Rational Development of Histone Deacetylase Inhibitors as Anti-cancer Agents: A Review

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Abstract

The epigenome is defined by DNA methylation patterns and the associated post-translational modifications of histones. This histone code determines the expression status of individual genes dependent upon their localization on the chromatin. The histone deacetylases (HDACs) play a major role in keeping the balance between the acetylated and deacetylated states of chromatin and eventually regulate gene expression. Recent developments in understanding the cancer cell cycle, specifically the interplay with chromatin control, are providing opportunities for developing mechanism-based therapeutic drugs. Inhibitors of HDACs are under considerable exploration non-clinically and in the clinic, in part due to their potential roles in reversing the silenced genes in transformed tumor cells by modulating transcriptional processes. This review is an effort to summarize the non-clinical and clinical status of HDAC inhibitors currently under development in anticancer therapy.
Introduction

In eukaryotic cells, DNA has been conserved over evolution in a condensed and densely packed higher order structure called chromatin. Chromatin, present in the interphase nucleus, comprises of regular repeating units of nucleosomes, which represent the principal protein-nucleic acid relationship. The major components of chromatin are nucleic acids (DNA and RNA), which are negatively charged, associated proteins including histones that are positively charged at neutral pH and non-histone chromosomal proteins which are acidic at neutral pH. Within the nucleus, chromatin can exists in two different forms; heterochromatin, which is highly compact and transcriptionally inactive form, or euchromatin, which is loosely packed and is accessible to RNA polymerases for involvement in transcriptional processes and gene expression. A nucleosome is a complex of 146 nucleotide base pairs of DNA wrapped around the core histone octamer that helps organize chromatin. The histone octamer is composed of two copies of each of H2A, H2B, H3 and H4 proteins that are very basic mainly due to positively charged amino-terminal side chains rich in amino acid lysine. Post-translational and other changes in chromatin like acetylation/deacetylation at lysine residues, methylation at lysine or arginine residues, phosphorylation at serine resides, ubiquitylation at lysines and/or ADP ribosylation are mediated by chemical modification of various sites on N-terminal tail.(Marks et al., 2003; Marks et al., 2004; Marks et al., 2000)

The structural modification of histones is regulated mainly by acetylation/deacetylation of N-terminal tail and is crucial in modulating gene expression, as it affects the interaction of DNA with transcription-regulatory non-nucleosomal protein complexes. The balance between the acetylated/deacetylated states of histones is
mediated by two different sets of enzymes; histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs preferentially acetylate specific lysine substrates among other non-histone protein substrates and transcription factors, impacting DNA-binding properties and in turn, altering gene transcription. HDACs restore the positive charge on lysine residues by removing acetyl groups and thus are involved primarily in the repression of gene transcription by compacting chromatin structure. Thus, open lysine residues attach firmly to the phosphate backbone of the DNA, preventing transcription. In this tight conformation, transcription factors, regulatory complexes, and RNA polymerases cannot bind to the DNA. Acetylation relaxes the DNA conformation, making it accessible to transcription machinery. High levels of acetylation of core histones are seen in chromatin-containing genes, which are highly transcribed genes; those genes that are silent are associated with low levels of acetylation. Since inappropriate silencing of critical genes can result in one or both hits of tumor suppressor gene (TSG) inactivation in cancer, theoretically the reactivation of affected TSGs could have an enormous therapeutic value in preventing and treating cancer.(Thiagalingam et al., 2003)

Histone acetylases and deacetylases: classification and function

The equilibrium of steady state acetylation is tightly controlled by the antagonistic effect of both HATs and HDACs, which in turn regulates transcription status of not just histones but also of other substrates such as p53. (Grozinger and Schreiber, 2002) Several groups of proteins with HAT activity have been identified, including GNAT (Gcn5-related N-acetyl transferase) family, MYST (monocytic leukemia zinc finger protein)
group, TIP60 (TAT-interactive protein) and the p300/CBP (CREB-binding protein) family. HATs act as large multiprotein complexes containing other HATs, coactivators for transcription factors, and co-repressors. (Annunziato and Hansen, 2000; Chen et al., 2001; Gregory et al., 2001; Nakatani, 2001; Schreiber and Bernstein, 2002) HATs, which bind non-histone protein substrates and transcription factors, have been called factor acetyltransferases. Acetylation of these transcription factors also affects their DNA binding properties and gene transcription. (Gregoretti et al., 2004; Roth et al., 2001) HAT genes may be overexpressed, translocated, or mutated in both hematological and epithelial cancers. (Johnstone and Licht, 2003; Mahlknecht and Hoelzer, 2000; Timmermann et al., 2001) Translocations of HATs, CREB-binding protein (CBP), and p300 acetyltransferases, into genes have given rise to various hematological malignancies. (Fenrick and Hiebert, 1998; Pandolfi, 2001)

There are three major groups or classes of mammalian HDACs based on their structural homologies to the three distinct yeast HDACs: Rpd3 (class I), Hda1 (class II), and Sir2/Hst (class III). Class III HDACs consist of the large family of sirtuins (silent information regulators) (SIRs) that are evolutionarily distinct, with a unique enzymatic mechanism dependent on the cofactor NAD+, and are virtually unaffected by all HDAC inhibitors in current development. (Gray and Ekstrom, 2001; Imai et al., 2000) Class I and II HDACs contain an active site zinc as a critical component of their enzymatic pocket, have been extensively described to have an association with cancers, and are thought to be comparably inhibited by all HDAC inhibitors in development. The Rpd3 homologous class I include HDACs 1, 2, 3 and 8, are widely expressed in tissues and are primarily localized in the nucleus. Hda1 homologous class II HDACs 4, 5, 6, 7, 9a, 9b
and 10, are much larger in size, display limited tissue distribution and can shuttle between the nucleus and cytoplasm, suggesting different functions and cellular substrates from Class I HDACs. (Guardiola and Yao, 2002; Kao et al., 2001) HDACs 6 and 10 are unique as they have two catalytic domains, while HDACs 4, 8 and 9 are expressed to greater extent in tumor tissues and have been shown to be specifically involved in differentiation. (de Ruijter et al., 2003) There is some evidence that certain inhibitors display variable degree of HDAC specificity, and hence it would be imperative to identify differences in HDAC functions to better target and tailor specific drugs compounds. (Grozinger and Schreiber, 2002; Heltweg et al., 2004; Jung, 2001; Miller et al., 2003) HDACs usually interact as constituents of large protein complexes that downregulate genes through association with co-repressors; like nuclear receptor corepressor (NcoR), silencing mediator for retinoid and thyroid hormone receptor (SMRT), transcription factors, estrogen receptors (ER), p53, cell-cycle specific regulators like retinoblastoma (Rb), E2F and other HDACs, as well as histones, but they can also bind to their receptor directly. (Frye, 2000; Imai et al., 2000; Zhou et al., 2002)

Class III HDACs (sirtuins, SIR T1, 2, 3, 4, 5, 6 and 7) are not inhibited by Class I and II HDAC inhibitors, but instead are inhibited by nicotinamide (Vitamin B3). Nicotinamide inhibits an NAD-dependent p53 deacetylation induced by SIR2alpha, and also enhances the p53 acetylation levels in vivo. (Luo et al., 2001) Recently it has been shown that by restraining mammalian forkhead proteins, specifically foxo3a, SIRT1 also reduces apoptosis. (Motta et al., 2004) The inhibition of forkhead activity by SIRT1 parallels the effect of this deacetylase on the tumor suppressor p53. These results have significant implications regarding an important role for Sirtuins in modulating the
sensitivity of cells in p53-dependent apoptotic response and the possible effect in cancer therapy. (North et al., 2003; Schwer et al., 2002)

**Chromatin modification and Cancer**

DNA gene expression is controlled by an assembly of nucleoproteins that includes histones and other architectural components of chromatin, non-histone DNA-bound regulators, and additional chromatin-bound polypeptides. Changes in growth and differentiation leading to malignancy appear to occur by alterations in transcriptional control and gene silencing. It is becoming increasingly apparent that imbalances of both DNA methylation and histone acetylation may play an important role in cancer development and progression. (Jones and Baylin, 2002; Marks et al., 2001; Marks et al., 2004; Timmermann et al., 2001) Unlike normal cells, in cancer, changes in genome expression are associated with the remodeling of long regions of regulatory DNA, including promoters, enhancers, locus control regions, and insulators, into specific chromatin architecture. These specific changes in the DNA architecture result in a general molecular signature for a type of cancer and complement its DNA methylation-based component. The changes in the infrastructure of chromatin over a target promoter are more profound than those observed by these enzymes acting independently. (Davis and Brackmann, 2003; Wade, 2001) Apart from acetylation, histone tails undergo other modifications including methylation, phosphorylation, ubiquitylation and adenosine diphosphate ribosylation. These other areas of modifications (“histone code”) have not yet been explored enough to identify their roles in epigenetic modifications. (Bhalla and List, 2004)
Disruption of HAT and HDAC function is associated with the development of cancer and malignant cells target chromatin-remodeling pathways as a means of disrupting transcriptional regulation. (Mahlknecht and Hoelzer, 2000) Of the various hypotheses describing deregulation mechanisms, following three have been put forth frequently: i) disordered hyperacetylation could activate promoters that are normally repressed leading to inappropriate expression of proteins, ii) abnormally decreased acetylation levels of promoter regions could repress the expression of genes necessary for a certain phenotype and iii) mistargeted or aberrant recruitment of HAT/HDAC activity could act as a pathological trigger. Even though there have been no direct alterations in HDAC genes demonstrated in cancer, the association of HDACs with various oncogenes and tumor suppressor genes is now well-established, as is the potential for HDAC involvement in tumorigenesis. (Kristeleit et al., 2004)

Histone deacetylase inhibitors as anticancer agents

The findings of recruitment of HDAC enzymes in cancer have provided a rationale for using inhibition of HDAC activity to release transcriptional repression as a viable option towards achieving eventual therapeutic benefit. (Johnstone and Licht, 2003) Inhibition of HDAC function can release dysregulation of genes involved in cell cycle progression, differentiation and apoptosis. HDAC inhibitors block deacetylation function, causing cell cycle arrest, differentiation, and/or apoptosis of many tumors. (Pandolfi, 2001) Several HDAC inhibitors have exhibited potent antitumor activity in human xenograft models, suggesting their usefulness as novel cancer therapeutic agents. Several are currently in phase I/II clinical trials both in hematological malignancies and in solid
tumors. Compared to agents used initially, some newer agents are effective at nanomolar concentrations and are relatively less toxic. A wide range of structures inhibit activity of class I/II HDAC enzymes, and with a few exceptions these can be divided into structural classes including: (1) carboxylates (short chain fatty acids), (2) small-molecule hydroxamates, (3) electrophilic ketones (epoxides), (4) cyclic peptides and (5) benzamides and (6) other hybrid compounds. (Drummond et al., 2004) Table I describes the various compounds, their activities in cell lines and pre-clinical murine models and their current clinical status.

Comprehensive reviews on structure, medicinal chemistry and structure-activity relationships of more than 80 different HDAC inhibitors and analogues have been previously published or reviewed. (Arts et al., 2003; Bouchain and Delorme, 2003; Bouchain et al., 2003; Curtin and Glaser, 2003; Curtin et al., 2002; Drummond et al., 2004; Kim et al., 2003; Kouraklis and Theocharis, 2002; Kristeleit et al., 2004; Miller et al., 2003; Plumb et al., 2004; Remiszewski, 2002; Remiszewski, 2003; Remiszewski et al., 2002) Despite the variety of structural distinctiveness, all of these HDAC inhibitors can be broadly characterized by a common pharmacophore that includes key elements of inhibitor-enzyme interactions. (Miller et al., 2003) Most of these compounds were designed to have three basic components: a hydrophobic cap that blocks the entrance to active site, a polar site and a hydroxamic acid type zinc-binding active site separated by a hydrophobic spacer that has optimal length spanning the hydrophobic pocket on the enzyme. (Drummond et al., 2004)
Short chain fatty acids

Dimethyl sulfoxide was one of the first compounds identified to be active in transformed cell differentiation. As a result of this, several compounds were synthesized and screened for activity in differentiation, growth arrest and or apoptosis. (Marks et al., 2001) Valproic acid is effective in vitro as a HDAC inhibitor at relatively high (millimolar) concentrations and has much weaker affinity. It has been shown to selectively induce proteosomal degeneration of HDAC2 and is antiangiogenic in vitro and in vivo. (Eyal et al., 2004; Kramer et al., 2003; Michaelis et al., 2004) Valproic acid, a well-established anticonvulsant for seizures and bipolar disorders, has been shown to have antigrowth activity of human endometrial cells and also to inhibit proliferation and induce apoptosis in acute myeloid leukemia cells expressing ABCB1 (P-glycoprotein) and the multi-drug resistance protein ABCC1 (MRP1). (Takai et al., 2004; Tang et al., 2004) (Gurvich et al., 2004) Valproic acid has been recently shown to inhibit angiogenesis in vitro and in vivo and markedly effects genes relevant in proliferation and apoptosis. (Michaelis et al., 2004; Thelen et al., 2004)

Phenyl acetate (PA) can penetrate the CNS and when tested in solid tumors, showed antitumor effects mediated by histone acetylation. PA is a metabolite of phenylbutyrate (PB) after B-oxidation in the liver and kidney. (Carducci et al., 1996; Piscitelli et al., 1995) PB, a well studied member of the short chain fatty acids, can arrest cells in G1–G0 by inducing p21WAF1 and other cdk-2-associated cell cycle proteins, alter levels of expression of activation and chemotaxis proteins such as urokinase-plasminogen activator, induce apoptosis, inhibit telomerase, and increase MHC class I expression, in various tumor models. (Gore and Carducci, 2000) However, the short chain fatty acids
have a low potency due to their short side chains, limiting their contact with the catalytic pocket of HDACs. (Johnstone, 2002) In human CCRF-CEM, acute T-lymphoblastic leukemia cells, butyrate and other HDAC inhibitors caused G2/M cell cycle arrest as well as apoptotic cell death. (Bernhard et al., 1999) Butyrates induce histone acetylation and granulocyte maturation in AML, selectively inhibits growth in human prostate cancer and cervical carcinoma cells. (Finzer et al., 2003; Gozzini et al., 2003; Kuefer et al., 2004) Butyrates have been under extensive clinical evaluation in both hematological malignancies and solid tumors. Butanoic acid or its prodrug pivaloyl oxymethyl butyrate (AN-9) is currently undergoing clinical trial after it showed 10-fold more potent activity than SB in leukemia tumor cell lines. (Batova et al., 2002; Patnaik et al., 2002; Reid et al., 2004) The antineoplastic activity of AN-9 stems from rapid hydrolysis and release of butyrate, permitting efficient delivery to subcellular targets. (Zimra et al., 2000; Zimra et al., 1997) In spite of overall weak activity of SCFA, several agents have been studied clinically owing to their use for alternative medical conditions. (Gore et al., 2002; Gore et al., 2001; Melchior et al., 1999)

Hydroxamic acids

This is the broadest class of inhibitors with high affinity for HDAC, which inhibit both HDAC I and II. Inhibitors containing hydroxamic acid (HA) residues bind with high affinity to the HDAC catalytic site, blocking the access of the substrate to the zinc ion. (Finnin et al., 1999) The general structure of these substances consists of a hydrophobic linker that allows the hydroxamic acid moiety to chelate the cation at the bottom of the HDAC catalytic pocket, while the bulky part of the molecule acts as a cap for the tube.
Most of the chemicals in this group are very potent (functioning at nanomolar to micromolar concentrations *in vitro*) but are reversible inhibitors of class I/II HDACs.

Trichostatin A (TSA) was one of the first HDAC inhibitors to be described and is widely used as a reference in research in this field. (Yoshida et al., 2001; Yoshida et al., 1995) It was developed as an antifungal agent but is relatively unstable and due to its toxicity to patients and lack of specificity for certain HDACs motivated the search for other substances. (Jung, 2001; Jung et al., 1999) The design of many synthetic drugs has been inspired by the TSA structure (the aromatic "cap", hydroxamic acid functionality and hydrophobic linker between them). TSA blocks proliferation and triggers apoptosis in hepatocellular carcinoma cells, blocks cell cycle progression in HeLa cells and differentiation in ovarian cancer cells by changing p21 tumor suppressor gene and DNA-binding Id1 protein. (Herold et al., 2002; Hoshikawa et al., 1994; Strait et al., 2002) TSA has also been shown to suppress growth of pancreatic adenocarcinoma cells and ACHN renal cell carcinoma via cell cycle arrest in association with p27, or apoptosis. (Donadelli et al., 2003; Park et al., 2003) TSA is more sensitive in estrogen receptor alpha positive breast cancer cells in inhibiting HDAC. (Margueron et al., 2003)

Simple hydroxamic acid derivatives such as suberoylanilide hydroxamic acid (SAHA) and pyroxamide have activity at submicromolar concentrations. (Marks, 2004; Richon et al., 1998; Richon et al., 2001) SAHA is a second-generation polar-planar compound that induces growth arrest, differentiation and/or apoptosis and is under clinical investigation in both hematological and non-hematological malignancies. (Coffey et al., 2000; Munster et al., 2001; Richon et al., 1998; Richon et al., 1996) In studies with breast cancer cells, SAHA inhibited clonogenic growth and induced apoptosis, while in
malignant human hemotopoietic cells; SAHA induced marked toxicity but showed relatively minor maturation activity. (Huang and Pardee, 2000; Vrana et al., 1999) SAHA also showed antiproliferative and pro-apoptotic actions in several mouse xenografts and cancer cells including prostate, bladder carcinoma and myeloma. SAHA also induced the CDK inhibitor p21WAF1/Cip1 and the inhibitory activity was independent of p53 status. (Butler et al., 2000; Butler et al., 2002; Cohen et al., 2002; Gui et al., 2004; Richon et al., 2000) Pyroxamide is another compound in this class that induced terminal differentiation in murine erythroleukemic cells and caused growth inhibition in prostate carcinoma, bladder and neuroblastoma cells via apoptosis. (Butler et al., 2001; Kouraklis and Theocharis, 2002; Kutko et al., 2003) In experiments with SAHA and butyrates, there was model proposed in which induction of apoptosis in Bcr/Abl+ cells by HDAC inhibitors involves coordinate inactivation of the cytoprotective Raf/MEK/ERK pathway in conjunction with the ROS-dependent activation of JNK. (Yu et al., 2003)

Oxamflatin is another compound in the same class which induces transcriptional activation of junD causing cell cycle arrest and morphological changes similar to TSA. (Kim et al., 1999) Scriptaid was found to be one of the most potent analogues in a random search for substances that augment signal transduction pathways and when screened in human and animal tumor cells, showed similar antiproliferative effects as SAHA (Bouchain and Delorme, 2003; Su et al., 2000) NVP-LAQ824, a cinnamic HA has been shown to inhibit HDAC in vitro and cause transcriptional activation of p21 promoter in reporter gene assays as submicromolar concentrations in multiple myeloma. (Catley et al., 2003) NVP-LAQ824 like most other HDAC inhibitors, was selective in its action as it required longer exposure and higher concentrations to retard growth of
normal human fibroblasts. (Atadja et al., 2004) Another HA analogue, suberic bishydroxamate (SBHA) was shown to regulate expression of multiple apoptotic mediators and induce mitochondria-dependent apoptosis in melanoma cells. (Zhang et al., 2004) PXD101 is a novel hydroxamate-type inhibitor of HDAC activity in nanomolar ranges in leukemia cells. It was shown to delay growth for xenografts of cisplatin-resistant ovarian tumor cells and had marked increase in acetylation of histone and showed good antitumor activity (Plumb et al., 2003)

Newer compounds such as cyclic HA peptides (CHAPs), and are a structural combination of HA like TSA and the cyclic tetrapeptides like trapoxin, inhibit isoform selective HDACs at nanomolar concentrations. (Furumai et al., 2001; Nishino et al., 2003) One of the CHAP derivatives, inhibited growth in four of five human tumor lines implanted into nude mice and shows great promise as therapeutic agents with higher selective inhibition of HDAC. (Komatsu et al., 2001)

**Cyclic peptides**

Cyclic peptides having epoxyketone (epoxides) may act by chemically modifying an active site nucleophile with the epoxy group and forming H-bonds with ketone. These chemicals are supposed to trap HDACs through the reaction of the epoxide moiety with the zinc cation or an amino acid (forming a covalent attachment) in the binding pocket. However, the lability of the epoxide functionality prevents significant *in vivo* activity, which makes them of little pharmacologic interest. The only HDAC inhibitors in this set of compounds are a number of natural products with significant *in vitro* activity, such as Trapoxin A, B (TPX), depudecin and 2-amino 8-oxo-9, 10-epoxydecanoic acid (AOE). TPX is a hybrid molecule containing cyclic peptide (acts as hydrophobic cap) and
epoxyketone moiety that has shown irreversible inhibition of mammalian HDACs at nanomolar ranges. (Kijima et al., 1993; Komatsu et al., 2001; Kosugi et al., 1999) Cyclic tetrapeptides such as apicidin, which has an ethyl ketone moiety, as well as FK228 (FR901228, also referred to as depsipeptide), inhibit HDACs at nanomolar concentrations. Apicidin is a fungal metabolite that is able to inhibit HDACs and proliferation of tumor cells via induction of p21WAF1/Cip1 and gelsolin. (Han et al., 2000) It is postulated that apicidin interacts with the catalytic site and has been shown to inhibit cell proliferation in several human cancer cell lines due to its anti-invasive and anti-angiogenic activity. (Hong et al., 2003; Kim et al., 2004; Meinke et al., 2000; Singh et al., 2001; Singh et al., 2002) FK228 is a natural product derived from *Chromobacterium violaceum* that exhibits potent antitumor activity through currently unknown mechanism of action. (Piekarz and Bates, 2004) One hypothesis proposes that the disulfide bridge is reduced inside the cell or organism and the 4-mercaptobut-1-enyl residue then fits inside the HDAC catalytic pocket, chelating Zn$^{2+}$ in a manner similar to that of other inhibitors. In cultured cells, it is able to induce histone hyperacetylation and growth arrest at nanomolar concentrations. In human leukemia cells, FK228 had an IC50 at nanomolar concentrations and induced apoptosis in cells from patient with chronic lymphocytic leukemia. (Byrd et al., 1999; Sasakawa et al., 2002; Sasakawa et al., 2003; Sasakawa et al., 2003) In addition, depsipeptide has been shown to be antiangiogenic by modulating expression of c-myc and other regulatory genes. (Kwon et al., 2002) FK228 is currently undergoing extensive evaluation in clinical trials. (Byrd et al., 2004; Kwon et al., 2002; Marshall et al., 2002; Sandor et al., 2002)

**Benzamides**
The synthetic benzamide derivatives include structurally diverse group of compounds such as MS-275 and CI-994. CI-994 has shown efficacy in solid tumors in murine models but not inhibit HDAC directly. The mechanism of its action is unknown, but it appears to inhibit both histone deacetylation and cellular proliferation at the G1–S transition phase. (Graziano et al., 1997; LoRusso et al., 1996; Prakash et al., 2001) MS-275 and some of its derivatives inhibit HDACs in vitro at micromolar concentrations, but the mechanism is not clearly understood. It is believed that the diaminophenyl group is very important for the inhibitory behavior; probably, both amino functionalities chelate the metallic ion in the catalytic site. MS-275-associated HDAC-inhibitory activity is accompanied by an increase in expression of cyclin dependent kinase inhibitor p21WAF1/Cip1 and accumulation in G1-phase. (Saito et al., 1999; Suzuki et al., 1999) MS-275 displays anti-proliferative activity in several human cancer cell lines including breast, colorectal, leukemia, lung, ovary and pancreas. MS-275 suppressed growth of several pediatric cancer cell lines in dose dependent manner, as well as tumors transplanted in nude mice. (Jaboin et al., 2002) MS-275 and CI-994 are undergoing clinical trials. There are reports of novel nonhydroxamate sulfonamide anilides similar in structure to MS-275 are being synthesized and have shown lower toxicity and comparable antiproliferative activity. (Bouchain et al., 2003; Fournel et al., 2002) Currently, focus is on development of novel compounds based on core structures of HA or benzamide platform, which may have better HDAC inhibitory profile and lower toxicity compared to parent compounds.

Mode of action of HDAC inhibitors in cancer cells
Even though a number of HDAC inhibitors have shown considerable promise in pre-clinical models, the mechanism of action has not been fully evaluated. HDAC inhibitors are effective in affecting cell cycle arrest, apoptosis, anti-angiogenesis and differentiation in cultured and transformed cells from both hematological (leukemias, lymphomas and myelomas) and epithelial (breast, bladder, ovarian, prostate and lung) tumor sources. The change that occurs after treatment with HDAC inhibitors (growth arrest, terminal differentiation, or apoptosis) appears to be dependent upon the tumor cell rather than on the specific HDAC inhibitors used. (Bhalla and List, 2004) The HDAC family is divided into the Zn-dependent (Class I and Class II) and Zn-independent, NAD-dependent (Class III) enzymes. The Zn-dependent enzymes have been the focus of intense research, whilst the Class III has been recently implicated in acetylation and regulation of key cell cycle proteins such as p53. (Cheng et al., 2003) (McLaughlin and La Thangue, 2004) Interestingly, a number of studies have showed that HDAC inhibitors are relatively non-toxic to normal cells or tissues but exhibit selective cytotoxicity against a wide range of cancer cells. (Rosato and Grant, 2003; Zhu et al., 2001) It has been postulated that defective cell cycle checkpoint regulation of neoplastic cells may render them susceptible to HDAC inhibition-induced apoptosis. (Johnstone and Licht, 2003; Warrener et al., 2003)

As noted earlier, histone acetylation is known to precede gene transcription and among the genes that are consistently upregulated because their promoters are associated with acetylated histones is the cell cycle gene CDKN1A, which encodes cyclin-dependent kinase (CDK) inhibitor p21\(^{WAF1}\). Cyclin-dependent kinase inhibitor WAF1 inhibits cell-cycle progression by blocking CDK activity and the arrest of the cell cycle in G1 stage.
Most HDAC inhibitors namely, butyrates, TSA, depsipetide, oxamflatin, MS-275 and SAHA induce expression of p21. (Archer et al., 1998; Blagosklonny et al., 2002; Chai et al., 2000; Han et al., 2000; Huang et al., 2000; Lavelle et al., 2001; Saito et al., 1999; Sandor et al., 2000; Siavoshian et al., 2000; Sowa et al., 1999; Sowa et al., 1999; Vrana et al., 1999; Wang et al., 2002) Some cDNA microarray studies have shown that treatment with TSA or SAHA alters the expression of a selective subset of approximately 2% of cellular genes that are either upregulated or downregulated. (Chiba et al., 2004; Chiba et al., 2004; Mitsiades et al., 2003) The genes that are usually affected by these inhibitors are CDKN1A and CDKN2A where the latter encodes genes of cell cycle regulation such as p16, cyclin E and thioredoxin binding protein 2. (Huang and Pardee, 2000; Kim et al., 2000) Thus, gene promoters have specific sites, such as SP1, which bind HDAC containing transcription complexes and repress gene transcription. (Li and Wu, 2004; Yokota et al., 2004) Inhibition of HDACs will activate these silenced genes, contributing to growth arrest, differentiation and/or apoptosis of transformed cells. Treatment with HDAC inhibitors triggers both the intrinsic and sensitizes tumor cells to the death ligands that initiate the extrinsic pathway of apoptosis. (Bhalla and List, 2004) Several HDAC inhibitors, including SB, SAHA and MS-275 induce mitochondrial permeability transition where pro-apoptotic molecules such as cytochrome c, are released into the cytosol, resulting in eventual activation of caspase-dependent apoptotic cascades (both receptor and mitochondria-mediated). (Aron et al., 2003; Guo et al., 2004; Nguyen et al., 2003; Rosato et al., 2001) Upregulation and induction of a conformational change of the pro-apoptotic proteins are some of the HDAC inhibitor-induced upstream events that may trigger the mitochondrial pathway of apoptosis as is described for MS-275 and SB or as
is proposed in case of SAHA, may not required key caspases such as caspase-8 and caspase-3. (Lucas et al., 2004; Ruefli et al., 2001) Recently, reactive oxygen species (ROS) has been identified as a major cell death mechanism of several HDAC inhibitors. (Rosato et al., 2003; Ruefli et al., 2001) There is some evidence that HDAC inhibitors may induce acetylation of non-histone proteins such as the heat shock protein hsp90. Depsipeptide, SAHA and LAQ824 lower the threshold for apoptosis by inducing the acetylation hsp90 and thus affect oncoproteins such as Bcr-Abl and FLT-3. (Atadja et al., 2004; Yao et al., 2003) This eventually results in the inhibition of its chaperone association with important pro-survival client proteins such as Erk, Akt and c-Raf. (Yu et al., 2003) SAHA and oxamflatin were also shown to kill both ABCB1 positive and negative cells, whereas FK228 was shown to be substrate for ABCB1. (Peart et al., 2003) These data may provide insight into defining rational approaches to chemotherapy, where genetic profile of tumor is matched with functional profile to promote favorable clinical response.

Induction of the cell cycle inhibitor plays an important role in the induction of differentiation by HDAC inhibitors. SAHA and sodium butyrate were shown to induce differentiation of leukemia and breast cancer cells. (Gore et al., 2002; Gore et al., 2001) Induction of the expression of other molecules involved in differentiation, such as gelsolin, an actin binding protein involved in cell morphology and structural changes were observed during treatment with HDAC inhibitors. (Han et al., 2000; Hoshikawa et al., 1994; Kamitani et al., 2002; Mielnicki et al., 1999) In addition to pro-apoptotic and cytostatic activities, another mode of tumor regression following treatment with HDAC inhibitors may be by indirect inhibition of angiogenesis. In \textit{in vitro} models, depsipeptide
blocked potently the hypoxia-stimulated proliferation, invasion, migration, adhesion, and tube formation of bovine aortic endothelial cells. (Kwon et al., 2002) Effective concentrations were comparable to cytotoxic concentrations and there was an indication of possible modulation of gene transcription as evidenced by the expression of angiogenic-inhibiting factors such as von Hippel Lindau and neurofibromin 2 and the suppression of angiogenic-stimulating factors such as vascular endothelial growth factor (VEGF). (Mie Lee et al., 2003; Sasakawa et al., 2003) Other HDAC inhibitors like apicidin, TSA, butyrate and newer analogue LAQ824 were all shown to inhibit angiogenesis through VEGF inhibition. (Kim et al., 2004; Michaelis et al., 2004; Qian et al., 2004; Sawa et al., 2002; Williams, 2001) Such insights into the mechanisms by which HDAC inhibitors interfere with cancer cell growth and survival has prompted the search for combination strategies to optimize therapy.

Combination therapy of HDAC inhibitors with other drugs

Silencing of genes that affect growth and differentiation has been shown to occur by aberrant DNA methylation in the promoter region and by changes in chromatin structure that involve histone deacetylation. (Baylin et al., 2001; Herman and Baylin, 2003) DNA methylation and histone deacetylation appear to act as synergistic layers for the transcriptional silencing of genes in cancer.(Cameron et al., 1999; Zhu et al., 2001; Zhu and Otterson, 2003) Such findings have great implication in development of combination therapies.

Epigenetic mechanisms, such as DNA methylation and histone deacetylation, may also play a role in loss of estrogen receptor alpha (ER) expression in ER negative human
breast cancer cells. Previous studies showed that pharmacologic inhibition of these mechanisms using the DNA methyltransferase inhibitor, 5-aza-2’deoxycitidine (AZA), and TSA, resulted in expression of functional ER mRNA and protein. (Yang et al., 2001) Scriptaid, a novel TPX-HA analogue, inhibits tumor growth in vitro and in vivo and, in conjunction with AZA, acts to re-express functional ER. (Keen et al., 2003) In another study, TSA was shown to sensitize ER alpha negative antihormone-unresponsive breast cancer cells to tamoxifen treatment, by upregulating its activity. (Jang et al., 2004) The in vitro antineoplastic activity of 5-aza-2’deoxycitidine (AZA), in combination with TSA or depsipeptide, on the human myeloid leukemic cell lines produced a greater inhibition of growth and DNA synthesis and a greater loss of clonogenicity than either agent alone. (Shaker et al., 2003) Similar results were noted with PB and AZA combination in lymphoid leukemic cells. (Lemaire et al., 2004) Another study found that when AZA was combined with PB, murine lung tumor development was significantly reduced >50%, while no effect was observed with PB alone. (Belinsky et al., 2003)

Chromatin DNA is tightly packed, and hence accessibility to the drug target may reduce the efficiency of these anticancer drugs. When six cancer cell lines were pre-treated with TSA or SAHA followed by exposure to anticancer drugs like etoposide (VP-16), camptothecin, cisplatin, doxorubicin, 5-fluorouracil, cyclophosphamide or ellipcitine, that target chromatin DNA, there was >10 fold sensitization of cells for VP-16. The data suggested that loosening-up the chromatin structure by histone acetylation can increase efficiency of several anticancer agents. (Kim et al., 2003) SAHA significantly potentiated the DNA damage by topoisomerase II inhibitors; however synergy was dependent on the sequence of drug administration and expression of target. Pre-exposure of cells to SAHA
for 48 hours was synergistic, whereas shorter period of exposure abrogated synergy and pre-treatment with topoisomerase II inhibitor showed antagonistic effects. (Marchion et al., 2004)

Inhibition of cell survival signals and proliferation by using inhibitors of tyrosine kinase activity, in combination with HDAC inhibitors is another mechanism to induce differentiation and/or apoptosis. (Nimmanapalli and Bhalla, 2002) The cytotoxic effects following the introduction of SAHA with imatinib mesylate showed accumulation of acetylated histones H3 and H4, induction of p21 and p27 and following SAHA treatment there was a decline in the mRNA and protein levels of Bcr-Abl, resulting in G1 arrest and apoptosis of leukemic cells. Co-treatment with imatinib mesylate and SAHA caused significantly more down-regulation of tyrosine kinase activity of Bcr-Abl and apoptosis of these cells when compared to treatment with SAHA alone. These findings suggested that cotreatment with SAHA and imatinib mesylate or arsenic trioxide are cytotoxic to Bcr-Abl positive acute leukemia cells and these agents may be promising therapy against imatinib mesylate-refractory Bcr-Abl positive acute leukemia. (Nimmanapalli et al., 2003; Yu et al., 2003) Similar results were achieved on combined exposure of Bcr/Abl positive human myeloid leukemia cells to imatinib (STI571) and SAHA, leading to diverse perturbations in signaling and cell cycle-regulatory proteins, associated with a marked increase in mitochondrial damage and cell death. (Yu et al., 2003) SAHA and PB were also shown to synergistically induce apoptosis in human leukemic cells when co-treated with hsp90 antagonist 17-allylamino-17-demethoxygeldanamycin (17-AAG). (Rahmani et al., 2003) Similar cumulative inhibitory effects were noted on combined treatment of SB and flavopiridol, where interruption of HDAC mediated p21
(WAF1/Cip1) induction by flavopiridol potentiated apoptosis. (Rosato et al., 2002; Rosato et al., 2004) Recently, the same investigators showed that MS-275 acts synergistically with fludarabine to increase the apoptotic activity in leukemia cells. (Maggio et al., 2004) Moreover, proteasome inhibitor bortezomib interacts synergistically with SB or SAHA to cause oxidative injury and apoptosis in Bcr/Abl positive multiple myeloma and leukemia cells sensitive and resistant to imatinib. (Pei et al., 2004; Yu et al., 2003)

LAQ824 both lowers expression and promotes proteasomal degradation of Bcr-Abl and induces apoptosis of imatinib-sensitive or refractory chronic myelogenous leukemia-blast crisis cells. (Nimmanapalli et al., 2003) Recent studies show that LAQ824 can also promote degradation of mutant FLT-3 and induce apoptosis of AML cells carrying the mutated FLT-3. The addition of the Flt-3 kinase inhibitor PKC412 had a synergistic effect on apoptosis in AML cells with mutant FLT-3. (Bali et al., 2004) The combination of SAHA or LAQ824 with various cytotoxic agents like taxotere, trastuzumab, gemcitabine and epothilone B, enhanced the cytotoxic effects in breast cancer cells, while the combination of 5-fluorouracil and other chemotherapy agents with PB also enhanced the cytotoxic effects in colorectal cancer cells. (Fuino et al., 2003; Huang et al., 2000; Huang and Waxman, 1998) In two separate studies, SAHA also potentiated sensitizing melanoma cells to TNF-related apoptosis-inducing ligand (TRAIL) induced apoptosis by simultaneous activation of intrinsic and extrinsic pathways. (Rosato et al., 2003; Zhang et al., 2003) In another study, VA was shown to increase cellular sensitivity to estrogens, progestins and other hormone nuclear ligands, by functioning as activator of p42/p44 mitogen-activated protein kinase (MAPk). (Jansen
et al., 2004) TSA upregulated RECK glycoprotein that negatively regulates matrix metalloproteinases (MMPs) and inhibits tumor metastasis and angiogenesis by specifically inhibiting MMP-2. (Liu et al., 2003) Radiotherapy is an effective treatment for several cancers but causes cutaneous radiation syndrome. PB, TSA and VA were shown to decrease skin fibrosis and tumorigenesis by suppressing aberrant expression of TGF-beta and TNF-alpha. (Chung et al., 2004) In human gastric and colorectal cancer cells, depsipeptide, MS-275 and CBHA all augmented radiation-induced cell death. (Zhang et al., 2004) Moreover, HDAC inhibitors have shown synergism when combined with all-trans retinoic acid (ATRA) to overcome the block in differentiation due to specific translocations associated with acute promyelocytic leukemia. (Coffey et al., 2001; Coffey et al., 2000; He et al., 2001)

**Histone Deacetylase inhibitors in Clinical trials**

Based on promising preclinical data, several HDAC inhibitors are currently being investigated in early phase trials in humans, both as single agent and in combination with known cytotoxic compounds. HDAC inhibitors such as PA, PB, VA, AN-9, SAHA, LAQ824, pyroxamide, FK228, MS-275 and CI-994 are evaluated in patients with various metastatic or refractory solid tumors in advanced stages and those with hematological malignancies like acute myeloid leukemia (AML), acute lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), chronic lymphoid leukemia (CLL), or lymphomas. Details about phase of development, major toxicities, pharmacokinetics and preliminary data on clinical response of various HDAC inhibitors used as single agent or given in
combination with cytotoxic agents that are undergoing clinical development are summarized in Tables II and III, respectively.

Future Direction

The concept of mechanism-based therapeutic development of novel anticancer agents is now being recognized, since better targeting of abnormalities has shown to offer new directions. The HDAC inhibitors in clinical trials has shown encouraging antitumor effects, with well-tolerated safety profiles. There may be significant repercussions in success or failure of an anticancer agent, when targeting a specific subtype of HDAC without having a broader understanding of mechanism of action and the differential role each enzyme play in chromatin remodeling in cancer cells. Although, none of these agents in clinical trials were developed to be selective inhibitors of individual HDAC subtype, they do show some target selectivity. (McLaughlin and La Thangue, 2004) For example, MS-275 showed selective inhibition of HDAC1 and HDAC3, but was inactive against HDAC8. (Hu et al., 2003) Similarly, FK228 has activity against class I (HDAC1 and HDAC2) enzymes, but not against class II (HDAC4 and HDAC6). (Furumai et al., 2002) The challenge remains to develop specific inhibitors of class I HDACs that are primarily located within the nucleus and class II HDACs that are known to shuttle between nucleus and cytoplasm (Johnstone, 2002; Kao et al., 2001). Recent findings using siRNA techniques to understand HDAC isotypes as potential targets, suggested that class I HDAC enzymes may be more relevant targets for intervention in oncology. (Curtin and Glaser, 2003) In any case, chromatin modifying enzymes have provided an
increasingly validated therapeutic target and there is now compelling evidence that these compounds exhibit efficacy in human diseases.

Phase I and phase II clinical trials with HDAC inhibitors have been completed, and others are being initiated. Most of these have been able to identify suitable doses for treatment with relatively less toxicity and reasonable efficacy in various cancers. Remission appeared to be transient in some of the patient trials, suggesting a need for determination of dosing parameters.(Bhalla and List, 2004) Based on preliminary clinical data and the apparent cytostatic mechanism of action, most HDAC inhibitors, with the possibly exception of FK228 in the treatment of renal cell carcinoma, seem to fit more as combination treatment with existing chemotherapy regimens along with being used in other mechanism-based agents. Nonetheless, various questions still remain to be answered: 1) what role do altered HAT or HDAC activities have in conjunction with tumorigenesis? Is it a direct effect or is an epigenetic adaptive phenomenon?; 2) why are tumor cells more sensitive to HDAC inhibitors than normal cells and is there a possibility that there may be increased HAT/HDAC activity in tumors?; 3) is modification of histone(s) the only mechanism leading to anti-neoplastic effects or are there targets responsible that are yet undefined?; and 4) what is the target specificity of HDAC inhibitors? (Piekarz and Bates, 2004) Unraveling specific roles of HDAC isozymes during human tumorigenesis will provide further incentive for the development of more specific HDAC inhibitors, potentially those enhancing clinical activity as well as decreasing non-specific toxicities. Also, optimizing potential interactions with other rationally designed and integrated therapeutic agents remains a promising premise for exploration. In addition, there is a general current lack of knowledge on the
pharmacokinetics and biodistribution of various HDAC inhibitors studied clinically. Current evidence suggests that novel formulations and drug delivery strategies that allow better targeting may significantly enhance the therapeutic potential of HDAC inhibitors. (Drummond et al., 2004)

**Conclusion**

A wealth of recent data has become available suggesting that histone modification is a promising therapeutic strategy affecting many of the hallmark traits of cancer. (Hanahan and Weinberg, 2000) Drugs such as HDAC inhibitors that have pleiotropic actions in modulating multiple genes, pathways and biological features of malignancy, might prove to be suited for dealing with combinatorial oncogenic abnormalities seen with most cancer types. (Kristeleit et al., 2004) In addition to applications in oncology, manipulation of histones involved in other diseases such as Huntington’s disease and hepatic fibrosis, may be avenues for further explorations in other therapeutic areas. (Ferrante et al., 2003; Hockly et al., 2003; Penner et al., 1987) Although the clinical development of novel HDAC inhibitors seems certain, their actual value will greatly depend on identification of molecular and cellular predictors and elucidation of their mechanism of action as anticancer agents.

**Acknowledgments**

The authors would like to thank Richard Piekarz for critically reviewing this manuscript and for his helpful suggestions.


## Table I. Overview of HDAC inhibitors.

<table>
<thead>
<tr>
<th>Class</th>
<th>Example</th>
<th>Alias</th>
<th>In vitro cell culture activity (concentration)</th>
<th>In vivo pre-clinical activity (murine or human xenograft model)</th>
<th>Clinical phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboxylates (Short chain fatty acids)</td>
<td>PA</td>
<td>phenylacetate</td>
<td>Yes (µM)</td>
<td>Leukemia, glioblastoma</td>
<td>I/II</td>
</tr>
<tr>
<td></td>
<td>PB</td>
<td>sodium phenylbutyrate</td>
<td>Yes (µM)</td>
<td>Prostate, endometrial</td>
<td>I/II</td>
</tr>
<tr>
<td></td>
<td>VA</td>
<td>valproic acid</td>
<td>Yes (mM)</td>
<td>Brain, melanoma</td>
<td>I/II</td>
</tr>
<tr>
<td></td>
<td>AN-9</td>
<td>Pivanex, pivaloyl oxymethyl butyrate</td>
<td>Yes (µM)</td>
<td>NSCLC, leukemia</td>
<td>I/II</td>
</tr>
<tr>
<td>Hydroxamic acids (HA)</td>
<td>SAHA</td>
<td>suberonyl anilide hydroxamic acid</td>
<td>Yes (nM)</td>
<td>Lung, prostate, melanoma</td>
<td>I/II</td>
</tr>
<tr>
<td></td>
<td>CBHA</td>
<td>m-carboxycinnamic acid bishydroxamic acid</td>
<td>Yes</td>
<td>Neuroblastoma</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>SBHA</td>
<td>suberic bishydroxamic acid</td>
<td>Yes</td>
<td>Melanoma, sarcoma</td>
<td>-</td>
</tr>
<tr>
<td>Pyroxamide</td>
<td>-</td>
<td>Yes (µM)</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>TSA</td>
<td>trichostatin A</td>
<td>No alias</td>
<td>Yes (nM)</td>
<td>Cervical, hepatoma, melanoma</td>
<td>-</td>
</tr>
<tr>
<td>Oxamflatin</td>
<td>-</td>
<td>Yes (µM)</td>
<td></td>
<td>Melanoma</td>
<td>-</td>
</tr>
<tr>
<td>NVP-LAQ824</td>
<td>-</td>
<td>Yes (nM)</td>
<td></td>
<td>Colon, multiple myeloma</td>
<td>I</td>
</tr>
<tr>
<td>Electrophillic ketones (epoxides)</td>
<td>TPX</td>
<td>Trapoxin A &amp; B</td>
<td>Yes (nM)</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>AOE</td>
<td>2-amino 8-oxo-9,10-epoxydecanoic acid</td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Depudecin</td>
<td>-</td>
<td>Yes (mM)</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Cyclic peptides</td>
<td>Apicidin</td>
<td>-</td>
<td>Yes (nM)</td>
<td>Melanoma, leukemia</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>FK-228, FR901228</td>
<td>Depsipeptide</td>
<td>Yes (nM)</td>
<td>Melanoma, colon, sarcoma, fibrosarcoma, lung, gastric</td>
<td>I/II</td>
</tr>
<tr>
<td>Benzamides</td>
<td>MS-275</td>
<td>MS-27-275</td>
<td>Yes (µM)</td>
<td>Leukemia, colorectal, gastric, pancreatic, lung, ovarian</td>
<td>I/II</td>
</tr>
<tr>
<td>Other hybrid compounds</td>
<td>CI-994</td>
<td>N-acetyl dinaline</td>
<td>Yes (indirect effect)</td>
<td>Colorectal, pancreatic, mammary, prostate, sarcoma, leukemia</td>
<td>I</td>
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<tr>
<td>CHAPs</td>
<td></td>
<td>cyclic HA- peptides (TPX-TSA analogues)</td>
<td>Yes (nM)</td>
<td>Melanoma, lung, stomach, breast</td>
<td>-</td>
</tr>
<tr>
<td>Scriptaid</td>
<td></td>
<td>TPX-HA</td>
<td>Yes (nM)</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Tubacin</td>
<td>-</td>
<td>-</td>
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<td>JNJ162411 99</td>
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<tr>
<td>A-161906</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-CI-UCHA</td>
<td></td>
<td>6- (3-chlorophenylureido)caproic hydroxamic acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PXD101</td>
<td>-</td>
<td>Yes (nM)</td>
<td>Breast, prostate, ovarian, colon, NSCLC</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
Table II. HDAC inhibitors in clinical trials as single agents.

<table>
<thead>
<tr>
<th>Name (Ref)</th>
<th>Phase</th>
<th>N</th>
<th>Tumor type</th>
<th>Route of administration / dosing regimen</th>
<th>DLT and adverse events</th>
<th>PK results</th>
<th>Clinical response/outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA(Thibault et al., 1994)</td>
<td>I</td>
<td>17</td>
<td>Solid tumors</td>
<td>IV bolus (60-150 mg/kg), target level 200-400g/ml x 2 weeks</td>
<td>CNS depression, emesis, confusion, lethargy</td>
<td>Non-linear PK, evidence of drug induction, 99% PA converted to PG and eliminated in urine, CNS penetration</td>
<td>3/9 SD x 2 months in HRPC, 1/6 SD &gt;9 months in glioblastoma</td>
</tr>
<tr>
<td>PA(Thibault et al., 1995)</td>
<td>I</td>
<td>18</td>
<td>Solid tumors</td>
<td>IV 1 h infusion b.i.d. 125 and 150 mg/kg x 2 weeks every 4 weeks</td>
<td>CNS depression</td>
<td>PA induced own clearance (27%), MTD 125 mg/kg, Cmax 2500 g/ml</td>
<td>1 PR glioblastoma, 1 HRPC with 50% post-therapy PSA decline</td>
</tr>
<tr>
<td>PA(Chang et al., 2003; Chang et al., 1999)</td>
<td>II</td>
<td>43 &amp; 9</td>
<td>Recurrent malignant gliomas</td>
<td>IV infusion 400mg/kg/day, compared 2 schedules, 2 weeks every 2 weeks or 12-day every 2 days Max 450 mg/kg/day</td>
<td>Fatigue, somnolence, lethargy, disorientation, malaise, weakness, N/V &amp; granulocytopenia</td>
<td>No differences in plasma concentration between 2 treatments, no apparent induction of PA metabolism</td>
<td>For schedule 1, PR 3/40 (7.5%), SD in 7/40 patients (17.5%), PD &lt; 2 months 30/40 patients. For schedule 2, 1/7 SD, 6/7 PD</td>
</tr>
<tr>
<td>PB(Carducci et al., 2001)</td>
<td>I</td>
<td>24</td>
<td>Refractory solid tumors</td>
<td>IV infusion 120-h every 3 weeks, dose 150 – 515 mg/kg/day</td>
<td>Neurocortical somnolence, confusion, hypokalaemia, hyponatreemia, fatigue, nausea</td>
<td>MTD =410 mg/kg/day, plasma CL increased continuously after 24h, PA accumulated when Vmax was less than dosing rate</td>
<td>No CR, 2 SD, reduction in bone pain</td>
</tr>
<tr>
<td>PB(Gilbert et al., 2001)</td>
<td>I</td>
<td>28</td>
<td>Refractory solid tumors</td>
<td>Oral dose TID 9-45 g/day in 5 dose levels</td>
<td>Grade 1 – 2 dyspepsia, fatigue, neurocortical nausea, vomiting,</td>
<td>MTD 27 g/day, bioavailability 78%, biologically active</td>
<td>No CR, PR, 7 patients (25%) with SD &gt; 6 months</td>
</tr>
<tr>
<td>Compound</td>
<td>Phase</td>
<td>No.</td>
<td>Disease / Source</td>
<td>Dosing</td>
<td>Toxicities</td>
<td>MTD</td>
<td>Comments</td>
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</tr>
<tr>
<td>PB (Gore et al., 2001)</td>
<td>I</td>
<td>27</td>
<td>Myeloid dysplasia, AML</td>
<td>IV infusion for 7 days every 28 days</td>
<td>hypocalcaemia concentrations (0.5mM)</td>
<td>MTD 375 mg/kg/day</td>
<td>No CR, PR, hematological improvements, increased neutrophils in 3, decreased blasts in 3</td>
</tr>
<tr>
<td>AN-9 (Patnaik et al., 2002)</td>
<td>I</td>
<td>28</td>
<td>Advanced solid tumors</td>
<td>IV infusion, 6h x 5 days every 21 days at doses 0.047 - 3.3 g/m²/day</td>
<td>No DLT, nausea, vomiting, fatigue, vision disturbance, anorexia, fever</td>
<td>MTD 3.3 g/m²/day based on volume of maximum lipid formulation administrable</td>
<td>1 PR, no increase in fetal hemoglobin</td>
</tr>
<tr>
<td>AN-9 (Reid et al., 2004)</td>
<td>II</td>
<td>47</td>
<td>Refractory NSCLC</td>
<td>IV infusion, 2.34 g/m²/day over 6h x 3 days every 21 days</td>
<td>Grade 1-2 fatigue (34%), nausea (17%), dysgeusia (11%)</td>
<td>-</td>
<td>3/47 PR, 14 patients with SD &gt; 12 weeks (30%), median survival 6.2 months, 1-year survival of 26%</td>
</tr>
<tr>
<td>VA (A. Atmaca, 2004)</td>
<td>I</td>
<td>26</td>
<td>Progressive cancers</td>
<td>IV infusion 1 h split twice daily x 5 days every 2 weeks at 30-120 mg/kg/day</td>
<td>Grade 3/4 neurotoxicity, no severe hematological</td>
<td>MTD 60 mg/kg, PBMC showed hyperacetylation</td>
<td>Neurotoxicity is dose-limiting</td>
</tr>
<tr>
<td>SAHA (Kell y et al., 2003)</td>
<td>I</td>
<td>37</td>
<td>Solid tumor and hematologic malignancy (B))</td>
<td>IV infusion, (A) 2 h x 3 days every 3 weeks, at 75-900 mg/ m²/day (B) 2 h x 5 days every 1-3 weeks 300-900 mg/ m²/day for 3-15 days</td>
<td>(A) No DLT in 8/8, (B) Grade 3/4 thrombocytopenia and neutropenia in hematological patients</td>
<td>MTD on (B), 300 mg/ m²/day t1/2 = 21-58 min, AUC increased with dose, accumulation of acetylated histones in PBMC after 4 h at all dose levels</td>
<td>1 PR in refractory Hodgkin’s disease &amp; SD &gt; 6 months in 2 patients with bladder cancer</td>
</tr>
<tr>
<td>SAHA (G. Garcia-Manero, 2004)</td>
<td>I</td>
<td>15</td>
<td>Advanced refractory leukemias or MDS</td>
<td>Orally TID x 14 days every 21 days at 100-250 mg</td>
<td>No DLT, nausea, vomiting, diarrhea, anorexia, headache, fatigue, dyspepsia</td>
<td>Histone hyperacetylation at all dose levels</td>
<td>1 CR at dose level 3 after 2 courses, 2 AML, 1 MDS patient had decrease in marrow</td>
</tr>
<tr>
<td>Study</td>
<td>Phase</td>
<td>Patients</td>
<td>Treatment</td>
<td>Side Effects</td>
<td>Key Findings</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SAHA (Kelly WK, 2002)</td>
<td>I</td>
<td>39</td>
<td>Advanced cancers</td>
<td>Oral, daily or BID at 200-600 mg</td>
<td>Thrombocytopenia, fatigue</td>
<td>Prolonged plasma concentrations &lt;10 h with single dose</td>
<td></td>
</tr>
<tr>
<td>SAHA (G. Blumenschein, 2004)</td>
<td>II</td>
<td>13</td>
<td>SCCHN (metastatic head and neck cancers)</td>
<td>Oral, daily at 400 mg</td>
<td>No DLT, grade 3-4 thrombocytopenia, anemia, anorexia</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>FK-228 (Marshall et al., 2002)</td>
<td>I</td>
<td>33</td>
<td>Advanced cancers</td>
<td>IV infusion 4 h, weekly x 3 every with 1 week off at 1-17.7 mg/m²</td>
<td>Grade 3 thrombocytopenia, fatigue, nausea, vomiting, anorexia at dose above 5 mg/m², subtle ECG changes</td>
<td>MTD 13.3 mg/m²/day</td>
<td></td>
</tr>
<tr>
<td>FK-228 (Piekarz et al., 2001; Sandor et al., 2002)</td>
<td>I</td>
<td>37</td>
<td>Advanced or refractory cancers</td>
<td>IV infusion 4 h on days 1 and 5 every 21 days at dose 1-24.9 mg/m²</td>
<td>Grade 3 fatigue, nausea, vomiting, grade 4 thrombocytopenia, cardiac arrhythmia</td>
<td>MTD 17.8 mg/m² over 4 h over 4 h t1/2(a) =0.42 h; elimination t1/2(b) =8.1h, mean CL=11.6 L/h/m² , inhibition of cell cycle in PC-3 cells</td>
<td></td>
</tr>
<tr>
<td>FK-228 (Byrd et al., 2004)</td>
<td>I</td>
<td>20</td>
<td>CLL and AML</td>
<td>IV infusion on days 1, 8, 15 at 13 mg/m²</td>
<td>Fatigue, nausea, progressive constitutional</td>
<td>Increases in histone acetylation by 100%, p21 promoter H4 acetylation,</td>
<td></td>
</tr>
</tbody>
</table>

Blasts to < 10%

Prolonged duration of acetylated histones in PBMC (>10 h), objective response in patients with larynx, renal cancer and lymphoma

No PR or CR, 1 MR based on tumor shrinkage,

No increase of cardiac enzymes or decrease of ejection fraction

Increased acetylation of histones in Sezary cells, variable effect on histones after 7 h, 1 PR in colon cancer x 6 months, 1 CR in peripheral T-cell lymphoma, 3 PR in CTCL

No cardiotoxicity, need to explore other schedules due to
<p>| CI-994 (Prakash et al., 2001) | I 53 | Solid tumors | Orally on schedule (A) x 2 weeks, (B) x 8 weeks followed by 2 weeks rest | Schedule (A) thrombocytopenia, neutropenia, increased LFT, creatinine, (B) thrombocytopenia, nausea, vomiting | Schedule (A) MTD 15 mg/m²/day, no cumulative toxicities, (B) MTD 8 mg/m²/day, t1/2 = 7.4-14.1 h, inverse relationship between platelet nadir and AUC, low effect of food on absorption | Both schedules, 1 PR in NSCLC x 2 years, 3 SD in NSCLC, colorectal and renal cancer |
| MS-275 (Ryan, 2003) | I 30 | Solid tumors and lymphomas | Orally on schedule (A) daily x 28 days every 6 weeks, (B) weekly x 4, every 6 weeks at 2-12 mg/m² | Schedule (A) severe GI toxicity, (B) and (C) fatigue, nausea, vomiting, anxiety thrombocytopenia, headache | MTD on (A) 2 mg/m², (B) 10 mg/m², histone acetylation at all dose levels | Schedule (A) intolerable, 15 SD on (B), |
| MS-275 | I 17 | Hematologic malignancy | Orally q7 day for 4 weeks every at 4-10 mg/m² | Severe infections at 10 mg/m², thrombocytopenia, GI toxicity | MTD at 8 mg/m² |
| MS-275 (L. Gore, 2004) | I 21 | Solid tumors and lymphomas | Orally on schedule (A) 2-6 mg/m² biweekly, (B) 2 mg/m² twice weekly x 3 weeks with 1 week off, (C) 4 mg/m² weekly for 3 weeks with 1 week off | No drug related DLT, grade 1-3 hypophosphatemia, asthenia, nausea, anorexia | MTD not reached on (A), (B) not pursued, rapid absorption with Tmax 0.5-2h, dose-dependent increase in exposure, biphasic elimination with t1/2=100h | 1 PR on (A) in melanoma, 3 SD in Ewing’s sarcoma, rectal carcinoma and melanoma |
| LAQ824 (O. G. Ottmann) | I 21 | ALL, AML | IV infusion, 3h on days 1-3 of 21 day | Thrombocytopenia (cerebral bleeding), | MTD 36 mg/m², dose-proportional increase in | No QTc prolongation, ECG &lt;400 msec, 1CR |</p>
<table>
<thead>
<tr>
<th>2004</th>
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</tr>
</thead>
<tbody>
<tr>
<td>CLL, CML, MDS</td>
<td>cycle at 6-80 mg/m(^2) in 6 dose levels</td>
<td>grade 2 hyperbilirubinemia</td>
<td>exposure, t1/2=9-18 h, 1.5 fold accumulation at day 3, Cmax after 1.5h , not at end of infusion in &gt;50% patients, indicates non-linear PK</td>
<td>in M1 AML, 6 SD, histone acetylation at &gt;12 mg/m(^2) doses</td>
</tr>
<tr>
<td>LAQ824(E. K. Rowinsky, 2004)</td>
<td>I 28 Advanced solid tumors</td>
<td>IV infusion, 3h on days 1-3 of 21 day cycle at 6-100 mg/m(^2) in 7 dose levels</td>
<td>Grade 3/4 transient transaminitis, fatigue, hyperbilirubinemia, nausea, thrombocytopenia,</td>
<td>Dose-proportional increase in exposure,t1/2=8-14 h, Cmax after 1.5h , not at end of infusion in &gt;50% patients, indicates non-linear PK</td>
</tr>
<tr>
<td>LBH589(J. Beck, 2004)</td>
<td>I 13 Advanced solid tumors</td>
<td>IV infusion, 30 min either on (A) days 1-3 and 8-10 of 21 day cycle at 1.2 -7.2 mg/m(^2), (B) days 1-3 or 15-17 of 28 day cycle at 2.4-4.8 mg/m(^2)</td>
<td>Prolonged grade 2 thrombocytopenia in (A), grade 3 neutropenia, anemia, hypoglycemia</td>
<td>Exposure increased proportionally with dose, t1/2 =15-20 h</td>
</tr>
</tbody>
</table>

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Table III. HDAC inhibitors in combination therapy with other agents.

<table>
<thead>
<tr>
<th>Name (Ref)</th>
<th>Phase</th>
<th>N</th>
<th>Tumor type</th>
<th>Route of administration / dosing regimen</th>
<th>DLT and adverse events</th>
<th>PK results</th>
<th>Clinical response/outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI-994 + gemcitabine (Nemunaitis et al., 2003)</td>
<td>I</td>
<td>20</td>
<td>Advanced cancers</td>
<td>Gemcitabine IV infusion weekly x 3 with 1 week off at 1000 mg/m², CI-994 orally daily x 21 days escalating at 2-8 mg/m²</td>
<td>Grade 4 thrombocytopenia (30%) at 8 mg/m²</td>
<td>MTD 6 mg/m² oral x 21 days with 1000 mg/m² gemcitabine, rapid absorption, Cmax within 2 hours of dosing</td>
<td>2 MR, 12 SD with median 105 days, 4 PD</td>
</tr>
<tr>
<td>CI-994 + capecitabine (Undevia et al., 2004)</td>
<td>I</td>
<td>54</td>
<td>Advanced cancers</td>
<td>Schedule (A) IV capecitabine twice daily at 1650 mg/m²/day, CI-994, 2-10 mg/m² orally x 2 of 3 weeks, (B) CI-994 x 5 of 6 week, (C) capecitabine 2000 mg/m²/day, CI-994 orally x 2 of 3 weeks</td>
<td>Thrombocytopenia,</td>
<td>MTD 6 mg/m² (10 mg) with capecitabine 2000 mg/m²/day, PK of CI-994 unaltered by capecitabine</td>
<td>No correlation between BSA and PK parameters, platelet nadir best predicted by Cmax</td>
</tr>
<tr>
<td>CI-994 (A Wozniak, 1999)</td>
<td>II</td>
<td>32</td>
<td>NSCLC</td>
<td>Orally, daily at 8 mg/m²</td>
<td>Thrombocytopenia, fatigue, anorexia, nausea, vomiting, paresthesia</td>
<td>-</td>
<td>2 PR, 8 SD &gt; 8 weeks, median survival 30 weeks</td>
</tr>
<tr>
<td>CI-994 (J O'Shaughnessy, 1999)</td>
<td>II</td>
<td>48</td>
<td>Renal cell carcinoma</td>
<td>Orally, daily at 8 mg/m²</td>
<td>Thrombocytopenia, fatigue, anorexia, nausea, vomiting, paresthesia</td>
<td>-</td>
<td>26 SD for &gt;8 weeks, median survival = 48 weeks</td>
</tr>
<tr>
<td>Compound</td>
<td>Phase</td>
<td>Duration</td>
<td>Treatment</td>
<td>Toxicities</td>
<td>Response</td>
<td></td>
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<tr>
<td>CI-994 (Mark Zalupski, 2000)</td>
<td>II</td>
<td>17</td>
<td>Advanced pancreatic cancer</td>
<td>Orally, daily at 8 mg/m²</td>
<td>Thrombocytopenia, asthenia, anorexia</td>
<td>2 SD for 6 weeks, No objective response, cytostatic mechanism</td>
<td></td>
</tr>
<tr>
<td>CI-994 + carbo + paclitaxel</td>
<td>I</td>
<td>21</td>
<td>Refractory solid tumors</td>
<td>Oral CI-994 daily x 7 or 14 days every 21 days (4-6 mg/m²/day), Carbo every 21 days Paclitaxel 175-225 mg/m² every 21 days</td>
<td>DLT=neutropenia, thrombocytopenia, diarrhea &amp; weakness</td>
<td>MTD; CI-994 4 mg/m²/day with paclitaxel 200 mg/m², carbo</td>
<td></td>
</tr>
<tr>
<td>PB + AC</td>
<td>I</td>
<td>6</td>
<td>Solid tumors</td>
<td>AC 25 mg/m² o.d. days 1-14 PB 400 mg/kg/day Cl days 6 and 13 every 5 weeks</td>
<td>ND</td>
<td>ND</td>
<td>No change in pre or post – tumor specimens for methyltransferase or GST</td>
</tr>
<tr>
<td>PB + RA</td>
<td>-</td>
<td>5</td>
<td>APL</td>
<td>RA(30-90 mg/m²/day)+PB (150-400 mg/kg/day)</td>
<td>ND</td>
<td>ND</td>
<td>1/5 cytological CR</td>
</tr>
<tr>
<td>AN-9 + docetaxel (T. Reid, 2004)</td>
<td>II</td>
<td>12</td>
<td>Advanced NSCLC</td>
<td>AN-9 IV infusion 6h/day for days 1-3 at 1.5-2.5 g/m², docetaxel on day 4 at 75 mg/m², regimen repeated every 3 weeks</td>
<td>No DLT, adverse events unrelated to AN-9, grade 3 neutropenia due to docetaxel in 9 (75%) patients</td>
<td>MTD 2.5 g/m² with 75 mg/m² docetaxel</td>
<td>3 PR, decrease in tumor size,</td>
</tr>
</tbody>
</table>