetylase increases cytotoxicity to anticancer drugs targeting DNA, *Cancer Res.* **63** (2003), 7291–7300.

[114] M.I. Klisovic, E.A. Maghraby, M.R. Parthun, M. Guimond, A.R. Sklenar, S.P. Whitman, K.K. Chan, T. Murphy, J. Anon, K.J. Archer, L.J. Rush, C. Plass, M.R. Grever, J.C. Byrd and G. Marcucci, Depsipeptide (FR 901228) promotes histone acetylation, gene transcription, apoptosis and its activity is enhanced by DNA methyltransferase inhibitors in AML1/ETO-positive leukemic cells, *Leukemia* **17** (2003), 350–358.

[115] R.B. Klisovic, W. Blum, B. Hackanson, C. Kefauver, S. Liu, Z. Liu, K.K. Chan, C. Plass, M.R. Grever, J.C. Byrd and G. Marcucci, Updated results of a phase I study of low dose decitabine and valproic acid (VA) in patients with acute myeloid leukemia (AML): Gene reexpression, demethylation, and clinical response, in: *American Society of Clinical Oncology Annual Meeting*, 2006.

[116] R.D. Kornberg, Chromatin structure: a repeating unit of histones and DNA, *Science* **184** (1974), 868–871.

[117] R.D. Kornberg and J.O. Thomas, Chromatin structure; oligomers of the histones, *Science* **184** (1974), 865–868.

[118] R.S. Kristeleit, D. Tandy, P. Atadja, A. Patnaik, J. Scott, J.S. DeBono, I. Judson, S.B. Kaye, P. Workman and W. Aherne, Effects of the histone deacetylase inhibitor LAQ824 on histone acetylation, Hsp70 and c-Raf in peripheral blood lymphocytes from patients wih advanced solid tumours enrolled in a phase I clinical trial, in: *American Society of Clinical Oncology Annual Meeting*, USA, 2004.

[119] E.U. Kurz, S.E. Wilson, K.B. Leader, B.P. Sampey, W.P. Allan, J.C. Yalowich and D.J. Kroll, The histone deacetylase inhibitor sodium butyrate induces DNA topoisomerase II alpha expression and confers hypersensitivity to etoposide in human leukemic cell lines, *Mol. Cancer Ther.* **1** (2001), 121–131.

[120] H.J. Kwon, M.S. Kim, M.J. Kim, H. Nakajima and K.W. Kim, Histone deacetylase inhibitor FK228 inhibits tumor angiogenesis, *Int. J. Cancer* **97** (2002), 290–296.

[121] G. Lagger, D. O'Carroll, M. Rembold, H. Khier, J. Tischler, G. Weitzer, B. Schuettengruber, C. Hauser, R. Brunmeir, T. Jenuwein and C. Seiser, Essential function of histone deacetylase 1 in proliferation control and CDK inhibitor repression, *Embo J.* **21** (2002), 2672–2681.

[122] E. Langley, M. Pearson, M. Faretta, U.M. Bauer, R.A. Frye, S. Minucci, P.G. Pelicci and T. Kouzarides, Human SIR2 deacetylates p53 and antagonizes PML/p53-induced cellular senescence, *Embo J.* **21** (2002), 2383–2396.

[123] O.D. Lau, T.K. Kundu, R.E. Soccio, S. Ait-Si-Ali, E.M. Khalil, A. Vassilev, A.P. Wolffe, Y. Nakatani, R.G. Roeder and P.A. Cole, HATs off: selective synthetic inhibitors of the histone acetyltransferases p300 and PCAF, *Mol. Cell* **5** (2000), 589–595.

[124] F. Leoni, A. Zaliani, G. Bertolini, G. Porro, P. Pagani, P. Pozzi, G. Dona, G. Fossati, S. Sozzani, T. Azam, P. Bufler, G. Fantuzzi, I. Goncharov, S.H. Kim, B.J. Pomerantz, L.L. Reznikov, B. Siegmund, C.A. Dinarello and P. Mascagni, The antitumor histone deacetylase inhibitor suberoylanilide hydroxamic acid exhibits antiinflammatory properties via suppression of cytokines, *Proc. Natl. Acad. Sci. USA* **99** (2002), 2995–3000.

[125] Q. Lu, Y.T. Yang, C.S. Chen, M. Davis, J.C. Byrd, M.R. Etherton and A. Umar, $Zn2^{+}$-chelating motif-tethered short-chain fatty acids as a novel class of histone deacetylase inhibitors, *J. Med. Chem.* **47** (2004), 467–474.

[126] K. Luger, Structure and dynamic behavior of nucleosomes, *Curr. Opin. Genet. Dev.* **13** (2003), 127–135.

[127] B. Lutterbach, J.J. Westendorf, B. Linggi, A. Patten, M. Moniwa, J.R. Davie, K.D. Huynh, V.J. Bardwell, R.M. Lavinsky, M.G. Rosenfeld, C. Glass, E. Seto and S.W. Hiebert, ETO, a target of t(8;21) in acute leukemia, interacts with the N-CoR and mSin3 corepressors, *Mol. Cell Biol.* **18** (1998), 7176–7184.

[128] R. Maruyama, S. Toyooka, K.O. Toyooka, A.K. Virmani, S. Zochbauer-Muller, A.J. Farinas, J.D. Minna, J. McConnell, E.P. Frenkel and A.F. Gazdar, Aberrant promoter methylation profile of prostate cancers and its relationship to clinicopathological features, *Clin. Cancer Res.* **8** (2002), 514–519.

[129] S. McGraw, C. Robert, L. Massicotte and M.A. Sirard, Quantification of histone acetyltransferase and histone deacetylase transcripts during early bovine embryo development, *Biol. Reprod.* **68** (2003), 383–389.

[130] T.A. McKinsey and E.N. Olson, Dual roles of histone deacetylases in the control of cardiac growth, *Novartis Found Symp.* **259** (2004), 132–141; discussion 141–135, 163–139.

[131] R.W. Miller and J.H. Rubinstein, Tumors in Rubinstein–Taybi syndrome, *Am. J. Med. Genet.* **56** (1995), 112–115.

[132] S. Minucci, V. Horn, N. Bhattacharyya, V. Russanova, V.V. Ogryzko, L. Gabriele, B.H. Howard and K. Ozato, A histone deacetylase inhibitor potentiates retinoid receptor action in embryonal carcinoma cells, *Proc. Natl. Acad. Sci. USA* **94** (1997), 11295–11300.

[133] S. Minucci, M. Maccarana, M. Cioce, P. De Luca, V. Gelmetti, S. Segalla, L. Di Croce, S. Giavara, C. Matteucci, A. Gobbi, A. Bianchini, E. Colombo, I. Schiavoni, G. Badaracco, X. Hu, M.A. Lazar, N. Landsberger, C. Nervi and P.G. Pelicci, Oligomerization of RAR and AML1 transcription factors as a novel mechanism of oncogenic activation, *Mol. Cell* **5** (2000), 811–820.

[134] S. Minucci, C. Nervi, F. Lo Coco and P.G. Pelicci, Histone deacetylases: a common molecular target for differentiation treatment of acute myeloid leukemias?, *Oncogene* **20** (2001), 3110–3115.

[135] C.S. Mitsiades, N.S. Mitsiades, C.J. McMullan, V. Poulaki, R. Shringarpure, T. Hideshima, M. Akiyama, D. Chauhan, N. Munshi, X. Gu, C. Bailey, M. Joseph, T.A. Libermann, V.M. Richon, P.A. Marks and K.C. Anderson, Transcriptional signature of histone deacetylase inhibition in multiple myeloma: biological and clinical implications, *Proc. Natl. Acad. Sci. USA* **101** (2004), 540–545.

[136] G. Mizuguchi, X. Shen, J. Landry, W.H. Wu, S. Sen and C. Wu, ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex, *Science* **303** (2004), 343–348.

[137] O. Moradei, C.R. Maroun, I. Paquin and A. Vaisburg, Histone deacetylase inhibitors: latest developments, trends and prospects, *Curr. Med. Chem. Anticancer Agents* **5** (2005), 529–560.

[138] P.N. Munster, D.C. Marchion, E. Bicaku, M.L. Schmitt, B. Padilla, P. Stauffer, C. Garrett, A. Chiappori, D.M. Sullivan and A.I. Daud, Phase I trial of a sequence-specific combination of the HDAC inhibitor, valproic acid (VPA), and the topoisomerase II inhibitor, epirubicin, in advanced solid tumors: Clinical results and correlative studies, in: *American Society of Clinical Oncology Annual Meeting*, 2006.

[139] J. Murakami, J. Asaumi, Y. Maki, H. Tsujigiwa, M. Kuroda, N. Nagai, Y. Yanagi, T. Inoue, S. Kawasaki, N. Tanaka, N. Matsubara, and K. Kishi, Effects of demethylating agent 5-aza-2($'$)-deoxycytidine and histone deacetylase inhibitor FR901228 on maspin gene expression in oral cancer cell lines, *Oral Oncol.* **40** (2004), 597–603.

[140] M. Muraoka, M. Konishi, R. Kikuchi-Yanoshita, K. Tanaka, N. Shitara, J.M. Chong, T. Iwama and M. Miyaki, p300 gene alterations in colorectal and gastric carcinomas, *Oncogene* **12** (1996), 1565–1569.

[141] M.C. Myzak, P.A. Karplus, F.L. Chung and R.H. Dashwood, A novel mechanism of chemoprotection by sulforaphane: in-

hibition of histone deacetylase, *Cancer Res.* **64** (2004), 5767–5774.

[142] A.F. Neuwald and D. Landsman, GCN5-related histone N-acetyltransferases belong to a diverse superfamily that includes the yeast SPT10 protein, *Trends Biochem. Sci.* **22** (1997), 154–155.

[143] D.M. Nguyen, W.D. Schrump, G.A. Chen, W. Tsai, P. Nguyen, J.B. Trepel and D.S. Schrump, Abrogation of p21 expression by flavopiridol enhances depsipeptide-mediated apoptosis in malignant pleural mesothelioma cells, *Clin. Cancer Res.* **10** (2004), 1813–1825.

[144] R. Nimmanapalli, L. Fuino, P. Bali, M. Gasparetto, M. Glozak, J. Tao, L. Moscinski, C. Smith, J. Wu, R. Jove, P. Atadja and K. Bhalla, 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** (2003), 5126–5135.

[145] R. Nimmanapalli, L. Fuino, C. Stobaugh, V. Richon and K. Bhalla, Cotreatment with the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) enhances imatinib-induced apoptosis of Bcr-Abl-positive human acute leukemia cells, *Blood* **101** (2003), 3236–3239.

[146] A. Nudelman, E. Gnizi, Y. Katz, R. Azulai, M. Cohen-Ohana, R. Zhuk, S.R. Sampson, L. Langzam, E. Fibach, E. Prus, V. Pugach and A. Rephaeli, Prodrugs of butyric acid. Novel derivatives possessing increased aqueous solubility and potential for treating cancer and blood diseases, *Eur. J. Med. Chem.* **36**, (2001), 63–74.

[147] V.V. Ogryzko, R.L. Schiltz, V. Russanova, B.H. Howard and Y. Nakatani, The transcriptional coactivators p300 and CBP are histone acetyltransferases, *Cell* **87** (1996), 953–959.

[148] A.L. Olins and D.E. Olins, Spheroid chromatin units (v bodies), *Science* **183** (1974), 330–332.

[149] D.E. Olins and A.L. Olins, Chromatin history: our view from the bridge, *Nat. Rev. Mol. Cell Biol.* **4** (2003), 809–814.

[150] E. Olsen, Y.H. Kim, T. Kuzel, T.R. Pacheco, F. Foss, S. Parker, J.G. Wang, S.R. Frankel, L. Lis and M. Duvic, Vorinostat (suberoylanilide hydroxamic acid, SAHA) is clinically active in advanced cutaneous T-cell lymphoma (CTCL): Results of a phase IIb trial, in: *American Society of Clinical Oncology Annual Meeting*, 2006.

[151] P. Oudet, M. Gross-Bellard and P. Chambon, Electron microscopic and biochemical evidence that chromatin structure is a repeating unit, *Cell* **4** (1975), 281–300.

[152] K. Petrie, F. Guidez, L. Howell, L. Healy, S. Waxman, M. Greaves and A. Zelent, The histone deacetylase 9 gene encodes multiple protein isoforms, *J. Biol. Chem.* **278** (2003), 16059–16072.

[153] C.J. Phiel, F. Zhang, E.Y. Huang, M.G. Guenther, M.A. Lazar and P.S. Klein, Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen, *J. Biol. Chem.* **276** (2001), 36734–36741.

[154] A.C. Phillips and K.H. Vousden, Acetyltransferases and tumour suppression, *Breast Cancer Res.* **2** (2000), 244–246.

[155] R. Piekarz, R. Frye, B.M. Turner, J. Wright, J. Leonard, S. Allen and S. Bates, Update on the phase II trial and correlative studies of depsipeptide in patients with cutaneous T-cell lymphoma and relapsed peripheral T-cell lymphoma, in: *American Society of Clinical Oncology Annual Meeting*, USA, 2004.

[156] R. Pili, M.P. Kruszewski, B.W. Hager, J. Lantz and M.A. Carducci, Combination of phenylbutyrate and 13-cis retinoic acid inhibits prostate tumor growth and angiogenesis, *Cancer Res.* **61** (2001), 1477–1485.

[157] R. Pili, M. Rudek, S. Altiok, D. Qian, M. Zhao, R. Donehower, A. Anderson, M. Halter, H. McFarland, J. Zwiebel and M. Carducci, Phase 1 pharmacokinetic and pharmacodynamic study of the histone deacetylase inhibitor MS-275 in combination with 13-cis retinoic acid in patients with advanced solid tumors, in: *American Society of Clinical Oncology Annual Meeting*, 2006.

[158] J.A. Plumb, P.W. Finn, R.J. Williams, M.J. Bandara, M.R. Romero, C.J. Watkins, N.B. La Thangue and R. Brown, Pharmacodynamic response and inhibition of growth of human tumor xenografts by the novel histone deacetylase inhibitor PXD101, *Mol. Cancer Ther.* **2** (2003), 721–728.

[159] B. Polevoda and F. Sherman, The diversity of acetylated proteins, *Genome Biol.* **3** (2002), reviews 0006.

[160] M. Primeau, J. Gagnon and R.L. Momparler, 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** (2003), 177–184.

[161] K. Pruitt, R.L. Zinn, J.E. Ohm, K.M. McGarvey, S.H. Kang, D.N. Watkins, J.G. Herman and S.B. Baylin, Inhibition of SIRT1 reactivates silenced cancer genes without loss of promoter DNA hypermethylation, *PLoS Genet.* **2** (2006), e40.

[162] D.Z. Qian, X. Wang, S.K. Kachhap, Y. Kato, Y. Wei, L. Zhang, P. Atadja and R. Pili, The histone deacetylase inhibitor NVP-LAQ824 inhibits angiogenesis and has a greater antitumor effect in combination with the vascular endothelial growth factor receptor tyrosine kinase inhibitor PTK787/ZK222584, *Cancer Res.* **64** (2004), 6626–6634.

[163] L. Qiu, A. Burgess, D.P. Fairlie, H. Leonard, P.G. Parsons and B.G. Gabrielli, Histone deacetylase inhibitors trigger a G2 checkpoint in normal cells that is defective in tumor cells, *Mol. Biol. Cell* **11** (2000), 2069–2083.

[164] I. Rahman, J. Marwick and P. Kirkham, Redox modulation of chromatin remodeling: impact on histone acetylation and deacetylation, NF-kappaB and pro-inflammatory gene expression, *Biochem. Pharmacol.* **68** (2004), 1255–1267.

[165] M. Rahmani, C. Yu, Y. Dai, E. Reese, W. Ahmed, P. Dent and S. Grant, 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** (2003), 8420–8427.

[166] V. Ramakrishnan, J.T. Finch, V. Graziano, P.L. Lee and R.M. Sweet, Crystal structure of globular domain of histone H5 and its implications for nucleosome binding, *Nature* **362** (1993), 219–223.

[167] A. Rascle, J.A. Johnston and B. Amati, Deacetylase activity is required for recruitment of the basal transcription machinery and transactivation by STAT5, *Mol. Cell Biol.* **23** (2003), 4162–4173.

[168] T. Reid, F. Valone, W. Lipera, D. Irwin, W. Paroly, R. Natale, S. Sreedharan, H. Keer, B. Lum, F. Scappaticci and A. Bhatnagar, Phase II trial of the histone deacetylase inhibitor pivaloyloxymethyl butyrate (Pivanex, AN-9) in advanced non-small cell lung cancer, *Lung Cancer* **45** (2004), 381–386.

[169] V.M. Richon, T.W. Sandhoff, R.A. Rifkind and P.A. Marks, Histone deacetylase inhibitor selectively induces p21WAF1 expression and gene-associated histone acetylation, *Proc. Natl. Acad. Sci. USA* **97** (2000), 10014–10019.

[170] V.M. Richon, Y. Webb, R. Merger, T. Sheppard, B. Jursic, L. Ngo, F. Civoli, R. Breslow, R.A. Rifkind and P.A. Marks, Second generation hybrid polar compounds are potent inducers of transformed cell differentiation, *Proc. Natl. Acad. Sci. USA* **93** (1996), 5705–5708.

[171] D. Robyr, Y. Suka, I. Xenarios, S.K. Kurdistani, A. Wang, N. Suka and M. Grunstein, Microarray deacetylation maps determine genome-wide functions for yeast histone deacetylases, *Cell* **109** (2002), 437–446.

[172] S. Ropero, M.F. Fraga, E. Ballestar, R. Hamelin, H. Yamamoto, M. Boix-Chornet, R. Caballero, M. Alaminos, F. Setien, M.F. Paz, M. Herranz, J. Palacios, D. Arango, T.F. Orntoft, L.A. Aaltonen, S. Schwartz, Jr. and M. Esteller, A truncating mutation of HDAC2 in human cancers confers resistance to histone deacetylase inhibition, *Nat. Genet.* **38** (2006), 566–569.

[173] R.R. Rosato, J.A. Almenara, Y. Daiand and S. Grant, 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** (2003), 1273–1284.

[174] R.R. Rosato, J.A. Almenara and S. Grant, The histone deacetylase inhibitor MS-275 promotes differentiation or apoptosis in human leukemia cells through a process regulated by generation of reactive oxygen species and induction of p21CIP1/WAF1 1, *Cancer Res.* **63** (2003), 3637–3645.

[175] R.R. Rosato, J.A. Almenara, C. Yu and S. Grant, 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** (2004), 571–581.

[176] R.R. Rosato, Z. Wang, R.V. Gopalkrishnan, P.B. Fisher and S. Grant, 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** (2001), 181–191.

[177] S.Y. Roth, J.M. Denu and C.D. Allis, Histone acetyltransferases, *Annu. Rev. Biochem.* **70** (2001), 81–120.

[178] E.d.B.J. Rowinsky, D.J. Deangelo, A. van Oosterom, J. Morganroth, G.H. Laird, M. Dugan, W.J. Scott and O.G. Ottmann, Cardiac monitoring in phase I trials of a novel histone deacetylase (HDAC) inhibitor LAQ824 in patients with advanced solid tumors and hematologic malignancies, in: *American Society of Clinical Oncology Annual Meeting*, 2005.

[179] A.A. Ruefli, M.J. Ausserlechner, D. Bernhard, V.R. Sutton, K.M. Tainton, R. Kofler, M.J. Smyth and R.W. Johnstone, The histone deacetylase inhibitor and chemotherapeutic agent suberoylanilide hydroxamic acid (SAHA) induces a cell-death pathway characterized by cleavage of Bid and production of reactive oxygen species, *Proc. Natl. Acad. Sci. USA* **98** (2001), 10833–10838.

[180] Q.C. Ryan, D. Headlee, M. Acharya, A. Sparreboom, J.B. Trepel, J. Ye, W.D. Figg, K. Hwang, E.J. Chung, A. Murgo, G. Melillo, Y. Elsayed, M. Monga, M. Kalnitskiy, J. Zwiebel and E.A. Sausville, Phase I and pharmacokinetic study of MS-275, a histone deacetylase inhibitor, in patients with advanced and refractory solid tumors or lymphoma, *J. Clin. Oncol.* **23** (2005), 3912–3922.

[181] V. Sandor, A. Senderowicz, S. Mertins, D. Sackett, E. Sausville, M.V. Blagosklonny and S.E. Bates, P21-dependent g(1)arrest with downregulation of cyclin D1 and upregulation of cyclin E by the histone deacetylase inhibitor FR901228, *Br. J. Cancer* **83** (2000), 817–825.

[182] H. Santos-Rosa, E. Valls, T. Kouzarides and M. Martinez-Balbas, Mechanisms of P/CAF auto-acetylation, *Nucleic Acids Res.* **31** (2003), 4285–4292.

[183] D.B. Seligson, S. Horvath, T. Shi, H. Yu, S. Tze, M. Grunstein and S.K. Kurdistani, Global histone modification patterns predict risk of prostate cancer recurrence, *Nature* **435** (2005), 1262–1266.

[184] L.C.M. Siu, L. Pearce, M. Maclean, R. Sullivan, Z. Li, A. Kalita, E.X. Chen, J. Longstreth, R.E. Martell and G.K. Reid, Phase I study of isotype-selective histone deacetylase (HDAC) inhibitor MGCD0103 given as three-times weekly oral dose in patients (pts) with advanced solid tumors, in: *EORTC*, USA, 2005.

[185] A.O. Soriano, H. Yang, S. Verstovsek, W. Wierda, C. Koller, Z. Estrov, S. Ouzounian, H. Kantarjian, J. Issa and G. Garcia-Manero, Phase I/II study of the combination of 5-azacytidine(5-AC), all-trans retinoic acid (ATRA) and valproic Acid (VPA) in patients with myelodysplastic syndrome (MDS) and leukemia, in: *American Society of Clinical Oncology Annual Meeting*, 2006.

[186] N.L.V. Steele, J. Plumb, G. Attard, A. Rasmussen, P. Buhl-Jensen, R. Brown, S. Blagden, J. Evans and J. de Bono, A phase 1 pharmacokinetic (PK) and pharmacodynamic (PD) study of the histone deacetylase (HDAC) inhibitor PXD101 in patients (pts) with advanced solid tumours, in: *American Society of Clinical Oncology Annual Meeting*, 2005.

[187] B.D. Strahl and C.D. Allis, The language of covalent histone modifications, *Nature* **403** (2000), 41–45.

[188] N. Takai, J.C. Desmond, T. Kumagai, D. Gui, J.W. Said, S. Whittaker, I. Miyakawa and H.P. Koeffler, Histone deacetylase inhibitors have a profound antigrowth activity in endometrial cancer cells, *Clin. Cancer Res.* **10** (2004), 1141–1149.

[189] A. Thibault, M.R. Cooper, W.D. Figg, D.J. Venzon, A.O. Sartor, A.C. Tompkins, M.S. Weinberger, D.J. Headlee, N.A. McCall and D. Samid, A phase I and pharmacokinetic study of intravenous phenylacetate in patients with cancer, *Cancer Res.* **54** (1994), 1690–1694.

[190] S.G.F. Toma, L. Emionite, M. Grattarola and L. Vergani, Effects of HDACIs and retinoids on breast cancer cells, in: *American Society of Clinical Oncology Annual Meeting*, 2003.

[191] B.M. Turner, Decoding the nucleosome, *Cell* **75** (1993), 5–8.

[192] H. Vaghefi and K.E. Neet, Deacetylation of p53 after nerve growth factor treatment in PC12 cells as a post-translational modification mechanism of neurotrophin-induced tumor suppressor activation, *Oncogene* (2004).

[193] R.A. Varier, V. Swaminathan, K. Balasubramanyam and T.K. Kundu, Implications of small molecule activators and inhibitors of histone acetyltransferases in chromatin therapy, *Biochem. Pharmacol.* **68** (2004), 1215–1220.

[194] K. Vasudev, S. Das, U. Goswami and G. Tayal, Pharmacokinetics of valproic acid in patients with bipolar disorder, *J. Psychopharmacol.* **15** (2001), 187–190.

[195] A. Vasudevan, Z. Ji, R.R. Frey, C.K. Wada, D. Steinman, H.R. Heyman, Y. Guo, M.L. Curtin, J. Guo, J. Li, L. Pease, K.B. Glaser, P.A. Marcotte, J.J. Bouska, S.K. Davidsen, M.R. Michaelides, Y. Dai, L.J. Pease, M.J. Staver, R.Q. Wei, D.H. Albert, R.B. Garland, J.H. Holms, P.L. Richardson, S.S. Murphy, P. Tapang, T.J. Magoc, J.F. Waring, J. Stender, R.G. Ulrich, M.E. Aakre, D.W. Morgan, G. Sheppard, K.D. Stewart, J. Pollock, P. Lee, C.Z. O'Connor, S.N. Anderson, D.J. Mussatto, C.W. Wegner, and H.L. Moses, Heterocyclic ketones as inhibitors of histone deacetylase, *Bioorg. Med. Chem. Lett.* **13** (2003), 3909–3913.

[196] H. Vaziri, S.K. Dessain, E. Ng Eaton, S.I. Imai, R.A. Frye, T.K. Pandita, L. Guarente and R.A. Weinberg, hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase, *Cell* **107** (2001), 149–159.

[197] E. Verdin, F. Dequiedt and H.G. Kasler, Class II histone deacetylases: versatile regulators, *Trends Genet.* **19** (2003), 286–293.

[198] D.M. Vigushin, S. Ali, P.E. Pace, N. Mirsaidi, K. Ito, I. Adcock and R.C. Coombes, Trichostatin A is a histone deacetylase inhibitor with potent antitumor activity against breast cancer in vivo, *Clin. Cancer Res.* **7** (2001), 971–976.

[199] J.A. Vrana, R.H. Decker, C.R. Johnson, Z. Wang, W.D. Jarvis, V.M. Richon, M. Ehinger, P.B. Fisher and S. Grant, Induction of apoptosis in U937 human leukemia cells by suberoylanilide hydroxamic acid (SAHA) proceeds through pathways that are regulated by Bcl-2/Bcl-XL, c-Jun, and p21CIP1, but independent of p53, *Oncogene* **18** (1999), 7016–7025.

[200] D. Waltregny, W. Glenisson, S.L. Tran, B.J. North, E. Verdin, A. Colige and V. Castronovo, Histone deacetylase HDAC8 associates with smooth muscle alpha-actin and is essential for smooth muscle cell contractility, *FASEB J.* **19** (2005), 966–968.

[201] D. Waltregny, B. North, F. Van Mellaert, J. de Leval, E. Verdin and V. Castronovo, Screening of histone deacetylases (HDAC) expression in human prostate cancer reveals distinct class I HDAC profiles between epithelial and stromal cells, *Eur. J. Histochem.* **48** (2004), 273–290.

[202] J. Wang, Y. Saunthararajah, R.L. Redner and J.M. Liu, Inhibitors of histone deacetylase relieve ETO-mediated repression and induce differentiation of AML1-ETO leukemia cells, *Cancer Res.* **59** (1999), 2766–2769.

[203] R.P. Warrell, Jr., L.Z. He, V. Richon, E. Calleja and P.P. Pandolfi, Therapeutic targeting of transcription in acute promyelocytic leukemia by use of an inhibitor of histone deacetylase, *J. Natl. Cancer Inst.* **90** (1998), 1621–1625.

[204] R. Warrener, H. Beamish, A. Burgess, N.J. Waterhouse, N. Giles, D. Fairlie and B. Gabrielli, Tumor cell-selective cytotoxicity by targeting cell cycle checkpoints, *FASEB J.* **17** (2003), 1550–1552.

[205] J.H. Waterborg, Dynamics of histone acetylation in vivo. A function for acetylation turnover?, *Biochem. Cell Biol.* **80** (2002), 363–378.

[206] A.P. Wolffe and J.J. Hayes, Chromatin disruption and modification, *Nucleic Acids Res.* **27** (1999), 711–720.

[207] W. Xu, D.G. Edmondson and S.Y. Roth, Mammalian GCN5 and P/CAF acetyltransferases have homologous amino-terminal domains important for recognition of nucleosomal substrates, *Mol. Cell Biol.* **18** (1998), 5659–5669.

[208] W.S. Xu, G. Perez, L. Ngo, C.Y. Gui and P.A. Marks, Induction of polyploidy by histone deacetylase inhibitor: a pathway for antitumor effects, *Cancer Res.* **65** (2005), 7832–7839.

[209] X. Yang, A.T. Ferguson, S.J. Nass, D.L. Phillips, K.A. Butash, S.M. Wang, J.G. Herman and N.E. Davidson, Transcriptional activation of estrogen receptor alpha in human breast cancer cells by histone deacetylase inhibition, *Cancer Res.* **60** (2000), 6890–6894.

[210] S. Yegnasubramanian, J. Kowalski, M.L. Gonzalgo, M. Zahurak, S. Piantadosi, P.C. Walsh, G.S. Bova, A.M. De Marzo, W.B. Isaacs and W.G. Nelson, Hypermethylation of CpG islands in primary and metastatic human prostate cancer, *Cancer Res.* **64** (2004), 1975–1986.

[211] M. Yoshida, M. Kijima, M. Akita and T. Beppu, Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A, *J. Biol. Chem.* **265** (1990), 17174–17179.

[212] C. Yu, M. Rahmani, J. Almenara, M. Subler, G. Krystal, D. Conrad, L. Varticovski, P. Dent and S. Grant, Histone deacetylase inhibitors promote STI571-mediated apoptosis in STI571-sensitive and -resistant Bcr/Abl+ human myeloid leukemia cells, *Cancer Res.* **63** (2003), 2118–2126.

[213] C. Yu, M. Rahmani, D. Conrad, M. Subler, P. Dent and S. Grant, The proteasome inhibitor bortezomib interacts synergistically with histone deacetylase inhibitors to induce apoptosis in Bcr/Abl+ cells sensitive and resistant to STI571, *Blood* **102** (2003), 3765–3774.

[214] J. Yu, Y. Li, T. Ishizuka, M.G. Guenther and M.A. Lazar, A SANT motif in the SMRT corepressor interprets the histone code and promotes histone deacetylation, *Embo J.* **22** (2003), 3403–3410.

[215] X. Yu, Z.S. Guo, M.G. Marcu, L. Neckers, D.M. Nguyen, G.A. Chen and D.S. Schrump, Modulation of p53, ErbB1, ErbB2, and Raf-1 expression in lung cancer cells by depsipeptide FR901228, *J. Natl. Cancer Inst.* **94** (2002), 504–513.

[216] L. Zeng, Y. Zhang, S. Chien, X. Liu and J.Y. Shyy, The role of p53 deacetylation in p21Waf1 regulation by laminar flow, *J. Biol. Chem.* **278**, (2003), 24594–24599.

[217] X.D. Zhang, S.K. Gillespie, J.M. Borrow and P. Hersey, The histone deacetylase inhibitor suberic bishydroxamate: a potential sensitizer of melanoma to TNF-related apoptosis-inducing ligand (TRAIL) induced apoptosis, *Biochem. Pharmacol.* **66** (2003), 1537–1545.

[218] Y. Zhang, M. Adachi, X. Zhao, R. Kawamura and K. Imai, Histone deacetylase inhibitors FK228, N-(2-aminophenyl)-4-[N-(pyridin-3-yl-methoxycarbonyl)aminomethyl]benzamide and m-carboxycinnamic acid bis-hydrox-

amide augment radiation-induced cell death in gastrointestinal adenocarcinoma cells, *Int. J. Cancer* **110** (2004), 301–308.

[219] Y. Zheng, P.R. Thompson, M. Cebrat, L. Wang, M.K. Devlin, R.M. Alani and P.A. Cole, Selective HAT inhibitors as mechanistic tools for protein acetylation, *Methods Enzymol.* **376**, (2004), 188–199.

[220] P. Zhu, E. Martin, J. Mengwasser, P. Schlag, K.P. Janssen and M. Gottlicher, Induction of HDAC2 expression upon loss of APC in colorectal tumorigenesis, *Cancer Cell* **5** (2004), 455–463.

[221] W.G. Zhu and G.A. Otterson, The interaction of histone deacetylase inhibitors and DNA methyltransferase inhibitors in the treatment of human cancer cells, *Curr. Med. Chem. Anti-Canc. Agents* **3** (2003), 187–199.