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REVIEW

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Histone Deacetylase Inhibitors: Emerging Mechanisms of Resistance

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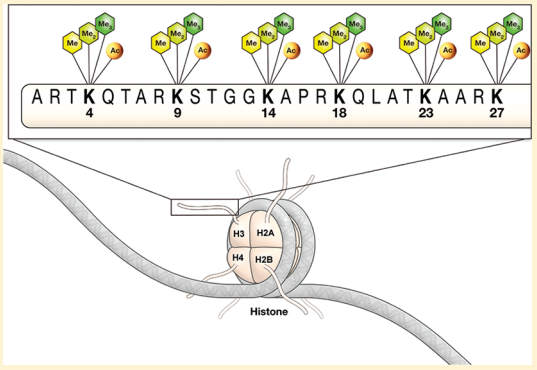
ABSTRACT: The histone deacetylase inhibitors (HDIs) have shown promise in the treatment of a number of hematologic malignancies, leading to the approval of vorinostat and romidepsin for the treatment of cutaneous T-cell lymphoma and romidepsin for the treatment of peripheral T-cell lymphoma by the U.S. Food and Drug Administration. Despite these promising results, clinical trials with the HDIs in solid tumors have not met with success. Examining mechanisms of resistance to HDIs may lead to strategies that increase their therapeutic potential in solid tumors. However, relatively few examples of drug-selected cell lines exist, and mechanisms of resistance have not been studied in depth. Very few clinical translational studies have evaluated resistance mechanisms. In the current review, we summarize many of the purported mechanisms of action of the HDIs in clinical trials and examine some of the emerging resistance mechanisms.



KEYWORDS: histone deacetylase inhibitor, resistance, romidepsin, vorinostat, panobinostat

INTRODUCTION

In the nucleus, DNA is wound around four core histone proteins (H2A, H2B, H3 and H4, see Figure 1) to form the nucleosomes, which, when compacted, form the condensed structure of chromatin. Each histone protein in the nucleosome has a lysine-rich tail that extends outside of the nucleosome and the accessibility of DNA within the nucleosome is, in part, controlled by modifications of the tail. Histones can be modified in a number of ways, including acetylation, methylation, phosphorylation, ubiquitination, sumoylation and citrullination. As shown in Figure 1, the lysines of the histone tails can be modified by methylation and acetylation. Acetylation is controlled by two enzymes: histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs transfer acetyl groups to the lysine residues of the histones, which neutralizes the positively charged lysines, decreasing attraction of the negatively charged DNA, thereby resulting in greater access by transcription factors and RNA polymerase. HDACs, on the other hand, remove acetyl groups, resulting in decreased access to DNA.[1] Changes in global histone acetylation were found to be associated with tumorigenesis in some disease models.[2−4]

Most of the human HATs function as coactivators of transcription.[2] The most studied function of this group of proteins is the acetylation of the histone tails, although they can also acetylate various cellular transcription factors.[2] Similarly, while HDACs are primarily known for their ability to deacetylate histones, they have also been shown to deacetylate other proteins such as tubulin, p53, Hsp90, Bcl-2, and Ku70.[5,6] Based on their similarity to yeast HDAC proteins, human HDACs are divided into four classes (compiled from refs 1 and 7):

Class I. HDACs 1−3 and 8, 40−55 kDa proteins, belong to this class and are ubiquitously expressed in human tissues. HDACs 1, 2, and 3 are localized to the nucleus while HDAC8 is located in both the cytoplasm and the nucleus. This class of HDACs shares a structural similarity with the yeast transcription factor Rpd-3.

Class IIA, IIB. HDACs 4, 5, 7, and 9 make up class IIA and HDACs 6 and 10 make up the class IIB HDACs, all of which are 70−130 kDa proteins. They share a structural similarity with the yeast HDA1 deacetylase. HDAC6 is unique in that it is a cytoplasmic HDAC that does not deacetylate histones.

Class III. This group of HDACs, known as the sirtuins, is made up of HDACs structurally similar to the yeast SirT2, and requires NAD^+ as a cofactor for enzymatic activity.

Class IV. The only known HDAC of this class is HDAC11. Relatively little is known about this HDAC, which is localized to the nucleus.

HISTONE DEACETYLASE INHIBITORS

The histone deacetylase inhibitors (HDIs) are a promising class of chemotherapeutic agents that have been added to the anticancer armamentarium. HDIs prevent the deacetylase activity of

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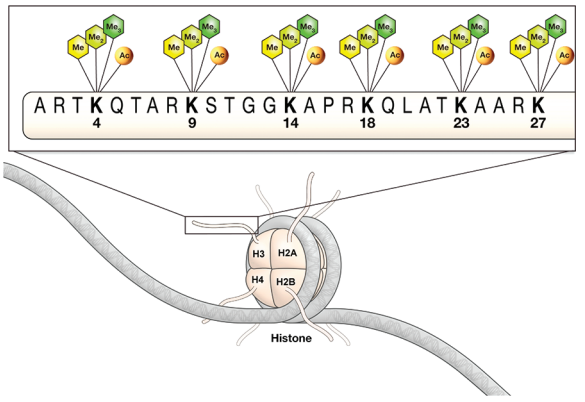

Figure 1. Modification of lysines in histone tails. DNA is wound around four core histone proteins: H2A, H2B, H3 and H4. Each of the histones possess lysine-rich tails and accessibility of the DNA is controlled by modifications to the tail. Lysines can either be multiply methylated or acetylated. Methylation and deacetylation of lysines both contribute to a more condensed chromatin structure, preventing transcription of genes. Demethylation and acetylation promote a more open chromatin structure allowing for increased gene transcription.

Table 1. Partial List of Histone Deacetylase Inhibitors Currently in Clinical Trials for the Treatment of Cancer

class	compound
aliphatic acids	valproic acid
	AR-42 (OSU-HDAC42)
hydroxamic acids	vorinostat (suberoylanilide hydroxamic acid, SAHA)[a]
	belinostat (PXD101)[b]
	dacinosat (LAQ824)
	panobinostat (LBH589)
	resminostat (4SC-201)
	PCI-24781
	SB939
	CHR2845
	CHR3996
	JNJ-26481585
benzamides	entinostat (MS-275)
	mocetinostat (MGCD0103)
	4SC-202
cyclic peptides	romidepsin (depsipeptide, FK228, FR901228)[a]

[a] Currently FDA approved. [b] Currently in registration trials.

HDACs, leading to unrestricted HAT activity and increased gene transcription. Several HDAC inhibitors are currently in clinical trials both in monotherapy and in combination therapy with other antitumor drugs. The HDIs currently in clinical trials fall primarily into the short-chain fatty acid class, the hydroxamate class, the cyclic peptide class and the benzamide class. A partial listing of these HDIs is provided in Table 1, and structures for several are provided in Figure 2. The HDACs that are targets for the HDIs discussed are all zinc-dependent enzymes. To date, most of the responses using HDAC inhibitors as single agents were observed in advanced hematological cancers and only very few were observed in solid tumors. In hematological malignancies, clinical efficacy has been observed in cutaneous T-cell lymphoma (CTCL), peripheral T cell-lymphoma (PTCL), and

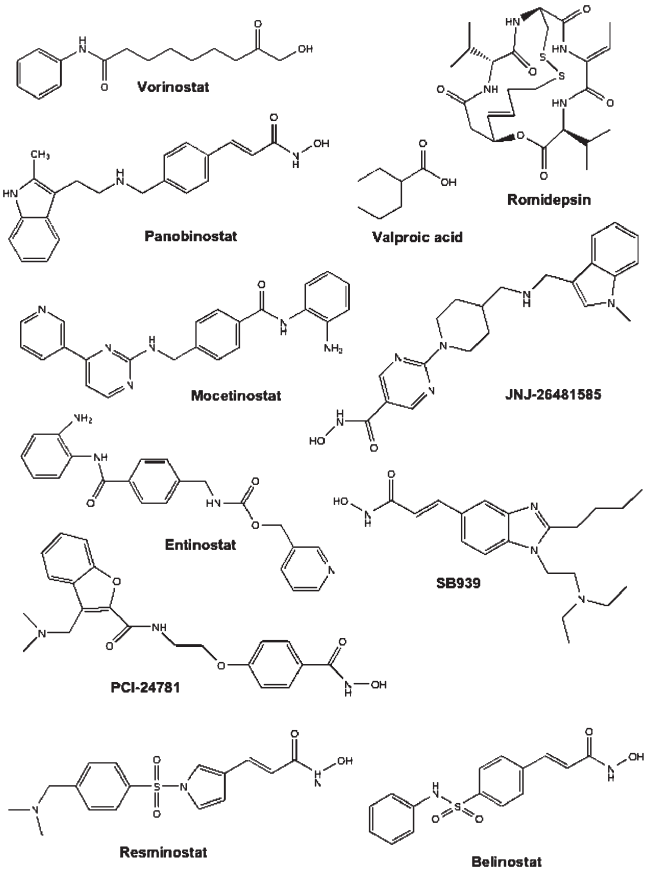

Figure 2. Structures of some of the histone deacetylase inhibitors currently in clinical trials.

Hodgkin and non-Hodgkin lymphoma, while only a few responses were observed in patients with myeloid malignancies.[8]

Some of the HDAC inhibitors that are being studied in clinical trials have demonstrated therapeutic potential in CTCL and other malignancies and are detailed below:

Vorinostat (Suberoylanilide Hydroxamic Acid, SAHA). An orally available, pan-HDAC inhibitor, vorinostat was found in *in vitro* studies to facilitate transcription of genes associated with growth arrest, differentiation, and apoptosis.[9,10] Responses in patients with refractory CTCL led to the approval of vorinostat in 2006 by the Food and Drug Administration (FDA) for the treatment of patients with relapsed or refractory CTCL.[11] In the registration trial, patients were treated with 400 mg daily of oral vorinostat, with an overall response rate of approximately 30% and a response duration of over 6 months.[11] Promising results have also been observed in follicular lymphoma and marginal zone lymphoma.[12] Combinations with the proteasome inhibitor bortezomib in the treatment of multiple myeloma have also seen clinical success.[13] In contrast, single agent trials with vorinostat for the treatment of most solid tumors have not met with success.[14]

Romidepsin (FK228, FR901228, NSC630176, Depsipeptide). Romidepsin is unique among the HDIs in that it is actually a prodrug; the disulfide bond of romidepsin must be reduced to yield the active form.[15] Most studies seem to suggest that romidepsin is an inhibitor of class I HDACs,[16] but some studies also find that romidepsin treatment leads to Hsp90 acetylation, leading to speculation that it might somehow affect HDAC6.[17] In 2001, we first reported the efficacy of romidepsin in a phase

I trial where partial responses (PRs) were observed in three patients with CTCL and a complete response (CR) was observed in one patient with PTCL.[18] These early successes in T-cell lymphoma led to two registration trials, culminating in the approval of romidepsin in November 2009 for the treatment of CTCL patients who had received at least one prior systemic therapy.[8] In the trial sponsored by the National Cancer Institute, among 71 patients with CTCL, the overall response rate was 34% with a median duration of response of 13.7 months.[19] In a second, independent, international trial of 96 patients with CTCL, the overall response rate was 38% and the median duration of response was 15 months.[20] In PTCL, an overall response rate of 38% was observed with a median duration of response of 8.9 months in a number of subtypes.[21] Romidepsin was recently approved by the FDA for the treatment of patients diagnosed with PTCL. As with vorinostat, results in solid tumors have been disappointing.[22−24]

Panobinostat (LBH589). The first clinical trials with this pan-HDAC inhibitor were conducted in patients with acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and myelodysplastic syndrome (MDS).[25] Panobinostat is currently in phase I and II clinical trials, with the most significant antitumor activity of this drug observed in patients with refractory CTCL and other hematologic malignancies.[26,27] As with the other HDIs, panobinostat has not been successful in solid tumor clinical trials,[26] so that the overall activity in T-cell lymphoma and inactivity in solid tumors appears to be a class effect.

Belinostat (PXD101). Belinostat is another pan-HDAC inhibitor that has been studied in multiple clinical trials as a single agent or in combination with chemotherapeutic agents. A phase I trial of this drug reported disease stabilization in patients with hematological malignancies.[28] Like romidepsin, belinostat has produced responses in patients diagnosed with PTCL.[29] Preliminary results from a phase II trial in lymphoma have been promising.[30]

Other HDIs. Less clinical data are available for some of the other HDIs. Some clinical activity was observed with valproic acid in pediatric patients with central nervous system tumors,[31] but not in a phase I trial for prostate cancer.[32] In a phase I trial, entinostat, an HDAC1-specific inhibitor, produced a partial remission in a patient with melanoma;[33] however, a subsequent phase II trial did not yield any objective responses.[34] Mocetinostat is a class I selective HDI that has demonstrated clinical activity in non-Hodgkin lymphoma and in relapsed or refractory Hodgkin lymphoma.[35] One patient with myelodysplastic syndrome and two patients with AML responded to single-agent mocetinostat in a phase I trial in leukemia,[36] while a phase II trial of mocetinostat in patients with chronic lymphocytic leukemia did not yield any responses.[37] A phase I trial of dacinostat in solid tumors demonstrated degradation of CRAF, an Hsp90 client protein, and increased histone acetylation in circulating peripheral blood mononuclear cells, but no responses were observed.[38]

MECHANISMS OF ACTION

One global effect of HDI treatment is an increase in histone acetylation. In clinical trials of HDIs, increased histone acetylation has been monitored in circulating peripheral blood mononuclear cells as a surrogate marker for the inhibition of HDACs.[39] However, this effect alone is apparently inadequate to confer activity, as solid tumor trials have demonstrated increased histone acetylation in tumor samples despite little clinical

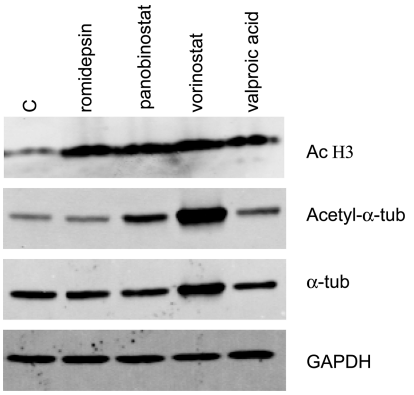

Figure 3. Histone deacetylase inhibitors all cause increased histone acetylation but differentially cause tubulin acetylation. HEK293 (Flp-In-239) cells were treated with 46 nM romidepsin, 100 nM panobinostat, 10 μM vorinostat or 1 mM valproic acid for 24 h after which protein was extracted, subjected to electrophoresis, and transferred to a PVDF membrane. The membrane was subsequently probed for acetylated histone H3 (AcH3), acetylated α-tubulin (acetyl-α-tub), total α-tubulin (α-tub) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). While all of the HDIs were able to induce histone acetylation, only panobinostat and vorinostat were able to cause increased tubulin acetylation, suggesting that these HDIs also target HDAC6.

effect.[40,41] In the search for the mechanism of action of HDIs, several have been suggested, including cell cycle arrest, activation of apoptotic pathways, induction of autophagy, reactive oxygen species generation, Hsp90 inhibition, and disruption of the aggresome pathway.[1,7,14] We elaborate on several of these potential mechanisms of action below:

Alteration of Gene Expression. Studies with cDNA arrays have shown that treatment with HDIs such as sodium butyrate, entinostat, vorinostat or romidepsin leads to a 2-fold or greater change in the expression of approximately 7−10% of the genes examined.[1] HDI treatment was found to induce about as many genes as were repressed. The histone deacetylase inhibitors induce p21 expression,[42] leading to G1 cell cycle arrest, and frequently downregulate cyclin D and c-myc. Whether or not these gene expression changes result in cell death probably depends upon cellular context. For example, decreased expression of c-myc will probably be more important for myc-dependent cancers, such as Burkitt's lymphoma, than for cancers that do not rely on c-myc for survival.[43] Vorinostat treatment has been shown to lead to decreased cyclin D1 expression and cell death in mantle cell lymphoma cell lines.[44]

Degradation of Hsp90 Client Proteins. Hsp90 is required to stabilize a number of "client" proteins that play a role in cancer cell proliferation, such as the human epidermal growth factor receptor 2 (ErbB-2, Her-2) and epidermal growth factor receptor (ErbB-1, EGFR); the fusion oncogene Bcr-Abl; and signaling transduction molecules such as Akt.[45] HDAC6 has been shown to be a deacetylase of both tubulin and Hsp90.[46] Treatment of cells with compounds that are known to inhibit HDAC6 leads to increased tubulin acetylation, increased Hsp90 acetylation and degradation of Hsp90 client proteins, having much the same mechanism of action as Hsp90 inhibitors.

Romidepsin was one of the first HDIs found to induce Hsp90 acetylation and cause degradation of the Hsp90 client proteins EGFR, Her-2 and Raf-1.[17] In breast cancer cell lines overexpressing Her-2, treatment with vorinostat or panobinostat has been

shown to lead to increased Hsp90 acetylation, Her-2 degradation and cell death, and synergy has been demonstrated when the HDIs were combined with Hsp90 inhibitors or Her-2 inhibitors such as trastuzumab or lapatinib.[47,48] Similarly, treatment of lung cancer cell lines expressing mutant EGFR with panobinostat resulted in decreased EGFR expression and synergized with the EGFR inhibitors erlotinib or lapatinib.[48,49] Leukemia cells expressing the Bcr-Abl fusion protein are particularly sensitive to treatment with vorinostat, dacinostat, romidepsin or panobinostat and cotreatment with imatinib or nilotinib results in synergistic cell death.[50−53] Again, the ability of an HDI to inhibit HDAC6 would probably be effective only in cancers where proliferation is driven by Hsp90 client proteins, although cell death could also occur as a result of deacetylation of other HDAC6 substrates.[54]

It is not clear, however, whether HDAC6 is the sole deacetylase of Hsp90. Romidepsin is considered a rather weak inhibitor of HDAC6, as romidepsin treatment does not result in acetylation of tubulin,[42] yet has still been shown to cause acetylation of Hsp90.[17] As seen in Figure 3, treating HEK293 (Flp-In-293) human embryonic kidney cells with varying concentrations of romidepsin, vorinostat, panobinostat or valproic acid results in increased histone H3 acetylation, but only results in tubulin acetylation in cells treated with vorinostat and panobinostat. Additionally, entinostat treatment has been shown to result in apoptosis due to degradation of mutant FLT-3, another Hsp90 client protein, in leukemia cells that express the mutant protein despite the fact that entinostat does not inhibit HDAC6.[55] It has been suggested the HDAC1 mediates this effect.[56] One study has suggested that acetylation of Hsp70 mediates degradation of the Bcr-Abl fusion protein.[57] Further study is needed to determine how HDIs that are weak inhibitors of HDAC6 mediate apoptosis in cell lines where proliferation is driven by Hsp90 client proteins.

Increased Production of Reactive Oxygen Species (ROS). Several studies have suggested that the generation of reactive oxygen species (ROS) is a key event in HDI-induced cell death. ROS generated by HDIs leads to DNA damage, and the addition of free radical scavengers such as *N*-acetylcysteine during the time of HDI treatment has been shown to result in decreased ROS generation and decreased HDI-mediated cell death.[58−61] HDI treatment has also been shown to prevent repair of DNA damage.[62,63] Another mechanism by which HDIs increase ROS production is through downregulation of thioredoxin and upregulation of thioredoxin-binding protein 2 (TBP-2). Thioredoxin is a thiol reductase that acts as a scavenger of ROS, and TBP-2 has been shown to be a negative regulator of thioredoxin, decreasing its reducing activity.[64] Vorinostat treatment has been shown not only to increase TBP-2 expression but also to suppress thioredoxin expression.[65]

Alterations in the Apoptotic Pathway. Apoptosis proceeds either via the extrinsic or cell death receptor-mediated pathway or the intrinsic or mitochondria-mediated pathway. Several HDIs have been shown to facilitate death by the extrinsic pathway by causing an increase in the expression of TRAIL, DR-4, DR-5, Fas and FasL as well as a decrease in c-FLIP, a protein associated with resistance to TRAIL-mediated apoptosis.[66−68] Activation of the apoptotic machinery associated with the intrinsic pathway, by decreasing expression of the antiapoptotic proteins Bcl-2, Bcl-XL, Mcl-1 and survivin and increasing expression of the proapoptotic proteins Bax and Bim, seems to be a class effect of the HDIs.[47,49,58,69]

Table 2. Summary of Resistance Mechanisms to HDIs

- ABC transporter expression:
  - increased levels of Pgp/ABCB1 or MRP1/ABCC1 (only true for romidepsin)
- cell cycle proteins:
  - increased p21 expression
- thioredoxin expression:
  - increased thioredoxin levels resulting in decreased ROS-mediated DNA damage
- apoptosis-related proteins:
  - increased levels of antiapoptotic proteins such as Bcl-2 and Bcl-XL
  - inability to upregulate proapoptotic proteins such as Bim
- alterations in HDAC protein levels
- signaling proteins:
  - increased signaling via MAPK, PI3K or STAT3
- NFkB activation:
  - acetylation of p65

MECHANISMS OF RESISTANCE

As the clinical course of the HDIs is pursued, it becomes important to identify potential mechanisms of resistance so as to increase efficacy and identify potential drug combinations. Despite the fact that HDIs have been in development for several years, relatively few drug-selected cell lines have been developed. Most of these *in vitro* selections have been with romidepsin alone, where emergence of P-glycoprotein (Pgp) seems to be the dominant mechanism of resistance.[70−72] Non-Pgp mechanisms of resistance have only been observed when cells are selected with romidepsin in the presence of a Pgp inhibitor.[71] However, some mechanisms of resistance have been identified by transfection with purported resistance mechanisms. Summarized below as well as in Table 2 are some of the major mechanisms of resistance to HDIs that have been characterized.

As will be noted below, the search for resistance mechanisms has primarily centered on laboratory models, and sparse clinical data have been gathered. Most studies have detected histone acetylation, whether in peripheral blood mononuclear cells or in biopsy samples, and some have concluded that the presence of histone acetylation does not correlate with response to therapy.[73] Our data with romidepsin suggested otherwise, when we compared histone acetylation analyzed by an immunodot blot assay with response on a clinical trial in cutaneous and peripheral T-cell lymphoma. It appeared that higher and more durable levels of histone acetylation in peripheral blood mononuclear cells were associated with better clinical response.[39] These findings may have been unique to romidepsin, which, as a prodrug, requires reduction of the disulfide bond to an active form.[15]

ATP-Binding Cassette Transporters. Treatment with histone deacetylase inhibitors, such as sodium butyrate, was found several years ago to induce a more differentiated phenotype, accompanied by increased expression of the multidrug-resistance gene, *MDR1* (*ABCB1*), and its product, Pgp.[74,75] Trichostatin A treatment was also found to increase Pgp expression.[76] Romidepsin was first brought to our attention after it was identified as a Pgp substrate based on rhodamine efflux patterns in the NCI Anticancer Drug Screen and was also found to induce expression of Pgp.[77] Valproic acid and apicidin have also been shown to increase *ABCB1* expression;[78,79] thus upregulation of *ABCB1* resulting in increased Pgp expression is believed to be a class effect of histone deacetylase inhibitors.

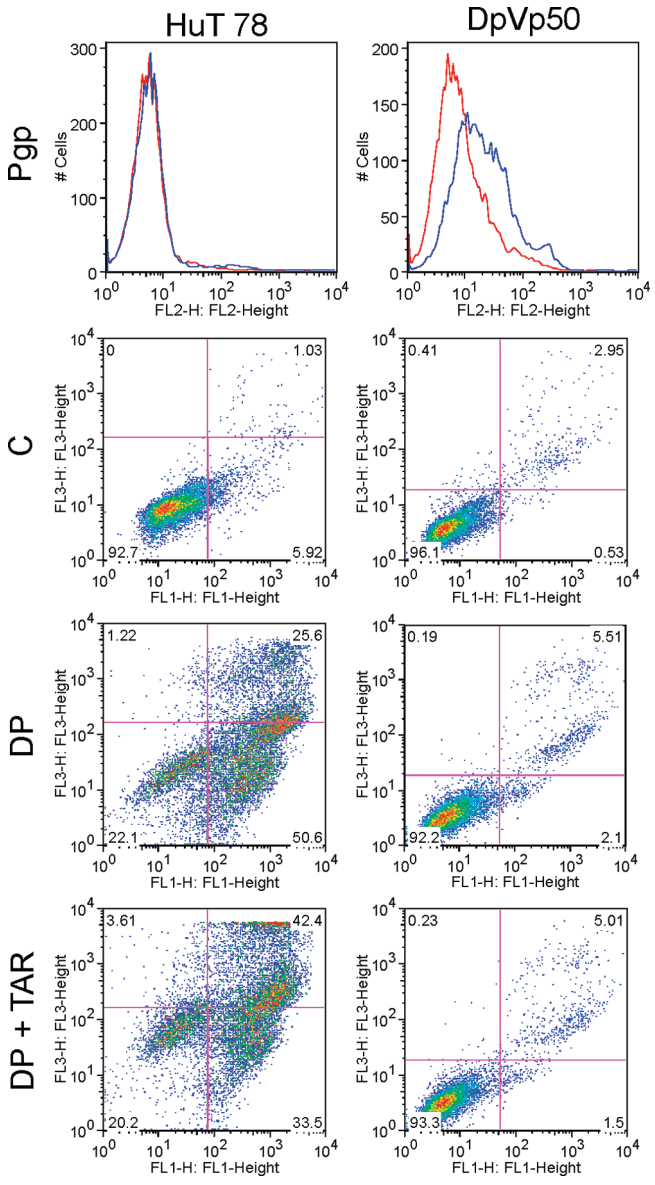

Figure 4. Resistance to romidepsin is not mediated by Pgp expression in HuT78 DpVp50 cells. HuT78 parental and DpVp50 cells were incubated with the Pgp-specific antibody, MRK-16 (blue histogram), or IgG negative control antibody (red histogram) for 30 min, after which cells were washed and incubated with phycoerythrin-labeled secondary antibody (top row, Pgp). While HuT78 parental cells are Pgp negative, the DpVp50 cells express low but detectable levels. Cells were also left untreated (second row, C) or were incubated with 50 ng/mL romidepsin for 48 h in the presence (third row, DP) or absence (bottom row DP + TAR) of 250 nM of the Pgp inhibitor tariquidar, after which cells were incubated with annexin V antibody and propidium iodide. Cells in the lower left quadrant are viable cells, while cells in the lower right quadrant are early apoptotic cells, and cells in the upper right quadrant are late apoptotic or necrotic cells. HuT parental cells readily undergo apoptosis after incubation with romidepsin either in the presence or in the absence of tariqudiar, as shown by the increase of cells in the upper and lower right quadrants. DpVp50 cells are resistant to romidepsin whether the inhibitor is added or not, suggesting a resistance mechanism that does not involve Pgp.

Romidepsin is unique among the HDIs in that it is also a substrate of Pgp, while other HDIs, such as vorinostat and belinostat, are not.[80,81] Cell lines expressing the multidrug resistance-associated protein-1 (*MRP1/ABCC1*) have also been found to be resistant to romidepsin, although less so than cells that express Pgp.[82] Cell lines selected for resistance to romidepsin express Pgp and are also resistant to other Pgp substrates such as vincristine or paclitaxel; resistance to romidepsin can be overcome by Pgp inhibitors.[70,72,83] Despite the major role of Pgp in romidepsin resistance in cancer cell lines, *ABCB1* gene expression in tumor biopsy samples from patients with CTCL enrolled on the NCI phase II trial did not appear to correlate with resistance to romidepsin treatment,[39] even though increased *ABCB1* expression has been observed in peripheral blood mononuclear cells and circulating tumor cells obtained from patients receiving depsipeptide.[70] Thus, other mechanisms of resistance are likely to play a role in the intrinsic resistance observed in clinical trials with HDIs.

In an attempt to identify non-Pgp mechanisms of resistance, we selected the HuT 78 cell line, derived from a patient with Sézary syndrome, with romidepsin in the presence of the Pgp inhibitor verapamil.[71] One of the resulting romidepsin-resistant cell lines, HuT DpVp50, is maintained in 50 ng/mL romidepsin in the presence of 5 μg/mL verapamil. As seen in Figure 4, although the DpVp50 cells express some Pgp, this is not the dominant mechanism of resistance; treatment of the resistant line with 50 ng/mL romidepsin for 48 h in the presence of the Pgp inhibitor tariquidar does not result in increased cell death compared to romidepsin treatment without tariquidar. HuT 78 parental cells, which do not express Pgp are exquisitely sensitive to romidepsin; accordingly, the addition of tariquidar does not have any effect on cytotoxicity. We are currently working to characterize the mechanism of resistance to romidepsin in these cell lines.

Cell Cycle Proteins. It has been postulated that the induction of p21, which is responsible for the G1 arrest caused by HDI treatment, might serve a protective role. We reported that, when p21-deficient HCT116 cells were treated with romidepsin, cells arrested only in G2 and were more sensitive to treatment compared to wild-type cells.[84] In accordance with that result, U937 leukemia cells transfected with a p21 antisense construct were found to be more sensitive to vorinostat treatment when compared to untransfected cells.[85] Additionally, cotreatment with flavopiridol has been shown to potentiate the cytotoxicty of romidepsin, sodium butyrate and vorinostat, due in part to the prevention of p21 upregulation.[86−88] Temisirolimus treatment has also been shown to decrease p21 expression in mantle cell lymphoma cell lines and to synergize with sublethal concentrations of vorinostat.[89] Clinical trials with vorinostat and temisirolimus or sirolimus are currently ongoing.

Increased Thioredoxin Levels. As mentioned above, thioredoxin is a scavenger of reactive oxygen and expression of TBP-2 has been shown to decrease its reductive capacity. Ungerstedt and colleagues found that high levels of thioredoxin in normal cells served to protect cells from ROS induced by HDI treatment.[90] Additionally, when HDI-sensitive transformed cells were transfected with a small-interfering RNA (siRNA) against thioredoxin, the cells exhibited higher ROS levels and increased cell death compared to untransfected cells.[90] Similarly, Chen and colleagues reported in romidepsin-treated, human lung cancer cells that thioredoxin expression negatively correlated with ROS generation and apoptosis,[91] supporting the idea that HDIs in combination with compounds that decrease thioredoxin expression or function may lead to increased activity.

Apoptosis-Related Proteins. Enforced expression of antiapoptotic proteins has been shown to prevent HDI-mediated cell death. High levels of Bcl-2 or Bcl-XL have been shown to confer resistance to treatment with vorinostat, dacinostat, panobinostat or oxamflatin, while only high levels of Bcl-2 were found to confer resistance to romidepsin treatment.[85,92,93] Bcl-2 expression has also been linked to sensitivity to panobinostat treatment in CTCL cell lines.[94] Increased apoptosis has been observed when HDIs are combined with Bcl-2 inhibitors such as ABT-737 or others in several model systems,[94−98] suggesting that the combination should be tested in the clinical setting.

When Shao and colleagues knocked down expression of the proapopototic Bax in panobinostat-sensitive, T-cell lymphoma cell lines, toxicity was diminished.[94] Similarly, knockdown of antiapoptotic Mcl-1 was found to potentiate HDI-mediated apoptosis in primary chronic lymphocytic leukemia cells and K562 cells.[99] Expression of Bim has also been shown to be required for romidepsin-mediated apoptosis in lung cancer cells.[100] Thus, in cells where these proapoptic proteins are silenced, HDI-mediated cell death might be blunted. Treatment with targeted therapeutics such as erlotinib or imatinib has been shown to increase levels of Bim in some model systems,[101,102] suggesting that combination with an HDI might be advantageous.

Alterations in HDAC Protein Levels. Another cell line selected for resistance to HDIs was generated by selection of the HL-60 leukemia cell line with dacinostat, resulting in the HL-60/LR cell line maintained in 200 nM dacinostat.[103] In addition to dacinostat, the resistant line was also highly cross-resistant to vorinostat, panobinostat and sodium butyrate.[103] While levels of BCL-XL and XIAP were attenuated, levels of Bim and Bax remained unchanged.[103] Interestingly, HL-60/LR cells expressed higher levels of HDACs 1, 2, and 4, but lacked expression of HDAC6 and had higher levels of Hsp90 acetylation compared to the parental line.[103] The resistant line also demonstrated collateral sensitivity to Hsp90 inhibitors.[103] A separate study demonstrated that increased HDAC1 expression prevented sodium butyrate-mediated toxicity in a melanoma cell line.[104] It is not clear whether this mechanism of resistance has clinical relevance; no studies have yet linked altered HDAC protein expression levels with clinical response to HDIs. However, HDAC2 expression levels were found to correlate with histone acetylation in a phase I trial of doxorubicin and vorinostat[105] in solid tumors and combined tamoxifen and vorinostat treatment in a phase II trial in breast cancer.[106]

Signaling Proteins. Activation of the mitogen-activated protein kinase (MAPK) or phosphoinositide 3-kinase (PI3K) pathways has increasingly been associated with resistance to HDIs. Combination of romidepsin with mitogen-activated protein kinase kinase (MEK) inhibitors has been shown to increase cell death, suggesting that activation of the MAPK pathway is an important mechanism of resistance to romidepsin.[107,108] In agreement with this hypothesis, Yu and colleagues found that enforced expression of constitutively active MEK1, but not Akt, in lung cancer cell lines reduced romidepsin-mediated cytotoxicity.[109] Other studies, however, seem to suggest that phosphorylated Akt is, in fact, an important resistance mechanism to romidepsin, as combination of romidepsin with inhibitors of Akt results in synergistic cytotoxicity.[110] Combination of panobinostat with compounds that abrogate MAPK and PI3K signaling has also been shown to result in synergistic cytotoxicity, possibly due to increased ROS.[111] Similarly, Jane and colleagues found that treatment of glioma cells with vandetanib inhibited both the MAPK and PI3K pathways and was synergistic with vorinsotat treatment.[112] Vorinostat in combination with PI3K inhibitors has shown promise in T-cell lymphoma cell line models.[113] These combination studies, while targeting different signaling molecules, do suggest that activation of one or more of these pathways may confer clinical resistance.

Activation of the signal transducer and activator of transcription (STAT) pathway has also been linked to vorinostat resistance. In a panel of almost 40 lymphoma cell lines, expression of STAT1, -3, and -5 was higher in cell lines that were more resistant to vorinostat compared to sensitive lines; phosphorylation levels of the STAT proteins were also higher in the resistant lines.[114] In a series of skin biopsy samples, patients with higher nuclear staining of phosphorylated STAT3 were more likely to be resistant to vorinostat treatment,[114] again implicating activation of signaling pathways in resistance to HDIs. Combined treatment with vorinostat and compounds shown to inhibit STAT3 phosphorylation, such as lestaurtinib,[115] may therefore increase efficacy in T-cell lymphoma.

NFκB Activation. Activation of the NFκB pathway is a hallmark of a number of cancers and leads to deactivation of the apoptotic pathway and increased cell survival.[116] Acetylation of the p65, or RelA, subunit of NFκB has been shown to increase the acitivity of NFκB,[117] and HDI treatment has been shown to result in increased p65 acetylation.[118,119] Constitutive activation of NFκB has been linked to resistance to HDIs in cell line models,[94] as has p65 acetylation caused by HDI treatment; combination of an HDI with an inhibitor of NFκB activation leads to synergistic cytotoxicity.[120] The increased cytotoxicity observed when HDIs are combined with proteasome inhibitors is believed to be due, at least in part, to decreased NFκB activity mediated by proteasome inhibitors.[121,122] However, while a phase II trial with vorinostat and bortezomib in multiple myeloma had a 42% response rate and elicited some responses from patients whose disease was refractory to bortezomib, response did not correlate with levels of NFκB or IκB^{13} in CD138+ bone marrow cells, casting some doubt on the relevance of NFκB status.

CONCLUSION

The histone deacetylase inhibitors have shown promise in the treatment of peripheral and cutaneous T-cell lymphomas. Their lack of success in clinical trials for solid tumors has been disappointing. As noted above, a long list of mechanisms of action has been compiled through *in vitro* studies. It can be concluded from this list that we still do not really understand how the HDIs work when they are effective, as in T-cell lymphomas. We have yet to validate a marker that predicts clinical response to HDI treatment. We also do not understand why they work in T-cell lymphomas and not in solid tumors. Perhaps in the context of the overall sensitivity of lymphomas to anticancer therapy, a decades-old observation, this is not so surprising. But the activity in T-cell lymphoma seems beyond that in other hematologic malignancies and is a class effect. It is likely that a dominant mechanism of action, not necessarily on the list of mechanisms outlined above, is responsible for the efficacy.

The insensitivity of solid tumors also appears to be a class effect. Unfortunately, *in vitro* models do not typically reflect the insensitivity of solid tumors in the clinic. This may be due in part to the rapid doubling time of cell lines in culture, and the epigenetic alterations that accompany that cell growth rate. Combined with the duration of exposure typically used in cell

culture sensitivity assays, responses to HDIs in the laboratory are homogeneous across different tumor types. The negative impact of this ubiquitous responsiveness is to divert those working in the field from focusing on a few candidate solid tumor types for targeted drug development.

One approach to overcoming resistance in solid tumors is to exploit the diverse effects of HDIs in solid tumors in combination therapies. Thus, if altered gene expression does not itself prompt cytotoxicity, it may allow promising combination therapies to be developed. Induction of the sodium iodide symporter and increased sensitivity of thyroid cancer cells to radioiodine uptake is one such example.[123] If altered handling of reactive oxygen species or reduced DNA repair is insufficient to induce cell death following HDI exposure, it may still allow for effective combination therapies with DNA damaging agents, whether chemotherapy or radiotherapy.[124,125] It is critical, however, in developing the rationale for combination studies that attention be paid to sequence of administration. For example, in small cell lung cancer cells, simultaneous exposure to an HDI and cisplatin or etoposide was more effective than sequential exposure.[126] Among the plethora of HDI effects on cells are likely to be some that are detrimental to cytotoxicity (e.g., p21 induction).

Further, the *in vitro* paradox also has the potential to yield misleading clinical directions from combination assays that require durations of exposure for efficacy that are not achievable in the clinic by any HDI developed to date. The length of the list of mechanisms of resistance generated from laboratory models confirms that we do not understand resistance, either. We can only hope that somewhere on those lists are the answers and that translational studies will help us separate the important mechanisms from those that are trivial. What we do know is that development of the HDIs is a major step in bringing epigenetic therapy to the anticancer armamentarium. We need to figure out how to exploit them more fully and in many more tumor types.

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REFERENCES

(1) Xu, W. S.; Parmigiani, R. B.; Marks, P. A. Histone deacetylase inhibitors: molecular mechanisms of action. *Oncogene* **2007**, *26* (37), 5541–52.

(2) Johnstone, R. W. Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. *Nat. Rev. Drug Discovery* **2002**, *1* (4), 287–99.

(3) Bianco-Miotto, T.; Chiam, K.; Buchanan, G.; Jindal, S.; Day, T. K.; Thomas, M.; Pickering, M. A.; O'Loughlin, M. A.; Ryan, N. K.; Raymond, W. A.; Horvath, L. G.; Kench, J. G.; Stricker, P. D.; Marshall, V. R.; Sutherland, R. L.; Henshall, S. M.; Gerald, W. L.; Scher, H. I.; Risbridger, G. P.; Clements, J. A.; Butler, L. M.; Tilley, W. D.; Horsfall, D. J.; Ricciardelli, C.; BioResource, A. P. C. Global levels of specific histone modifications and an epigenetic gene signature predict prostate cancer progression and development. *Cancer Epidemiol. Biomarkers Prev.* **2010**, *19* (10), 2611–22.

(4) Elsheikh, S.; Green, A.; Rakha, E.; Powe, D.; Ahmed, R.; Collins, H.; Soria, D.; Garibaldi, J.; Paish, C.; Ammar, A.; Grainge, M.; Ball, G.; Abdelghany, M.; Martinez-Pomares, L.; Heery, D.; Ellis, I. Global histone modifications in breast cancer correlate with tumor phenotypes, prognostic factors, and patient outcome. *Cancer Res.* **2009**, *69* (9), 3802–9.

(5) Choudhary, C.; Kumar, C.; Gnad, F.; Nielsen, M. L.; Rehman, M.; Walther, T. C.; Olsen, J. V.; Mann, M. Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* **2009**, *325* (5942), 834–40.

(6) Marks, P.; Xu, W. Histone deacetylase inhibitors: Potential in cancer therapy. *J. Cell. Biochem.* **2009**, *107* (4), 600–8.

(7) Schrump, D. Cytotoxicity mediated by histone deacetylase inhibitors in cancer cells: mechanisms and potential clinical implications. *Clin. Cancer Res.* **2009**, *15* (12), 3947–57.

(8) Mercurio, C.; Minucci, S.; Pelicci, P. G. Histone deacetylases and epigenetic therapies of hematological malignancies. *Pharmacol. Res.* **2010**, *62* (1), 18–34.

(9) Rasheed, W.; Bishton, M.; Johnstone, R. W.; Prince, H. M. Histone deacetylase inhibitors in lymphoma and solid malignancies. *Expert Rev. Anticancer Ther.* **2008**, *8* (3), 413–32.

(10) Marks, P. A.; Breslow, R. Dimethyl sulfoxide to vorinostat: development of this histone deacetylase inhibitor as an anticancer drug. *Nat. Biotechnol.* **2007**, *25* (1), 84–90.

(11) Olsen, E.; Kim, Y.; Kuzel, T.; Pacheco, T.; Foss, F.; Parker, S.; Frankel, S.; Chen, C.; Ricker, J.; Arduino, J.; Duvic, M. Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. *J. Clin. Oncol.* **2007**, *25* (21), 3109–15.

(12) Kirschbaum, M.; Frankel, P.; Popplewell, L.; Zain, J.; Delioukina, M.; Pullarkat, V.; Matsuoka, D.; Pulone, B.; Rotter, A. J.; Espinoza-Delgado, I.; Nademanee, A.; Forman, S. J.; Gandara, D.; Newman, E. Phase II study of >vorinostat for treatment of relapsed or refractory indolent non-Hodgkin's lymphoma and mantle cell lymphoma. *J. Clin. Oncol.* **2011**, *29* (9), 1198–203.

(13) Badros, A.; Burger, A.; Philip, S.; Niesvizky, R.; Kolla, S.; Goloubeva, O.; Harris, C.; Zwiebel, J.; Wright, J.; Espinoza-Delgado, I.; Baer, M.; Holleran, J.; Egorin, M.; Grant, S. Phase I study of vorinostat in combination with bortezomib for relapsed and refractory multiple myeloma. *Clin. Cancer Res.* **2009**, *15* (16), 5250–7.

(14) Lane, A.; Chabner, B. Histone deacetylase inhibitors in cancer therapy. *J. Clin. Oncol.* **2009**, *27* (32), 5459–68.

(15) Furumai, R.; Matsuyama, A.; Kobashi, N.; Lee, K. H.; Nishiyama, M.; Nakajima, H.; Tanaka, A.; Komatsu, Y.; Nishino, N.; Yoshida, M.; Horinouchi, S. FK228 (depsipeptide) as a natural prodrug that inhibits class I histone deacetylases. *Cancer Res.* **2002**, *62* (17), 4916–21.

(16) Bantscheff, M.; Hopf, C.; Savitski, M. M.; Dittmann, A.; Grandi, P.; Michon, A. M.; Schlegl, J.; Abraham, Y.; Becher, I.; Bergamini, G.; Boesche, M.; Delling, M.; Dümpelfeld, B.; Eberhard, D.; Huthmacher, C.; Mathieson, T.; Poeckel, D.; Reader, V.; Strunk, K.; Sweetman, G.; Kruse, U.; Neubauer, G.; Ramsden, N. G.; Drewes, G. Chemoproteomics profiling of HDAC inhibitors reveals selective targeting of HDAC complexes. *Nat. Biotechnol.* **2011**, *29* (3), 255–65.

(17) Yu, X.; Guo, Z. S.; Marcu, M. G.; Neckers, L.; Nguyen, D. M.; Chen, G. A.; Schrump, D. S. Modulation of p53, ErbB1, ErbB2, and Raf-1 expression in lung cancer cells by depsipeptide FR901228. *J. Natl. Cancer Inst.* **2002**, *94* (7), 504–13.

(18) Piekarz, R. L.; Robey, R.; Sandor, V.; Bakke, S.; Wilson, W. H.; Dahmoush, L.; Kingma, D. M.; Turner, M. L.; Altemus, R.; Bates, S. E. Inhibitor of histone deacetylation, depsipeptide (FR901228), in the treatment of peripheral and cutaneous T-cell lymphoma: a case report. *Blood* **2001**, *98* (9), 2865–8.

(19) Piekarz, R.; Frye, R.; Turner, M.; Wright, J.; Allen, S.; Kirschbaum, M.; Zain, J.; Prince, H.; Leonard, J.; Geskin, L.; Reeder, C.; Joske,

D.; Figg, W.; Gardner, E.; Steinberg, S.; Jaffe, E.; Stetler-Stevenson, M.; Lade, S.; Fojo, A.; Bates, S. Phase II multi-institutional trial of the histone deacetylase inhibitor romidepsin as monotherapy for patients with cutaneous T-cell lymphoma. *J. Clin. Oncol.* **2009**, *27* (32), 5410–7.

(20) Whittaker, S. J.; Demierre, M. F.; Kim, E. J.; Rook, A. H.; Lerner, A.; Duvic, M.; Scarisbrick, J.; Reddy, S.; Robak, T.; Becker, J. C.; Samtsov, A.; McCulloch, W.; Kim, Y. H. Final results from a multicenter, international, pivotal study of romidepsin in refractory cutaneous T-cell lymphoma. *J. Clin. Oncol.* **2010**, *28* (29), 4485–91.

(21) Piekarz, R. L.; Frye, R.; Prince, H. M.; Kirschbaum, M. H.; Zain, J.; Allen, S. L.; Jaffe, E. S.; Ling, A.; Turner, M.; Peer, C. J.; Figg, W. D.; Steinberg, S. M.; Smith, S.; Joske, D.; Lewis, I.; Hutchins, L.; Craig, M.; Fojo, A. T.; Wright, J. J.; Bates, S. E. Phase II trial of romidepsin in patients with peripheral T-cell lymphoma. *Blood* **2011**.

(22) Grant, C.; Rahman, F.; Piekarz, R.; Peer, C.; Frye, R.; Robey, R. W.; Gardner, E. R.; Figg, W. D.; Bates, S. E. Romidepsin: a new therapy for cutaneous T-cell lymphoma and a potential therapy for solid tumors. *Expert Rev. Anticancer Ther.* **2010**, *10* (7), 997–1008.

(23) Molife, L.; Attard, G.; Fong, P.; Karavasilis, V.; Reid, A.; Patterson, S.; Riggs, C. J.; Higano, C.; Stadler, W.; McCulloch, W.; Dearnaley, D.; Parker, C.; de Bono, J. Phase II, two-stage, single-arm trial of the histone deacetylase inhibitor (HDACi) romidepsin in metastatic castration-resistant prostate cancer (CRPC). *Ann. Oncol.* **2010**, *21* (1), 109–13.

(24) Stadler, W. M.; Margolin, K.; Ferber, S.; McCulloch, W.; Thompson, J. A. A phase II study of depsipeptide in refractory metastatic renal cell cancer. *Clin. Genitourin. Cancer* **2006**, *5* (1), 57–60.

(25) Giles, F.; Fischer, T.; Cortes, J.; Garcia-Manero, G.; Beck, J.; Ravandi, F.; Masson, E.; Rae, P.; Laird, G.; Sharma, S.; Kantarjian, H.; Dugan, M.; Albitar, M.; Bhalla, K. A phase I study of intravenous LBH589, a novel cinnamic hydroxamic acid analogue histone deacetylase inhibitor, in patients with refractory hematologic malignancies. *Clin. Cancer Res.* **2006**, *12* (15), 4628–35.

(26) Prince, H.; Bishton, M.; Johnstone, R. Panobinostat (LBH589): a potent pan-deacetylase inhibitor with promising activity against hematologic and solid tumors. *Future Oncol.* **2009**, *5* (5), 601–12.

(27) Dickinson, M.; Ritchie, D.; DeAngelo, D.; Spencer, A.; Ottmann, O.; Fischer, T.; Bhalla, K.; Liu, A.; Parker, K.; Scott, J.; Bishton, M.; Prince, H. Preliminary evidence of disease response to the pan deacetylase inhibitor panobinostat (LBH589) in refractory Hodgkin Lymphoma. *Br. J. Haematol.* **2009**, *147* (1), 97–101.

(28) Gimsing, P.; Hansen, M.; Knudsen, L.; Knoblauch, P.; Christensen, I.; Ooi, C.; Buhl-Jensen, P. A phase I clinical trial of the histone deacetylase inhibitor belinostat in patients with advanced hematological neoplasia. *Eur. J. Haematol.* **2008**, *81* (3), 170–6.

(29) Howman, R. A.; Prince, H. M. New drug therapies in peripheral T-cell lymphoma. *Expert Rev. Anticancer Ther.* **2011**, *11* (3), 457–72.

(30) Zain, J. M.; O'Connor, O.; Zinzani, P. L.; Norman, A.; de Nully Brown, P. Multicenter, open-label trial of PXD 101 in patients with relapsed/refractory peripheral T-cell lymphoma. *ASCO Meeting Abstracts* **2010**, *28* (15_suppl), e18565.

(31) Su, J. M.; Li, X. N.; Thompson, P.; Ou, C. N.; Ingle, A. M.; Russell, H.; Lau, C. C.; Adamson, P. C.; Blaney, S. M. Phase 1 study of valproic acid in pediatric patients with refractory solid or CNS tumors: a children's oncology group report. *Clin. Cancer Res.* **2011**, *17* (3), 589–97.

(32) Sharma, S.; Symanowski, J.; Wong, B.; Dino, P.; Manno, P.; Vogelzang, N. A Phase II Clinical Trial of Oral Valproic Acid in Patients with Castration-Resistant Prostate Cancers Using an Intensive Biomarker Sampling Strategy. *Transl Oncol* **2008**, *1* (3), 141–7.

(33) Gore, L.; Rothenberg, M.; O'Bryant, C.; Schultz, M.; Sandler, A.; Coffin, D.; McCoy, C.; Schott, A.; Scholz, C.; Eckhardt, S. A phase I and pharmacokinetic study of the oral histone deacetylase inhibitor, MS-275, in patients with refractory solid tumors and lymphomas. *Clin. Cancer Res.* **2008**, *14* (14), 4517–25.

(34) Hauschild, A.; Trefzer, U.; Garbe, C.; Kaehler, K.; Ugurel, S.; Kiecker, F.; Eigentler, T.; Krissel, H.; Schott, A.; Schadendorf, D. Multicenter phase II trial of the histone deacetylase inhibitor pyridylmethyl-N-{4-[(2-aminophenyl)-carbamoyl]-benzyl}-carbamate in pretreated metastatic melanoma. *Melanoma Res.* **2008**, *18* (4), 274–8.

(35) Boumber, Y.; Younes, A.; Garcia-Manero, G. Mocetinostat (MGCD0103): a review of an isotype-specific histone deacetylase inhibitor. *Expert Opin. Invest. Drugs* **2011**, *20* (6), 823–9.

(36) Garcia-Manero, G.; Assouline, S.; Cortes, J.; Estrov, Z.; Kantarjian, H.; Yang, H.; Newsome, W. M.; Miller, W. H.; Rousseau, C.; Kalita, A.; Bonfils, C.; Dubay, M.; Patterson, T. A.; Li, Z.; Besterman, J. M.; Reid, G.; Laille, E.; Martell, R. E.; Minden, M. Phase 1 study of the oral isotype specific histone deacetylase inhibitor MGCD0103 in leukemia. *Blood* **2008**, *112* (4), 981–9.

(37) Blum, K. A.; Advani, A.; Fernandez, L.; Van Der Jagt, R.; Brandwein, J.; Kambhampati, S.; Kassis, J.; Davis, M.; Bonfils, C.; Dubay, M.; Dumouchel, J.; Drouin, M.; Lucas, D. M.; Martell, R. E.; Byrd, J. C. Phase II study of the histone deacetylase inhibitor MGCD0103 in patients with previously treated chronic lymphocytic leukaemia. *Br. J. Haematol.* **2009**, *147* (4), 507–14.

(38) de Bono, J. S.; Kristeleit, R.; Tolcher, A.; Fong, P.; Pacey, S.; Karavasilis, V.; Mita, M.; Shaw, H.; Workman, P.; Kaye, S.; Rowinsky, E. K.; Aherne, W.; Atadja, P.; Scott, J. W.; Patnaik, A. Phase I pharmacokinetic and pharmacodynamic study of LAQ824, a hydroxamate histone deacetylase inhibitor with a heat shock protein-90 inhibitory profile, in patients with advanced solid tumors. *Clin. Cancer Res.* **2008**, *14* (20), 6663–73.

(39) Bates, S.; Zhan, Z.; Steadman, K.; Obrzut, T.; Luchenko, V.; Frye, R.; Robey, R.; Turner, M.; Gardner, E.; Figg, W.; Steinberg, S.; Ling, A.; Fojo, T.; To, K.; Piekarz, R. Laboratory correlates for a phase II trial of romidepsin in cutaneous and peripheral T-cell lymphoma. *Br. J. Haematol.* **2010**, *148* (2), 256–67.

(40) Schrump, D. S.; Fischette, M. R.; Nguyen, D. M.; Zhao, M.; Li, X.; Kunst, T. F.; Hancox, A.; Hong, J. A.; Chen, G. A.; Kruchin, E.; Wright, J. J.; Rosing, D. R.; Sparreboom, A.; Figg, W. D.; Steinberg, S. M. Clinical and molecular responses in lung cancer patients receiving Romidepsin. *Clin. Cancer Res.* **2008**, *14* (1), 188–98.

(41) Siu, L.; Pili, R.; Duran, I.; Messersmith, W.; Chen, E.; Sullivan, R.; MacLean, M.; King, S.; Brown, S.; Reid, G.; Li, Z.; Kalita, A.; Laille, E.; Besterman, J.; Martell, R.; Carducci, M. Phase I study of MGCD0103 given as a three-times-per-week oral dose in patients with advanced solid tumors. *J. Clin. Oncol.* **2008**, *26* (12), 1940–7.

(42) Blagosklonny, M. V.; Robey, R.; Sackett, D. L.; Du, L.; Traganos, F.; Darzynkiewicz, Z.; Fojo, T.; Bates, S. E. Histone deacetylase inhibitors all induce p21 but differentially cause tubulin acetylation, mitotic arrest, and cytotoxicity. *Mol. Cancer Ther.* **2002**, *1* (11), 937–41.

(43) Kretzner, L.; Scuto, A.; Dino, P. M.; Kowolik, C. M.; Wu, J.; Ventura, P.; Jove, R.; Forman, S. J.; Yen, Y.; Kirschbaum, M. H. Combining Histone Deacetylase Inhibitor Vorinostat with Aurora Kinase Inhibitors Enhances Lymphoma Cell Killing with Repression of c-Myc, hTERT, and microRNA Levels. *Cancer Res.* **2011**, *71* (11), 3912–20.

(44) Kawamata, N.; Chen, J.; Koeffler, H. P. Suberoylanilide hydroxamic acid (SAHA; vorinostat) suppresses translation of cyclin D1 in mantle cell lymphoma cells. *Blood* **2007**, *110* (7), 2667–73.

(45) Trepel, J.; Mollapour, M.; Giaccone, G.; Neckers, L. Targeting the dynamic HSP90 complex in cancer. *Nat. Rev. Cancer* **2010**, *10* (8), 537–49.

(46) Aldana-Masangkay, G. I.; Sakamoto, K. M. The role of HDAC6 in cancer. *J. Biomed. Biotechnol.* **2011**, *2011*, 875824.

(47) Bali, P.; Pranpat, M.; Swaby, R.; Fiskus, W.; Yamaguchi, H.; Balasis, M.; Rocha, K.; Wang, H.; Richon, V.; Bhalla, K. Activity of suberoylanilide hydroxamic Acid against human breast cancer cells with amplification of her-2. *Clin. Cancer Res.* **2005**, *11* (17), 6382–9.

(48) Labonte, M. J.; Wilson, P. M.; Fazzone, W.; Russell, J.; Louie, S. G.; El-Khoueiry, A.; Lenz, H. J.; Ladner, R. D. The Dual EGFR/HER2 Inhibitor Lapatinib Synergistically Enhances the Antitumor Activity of the Histone Deacetylase Inhibitor Panobinostat in Colorectal Cancer Models. *Cancer Res.* **2011**, *71* (10), 3635–48.

(49) Edwards, A.; Li, J.; Atadja, P.; Bhalla, K.; Haura, E. Effect of the histone deacetylase inhibitor LBH589 against epidermal growth factor receptor-dependent human lung cancer cells. *Mol. Cancer Ther.* **2007**, *6* (9), 2515–24.

(50) Nimmanapalli, R.; Fuino, L.; Bali, P.; Gasparetto, M.; Glozak, M.; Tao, J.; Moscinski, L.; Smith, C.; Wu, J.; Jove, R.; Atadja, P.; Bhalla, K. 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.* **2003**, *63* (16), 5126–35.

(51) Nimmanapalli, R.; Fuino, L.; Stobaugh, C.; Richon, V.; Bhalla, K. Cotreatment with the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) enhances imatinib-induced apoptosis of Bcr-Abl-positive human acute leukemia cells. *Blood* **2003**, *101* (8), 3236–9.

(52) Okabe, S.; Tauchi, T.; Nakajima, A.; Sashida, G.; Gotoh, A.; Broxmeyer, H.; Ohyashiki, J.; Ohyashiki, K. Depsipeptide (FK228) preferentially induces apoptosis in BCR/ABL-expressing cell lines and cells from patients with chronic myelogenous leukemia in blast crisis. *Stem Cells Dev.* **2007**, *16* (3), 503–14.

(53) Fiskus, W.; Pranpat, M.; Bali, P.; Balasis, M.; Kumaraswamy, S.; Boyapalle, S.; Rocha, K.; Wu, J.; Giles, F.; Manley, P. W.; Atadja, P.; Bhalla, K. Combined effects of novel tyrosine kinase inhibitor AMN107 and histone deacetylase inhibitor LBH589 against Bcr-Abl-expressing human leukemia cells. *Blood* **2006**, *108* (2), 645–52.

(54) Parmigiani, R. B.; Xu, W. S.; Venta-Perez, G.; Erdjument-Bromage, H.; Yaneva, M.; Tempst, P.; Marks, P. A. HDAC6 is a specific deacetylase of peroxiredoxins and is involved in redox regulation. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105* (28), 9633–8.

(55) Nishioka, C.; Ikezoe, T.; Yang, J.; Takeuchi, S.; Koeffler, H.; Yokoyama, A. MS-275, a novel histone deacetylase inhibitor with selectivity against HDAC1, induces degradation of FLT3 via inhibition of chaperone function of heat shock protein 90 in AML cells. *Leuk. Res.* **2008**, *32* (9), 1382–92.

(56) Zhou, Q.; Agoston, A. T.; Atadja, P.; Nelson, W. G.; Davidson, N. E. Inhibition of histone deacetylases promotes ubiquitin-dependent proteasomal degradation of DNA methyltransferase 1 in human breast cancer cells. *Mol. Cancer Res.* **2008**, *6* (5), 873–83.

(57) Wang, Y.; Wang, S.; Zhang, X.; Zhao, M.; Hou, C.; Xu, Y.; Du, Z.; Yu, X. FK228 inhibits Hsp90 chaperone function in K562 cells via hyperacetylation of Hsp70. *Biochem. Biophys. Res. Commun.* **2007**, *356* (4), 998–1003.

(58) Rosato, R. R.; Almenara, J. A.; Grant, S. 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.* **2003**, *63* (13), 3637–45.

(59) Yu, C.; Subler, M.; Rahmani, M.; Reese, E.; Krystal, G.; Conrad, D.; Dent, P.; Grant, S. Induction of apoptosis in BCR/ABL+ cells by histone deacetylase inhibitors involves reciprocal effects on the RAF/MEK/ERK and JNK pathways. *Cancer Biol. Ther.* **2003**, *2* (5), 544–51.

(60) Rosato, R. R.; Maggio, S. C.; Almenara, J. A.; Payne, S. G.; Atadja, P.; Spiegel, S.; Dent, P.; Grant, S. The histone deacetylase inhibitor LAQ824 induces human leukemia cell death through a process involving XIAP down-regulation, oxidative injury, and the acid sphingomyelinase-dependent generation of ceramide. *Mol. Pharmacol.* **2006**, *69* (1), 216–25.

(61) Lee, J. H.; Choy, M. L.; Ngo, L.; Foster, S. S.; Marks, P. A. Histone deacetylase inhibitor induces DNA damage, which normal but not transformed cells can repair. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107* (33), 14639–44.

(62) Robert, T.; Vanoli, F.; Chiolo, I.; Shubassi, G.; Bernstein, K. A.; Rothstein, R.; Botrugno, O. A.; Parazzoli, D.; Oldani, A.; Minucci, S.; Foiani, M. HDACs link the DNA damage response, processing of double-strand breaks and autophagy. *Nature* **2011**, *471* (7336), 74–9.

(63) Miller, K. M.; Tjeertes, J. V.; Coates, J.; Legube, G.; Polo, S. E.; Britton, S.; Jackson, S. P. Human HDAC1 and HDAC2 function in the DNA-damage response to promote DNA nonhomologous end-joining. *Nat. Struct. Mol. Biol.* **2010**, *17* (9), 1144–51.

(64) Marks, P. A. Thioredoxin in cancer--role of histone deacetylase inhibitors. *Semin. Cancer Biol.* **2006**, *16* (6), 436–43.

(65) Butler, L. M.; Zhou, X.; Xu, W. S.; Scher, H. I.; Rifkind, R. A.; Marks, P. A.; Richon, V. M. The histone deacetylase inhibitor SAHA arrests cancer cell growth, up-regulates thioredoxin-binding protein-2, and down-regulates thioredoxin. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99* (18), 11700–5.

(66) Guo, F.; Sigua, C.; Tao, J.; Bali, P.; George, P.; Li, Y.; Wittmann, S.; Moscinski, L.; Atadja, P.; Bhalla, K. 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.* **2004**, *64* (7), 2580–9.

(67) Kauh, J.; Fan, S.; Xia, M.; Yue, P.; Yang, L.; Khuri, F. R.; Sun, S. Y. c-FLIP degradation mediates sensitization of pancreatic cancer cells to TRAIL-induced apoptosis by the histone deacetylase inhibitor LBH589. *PLoS One* **2010**, *5* (4), e10376.

(68) Yeh, C. C.; Deng, Y. T.; Sha, D. Y.; Hsiao, M.; Kuo, M. Y. Suberoylanilide hydroxamic acid sensitizes human oral cancer cells to TRAIL-induced apoptosis through increase DR5 expression. *Mol. Cancer Ther.* **2009**, *8* (9), 2718–25.

(69) Jiang, X.; Tsang, Y. H.; Yu, Q. c-Myc overexpression sensitizes Bim-mediated Bax activation for apoptosis induced by histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) through regulating Bcl-2/Bcl-xL expression. *Int. J. Biochem. Cell Biol.* **2007**, *39* (5), 1016–25.

(70) Robey, R. W.; Zhan, Z.; Piekarz, R. L.; Kayastha, G. L.; Fojo, T.; Bates, S. E. Increased MDR1 expression in normal and malignant peripheral blood mononuclear cells obtained from patients receiving depsipeptide (FR901228, FK228, NSC630176). *Clin. Cancer Res.* **2006**, *12* (5), 1547–55.

(71) Piekarz, R. L.; Robey, R. W.; Zhan, Z.; Kayastha, G.; Sayah, A.; Abdeldaim, A. H.; Torrico, S.; Bates, S. E. T-cell lymphoma as a model for the use of histone deacetylase inhibitors in cancer therapy: impact of depsipeptide on molecular markers, therapeutic targets, and mechanisms of resistance. *Blood* **2004**, *103* (12), 4636–43.

(72) Xiao, J. J.; Huang, Y.; Dai, Z.; Sadee, W.; Chen, J.; Liu, S.; Marcucci, G.; Byrd, J.; Covey, J. M.; Wright, J.; Grever, M.; Chan, K. K. Chemoresistance to Depsipeptide FK228 [(E)-(1S,4S,10S,21R)-7-[(Z)-Ethylidene]-4,21-diisopropyl-2-oxa-12,13-dithi a-5,8,20,23-tetraazabicyclo[8,7,6]-tricos-16-ene-3,6,9,22-pentanone] Is Mediated by Reversible MDR1 Induction in Human Cancer Cell Lines. *J. Pharmacol. Exp. Ther.* **2005**, *314* (1), 467–75.

(73) Prince, H.; Bishton, M.; Harrison, S. Clinical studies of histone deacetylase inhibitors. *Clin. Cancer Res.* **2009**, *15* (12), 3958–69.

(74) Mickley, L. A.; Bates, S. E.; Richert, N. D.; Currier, S.; Tanaka, S.; Foss, F.; Rosen, N.; Fojo, A. T. Modulation of the expression of a multidrug resistance gene (mdr-1/P-glycoprotein) by differentiating agents. *J. Biol. Chem.* **1989**, *264* (30), 18031–40.

(75) Frommel, T. O.; Coon, J. S.; Tsuruo, T.; Roninson, I. B. Variable effects of sodium butyrate on the expression and function of the mdr-1 (P-glycoprotein) gene in colon carcinoma cell lines. *Int. J. Cancer* **1993**, *55*, 297–302.

(76) Jin, S.; Scotto, K. W. Transcriptional regulation of the MDR1 gene by histone acetyltransferase and deacetylase is mediated by NF-Y. *Mol. Cell. Biol.* **1998**, *18* (7), 4377–84.

(77) Lee, J. S.; Paull, K.; Alvarez, M.; Hose, C.; Monks, A.; Grever, M.; Fojo, A. T.; Bates, S. E. Rhodamine efflux patterns predict P-glycoprotein substrates in the National Cancer Institute Drug Screen. *Mol. Pharmacol.* **1994**, *46*, 627–638.

(78) Cerveny, L.; Svecova, L.; Anzenbacherova, E.; Vrzal, R.; Staud, F.; Dvorak, Z.; Ulrichova, J.; Anzenbacher, P.; Pavek, P. Valproic acid induces CYP3A4 and MDR1 gene expression by activation of constitutive androstane receptor and pregnane X receptor pathways. *Drug Metab. Dispos.* **2007**, *35* (7), 1032–41.

(79) Kim, Y. K.; Kim, N. H.; Hwang, J. W.; Song, Y. J.; Park, Y. S.; Seo, D. W.; Lee, H. Y.; Choi, W. S.; Han, J. W.; Kim, S. N. Histone deacetylase inhibitor apicidin-mediated drug resistance: involvement of P-glycoprotein. *Biochem. Biophys. Res. Commun.* **2008**, *368* (4), 959–64.

(80) Peart, M. J.; Tainton, K. M.; Ruefli, A. A.; Dear, A. E.; Sedelies, K. A.; O'Reilly, L. A.; Waterhouse, N. J.; Trapani, J. A.; Johnstone, R. W.

Novel mechanisms of apoptosis induced by histone deacetylase inhibitors. *Cancer Res.* **2003**, *63* (15), 4460–71.

(81) Qian, X.; LaRochelle, W. J.; Ara, G.; Wu, F.; Petersen, K. D.; Thougaard, A.; Sehested, M.; Lichenstein, H. S.; Jeffers, M. Activity of PXD101, a histone deacetylase inhibitor, in preclinical ovarian cancer studies. *Mol. Cancer Ther.* **2006**, *5* (8), 2086–95.

(82) Xiao, J. J.; Foraker, A. B.; Swaan, P. W.; Liu, S.; Huang, Y.; Dai, Z.; Chen, J.; Sadee, W.; Byrd, J.; Marcucci, G.; Chan, K. K. Efflux of depsipeptide FK228 (FR901228, NSC-630176) is mediated by P-glycoprotein and multidrug resistance-associated protein 1. *J. Pharmacol. Exp. Ther.* **2005**, *313* (1), 268–76.

(83) Yamada, H.; Arakawa, Y.; Saito, S.; Agawa, M.; Kano, Y.; Horiguchi-Yamada, J. Depsipeptide-resistant KU812 cells show reversible P-glycoprotein expression, hyper-acetylated histones, and modulated gene expression profile. *Leuk. Res.* **2006**, *30* (6), 723–34.

(84) Sandor, V.; Senderowicz, A.; Mertins, S.; Sackett, D.; Sausville, E.; Blagosklonny, M. V.; Bates, S. E. P21-dependent g(1)arrest with downregulation of cyclin D1 and upregulation of cyclin E by the histone deacetylase inhibitor FR901228. *Br. J. Cancer* **2000**, *83* (6), 817–25.

(85) Vrana, J.; Decker, R.; Johnson, C.; Wang, Z.; Jarvis, W.; Richon, V.; Ehinger, M.; Fisher, P.; Grant, S. 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* **1999**, *18* (50), 7016–25.

(86) Nguyen, D. M.; Schrump, W. D.; Tsai, W. S.; Chen, A.; Stewart, J. H. t.; Steiner, F.; Schrump, D. S. Enhancement of depsipeptide-mediated apoptosis of lung or esophageal cancer cells by flavopiridol: activation of the mitochondria-dependent death-signaling pathway. *J. Thorac. Cardiovasc. Surg.* **2003**, *125* (5), 1132–42.

(87) Rosato, R. R.; Almenara, J. A.; Yu, C.; Grant, S. 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.* **2004**, *65* (3), 571–81.

(88) Almenara, J.; Rosato, R.; Grant, S. Synergistic induction of mitochondrial damage and apoptosis in human leukemia cells by flavopiridol and the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA). *Leukemia* **2002**, *16* (7), 1331–43.

(89) Yazbeck, V. Y.; Buglio, D.; Georgakis, G. V.; Li, Y.; Iwado, E.; Romaguera, J. E.; Kondo, S.; Younes, A. Temsirolimus downregulates p21 without altering cyclin D1 expression and induces autophagy and synergizes with vorinostat in mantle cell lymphoma. *Exp. Hematol.* **2008**, *36* (4), 443–50.

(90) Ungerstedt, J.; Sowa, Y.; Xu, W.; Shao, Y.; Dokmanovic, M.; Perez, G.; Ngo, L.; Holmgren, A.; Jiang, X.; Marks, P. Role of thioredoxin in the response of normal and transformed cells to histone deacetylase inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102* (3), 673–8.

(91) Chen, G.; Li, A.; Zhao, M.; Gao, Y.; Zhou, T.; Xu, Y.; Du, Z.; Zhang, X.; Yu, X. Proteomic analysis identifies protein targets responsible for depsipeptide sensitivity in tumor cells. *J. Proteome Res.* **2008**, *7* (7), 2733–42.

(92) Ellis, L.; Bots, M.; Lindemann, R. K.; Bolden, J. E.; Newbold, A.; Cluse, L. A.; Scott, C. L.; Strasser, A.; Atadja, P.; Lowe, S. W.; Johnstone, R. W. The histone deacetylase inhibitors LAQ824 and LBH589 do not require death receptor signaling or a functional apoptosome to mediate tumor cell death or therapeutic efficacy. *Blood* **2009**, *114* (2), 380–93.

(93) Newbold, A.; Lindemann, R. K.; Cluse, L. A.; Whitecross, K. F.; Dear, A. E.; Johnstone, R. W. Characterisation of the novel apoptotic and therapeutic activities of the histone deacetylase inhibitor romidepsin. *Mol. Cancer Ther.* **2008**, *7* (5), 1066–79.

(94) Shao, W.; Growney, J. D.; Feng, Y.; O'Connor, G.; Pu, M.; Zhu, W.; Yao, Y. M.; Kwon, P.; Fawell, S.; Atadja, P. Activity of deacetylase inhibitor panobinostat (LBH589) in cutaneous T-cell lymphoma models: Defining molecular mechanisms of resistance. *Int. J. Cancer* **2010**, *127* (9), 2199–208.

(95) Wiegmans, A. P.; Alsop, A. E.; Bots, M.; Cluse, L. A.; Williams, S. P.; Banks, K. M.; Ralli, R.; Scott, C. L.; Frenzel, A.; Villunger, A.; Johnstone, R. W. Deciphering the Molecular Events Necessary for Synergistic Tumor Cell Apoptosis Mediated by the Histone Deacetylase Inhibitor Vorinostat and the BH3Mimetic ABT-737. *Cancer Res.* **2011**, *71* (10), 3603–15.

(96) Xu, W.; Ngo, L.; Perez, G.; Dokmanovic, M.; Marks, P. A. Intrinsic apoptotic and thioredoxin pathways in human prostate cancer cell response to histone deacetylase inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103* (42), 15540–5.

(97) Whitecross, K. F.; Alsop, A. E.; Cluse, L. A.; Wiegmans, A.; Banks, K. M.; Coomans, C.; Peart, M. J.; Newbold, A.; Lindemann, R. K.; Johnstone, R. W. Defining the target specificity of ABT-737 and synergistic antitumor activities in combination with histone deacetylase inhibitors. *Blood* **2009**, *113* (9), 1982–91.

(98) Wei, Y.; Kadia, T.; Tong, W.; Zhang, M.; Jia, Y.; Yang, H.; Hu, Y.; Tambaro, F. P.; Viallet, J.; O'Brien, S.; Garcia-Manero, G. The combination of a histone deacetylase inhibitor with the Bcl-2 homology domain-3 mimetic GX15−070 has synergistic antileukemia activity by activating both apoptosis and autophagy. *Clin. Cancer Res.* **2010**, *16* (15), 3923–32.

(99) Inoue, S.; Walewska, R.; Dyer, M. J.; Cohen, G. M. Down-regulation of Mcl-1 potentiates HDACi-mediated apoptosis in leukemic cells. *Leukemia* **2008**, *22* (4), 819–25.

(100) Yang, Y.; Zhao, Y.; Liao, W.; Yang, J.; Wu, L.; Zheng, Z.; Yu, Y.; Zhou, W.; Li, L.; Feng, J.; Wang, H.; Zhu, W. G. Acetylation of FoxO1 activates Bim expression to induce apoptosis in response to histone deacetylase inhibitor depsipeptide treatment. *Neoplasia* **2009**, *11* (4), 313–24.

(101) Gong, Y.; Somwar, R.; Politi, K.; Balak, M.; Chmielecki, J.; Jiang, X.; Pao, W. Induction of BIM is essential for apoptosis triggered by EGFR kinase inhibitors in mutant EGFR-dependent lung adenocarcinomas. *PLoS Med.* **2007**, *4* (10), e294.

(102) Kuroda, J.; Puthalakath, H.; Cragg, M. S.; Kelly, P. N.; Bouillet, P.; Huang, D. C.; Kimura, S.; Ottmann, O. G.; Druker, B. J.; Villunger, A.; Roberts, A. W.; Strasser, A. Bim and Bad mediate imatinib-induced killing of Bcr/Abl+ leukemic cells, and resistance due to their loss is overcome by a BH3 mimetic. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103* (40), 14907–12.

(103) Fiskus, W.; Rao, R.; Fernandez, P.; Herger, B.; Yang, Y.; Chen, J.; Kolhe, R.; Mandawat, A.; Wang, Y.; Joshi, R.; Eaton, K.; Lee, P.; Atadja, P.; Peiper, S.; Bhalla, K. Molecular and biologic characterization and drug sensitivity of pan-histone deacetylase inhibitor-resistant acute myeloid leukemia cells. *Blood* **2008**, *112* (7), 2896–905.

(104) Bandyopadhyay, D.; Mishra, A.; Medrano, E. E. Overexpression of histone deacetylase 1 confers resistance to sodium butyrate-mediated apoptosis in melanoma cells through a p53-mediated pathway. *Cancer Res.* **2004**, *64* (21), 7706–10.

(105) Munster, P.; Marchion, D.; Thomas, S.; Egorin, M.; Minton, S.; Springett, G.; Lee, J.; Simon, G.; Chiappori, A.; Sullivan, D.; Daud, A. Phase I trial of vorinostat and doxorubicin in solid tumours: histone deacetylase 2 expression as a predictive marker. *Br. J. Cancer* **2009**, *101* (7), 1044–50.

(106) Munster, P. N.; Thurn, K. T.; Thomas, S.; Raha, P.; Lacevic, M.; Miller, A.; Melisko, M.; Ismail-Khan, R.; Rugo, H.; Moasser, M.; Minton, S. E. A phase II study of the histone deacetylase inhibitor vorinostat combined with tamoxifen for the treatment of patients with hormone therapy-resistant breast cancer. *Br. J. Cancer* **2011**, *104* (12), 1828–35.

(107) Matsubara, H.; Watanabe, M.; Imai, T.; Yui, Y.; Mizushima, Y.; Hiraumi, Y.; Kamitsuji, Y.; Watanabe, K.; Nishijo, K.; Toguchida, J.; Nakahata, T.; Adachi, S. Involvement of extracellular signal-regulated kinase activation in human osteosarcoma cell resistance to the histone deacetylase inhibitor FK228 [(1S,4S,7Z,10S,16E,21R)-7-ethylidene-4,21-bis(propan-2-yl)-2-oxa-12,13-dithia-5,8,20,23-tetraazabicyclo[8.7.6]-tricos-16-ene-3,6,9,19,22-pentone]. *J. Pharmacol. Exp. Ther.* **2009**, *328* (3), 839–48.

(108) Ozaki, K.; Minoda, A.; Kishikawa, F.; Kohno, M. Blockade of the ERK pathway markedly sensitizes tumor cells to HDAC inhibitor-induced cell death. *Biochem. Biophys. Res. Commun.* **2006**, *339* (4), 1171–7.

(109) Yu, X.; Wang, S.; Chen, G.; Hou, C.; Zhao, M.; Hong, J.; Nguyen, D.; Schrump, D. Apoptosis induced by depsipeptide FK228 coincides with inhibition of survival signaling in lung cancer cells. *Cancer J.* **2007**, *13* (2), 105–13.

(110) Kodani, M.; Igishi, T.; Matsumoto, S.; Chikumi, H.; Shigeoka, Y.; Nakanishi, H.; Morita, M.; Yasuda, K.; Hitsuda, Y.; Shimizu, E. Suppression of phosphatidylinositol 3-kinase/Akt signaling pathway is a determinant of the sensitivity to a novel histone deacetylase inhibitor, FK228, in lung adenocarcinoma cells. *Oncol. Rep.* **2005**, *13* (3), 477–83.

(111) Yu, C.; Friday, B. B.; Lai, J. P.; McCollum, A.; Atadja, P.; Roberts, L. R.; Adjei, A. A. Abrogation of MAPK and Akt signaling by AEE788 synergistically potentiates histone deacetylase inhibitor-induced apoptosis through reactive oxygen species generation. *Clin. Cancer Res.* **2007**, *13* (4), 1140–8.

(112) Jane, E. P.; Premkumar, D. R.; Addo-Yobo, S. O.; Pollack, I. F. Abrogation of mitogen-activated protein kinase and Akt signaling by vandetanib synergistically potentiates histone deacetylase inhibitor-induced apoptosis in human glioma cells. *J. Pharmacol. Exp. Ther.* **2009**, *331* (1), 327–37.

(113) Wozniak, M. B.; Villuendas, R.; Bischoff, J. R.; Aparicio, C. B.; Martínez Leal, J. F.; de La Cueva, P.; Rodriguez, M. E.; Herreros, B.; Martin-Perez, D.; Longo, M. I.; Herrera, M.; Piris, M. A.; Ortiz-Romero, P. L. Vorinostat interferes with the signaling transduction pathway of T-cell receptor and synergizes with phosphoinositide-3 kinase inhibitors in cutaneous T-cell lymphoma. *Haematologica* **2010**, *95* (4), 613–21.

(114) Fantin, V.; Loboda, A.; Paweletz, C.; Hendrickson, R.; Pierce, J.; Roth, J.; Li, L.; Gooden, F.; Korenchuk, S.; Hou, X.; Harrington, E.; Randolph, S.; Reilly, J.; Ware, C.; Kadin, M.; Frankel, S.; Richon, V. Constitutive activation of signal transducers and activators of transcription predicts vorinostat resistance in cutaneous T-cell lymphoma. *Cancer Res.* **2008**, *68* (10), 3785–94.

(115) Diaz, T.; Navarro, A.; Ferrer, G.; Gel, B.; Gaya, A.; Artells, R.; Bellosillo, B.; Garcia-Garcia, M.; Serrano, S.; Martínez, A.; Monzo, M. Lestaurtinib inhibition of the Jak/STAT signaling pathway in hodgkin lymphoma inhibits proliferation and induces apoptosis. *PLoS One* **2011**, *6* (4), e18856.

(116) Staudt, L. M. Oncogenic activation of NF-kappaB. *Cold Spring Harbor Perspect. Biol.* **2010**, *2* (6), a000109.

(117) Chen, Lf; Fischle, W.; Verdin, E.; Greene, W. C. Duration of nuclear NF-kappaB action regulated by reversible acetylation. *Science* **2001**, *293* (5535), 1653–7.

(118) Duan, J.; Friedman, J.; Nottingham, L.; Chen, Z.; Ara, G.; Van Waes, C. Nuclear factor-kappaB p65 small interfering RNA or proteasome inhibitor bortezomib sensitizes head and neck squamous cell carcinomas to classic histone deacetylase inhibitors and novel histone deacetylase inhibitor PXD101. *Mol. Cancer Ther.* **2007**, *6* (1), 37–50.

(119) Dai, Y.; Rahmani, M.; Dent, P.; Grant, S. Blockade of histone deacetylase inhibitor-induced RelA/p65 acetylation and NF-kappaB activation potentiates apoptosis in leukemia cells through a process mediated by oxidative damage, XIAP downregulation, and c-Jun N-terminal kinase 1 activation. *Mol. Cell. Biol.* **2005**, *25* (13), 5429–44.

(120) Rundall, B. K.; Denlinger, C. E.; Jones, D. R. Combined histone deacetylase and NF-kappaB inhibition sensitizes non-small cell lung cancer to cell death. *Surgery* **2004**, *136* (2), 416–25.

(121) Domingo-Domènech, J.; Pippa, R.; Tápia, M.; Gascón, P.; Bachs, O.; Bosch, M. Inactivation of NF-kappaB by proteasome inhibition contributes to increased apoptosis induced by histone deacetylase inhibitors in human breast cancer cells. *Breast Cancer Res. Treat.* **2008**, *112* (1), 53–62.

(122) Dai, Y.; Chen, S.; Kramer, L. B.; Funk, V. L.; Dent, P.; Grant, S. Interactions between bortezomib and romidepsin and belinostat in chronic lymphocytic leukemia cells. *Clin. Cancer Res.* **2008**, *14* (2), 549–58.

(123) Kitazono, M.; Robey, R.; Zhan, Z.; Sarlis, N. J.; Skarulis, M. C.; Aikou, T.; Bates, S.; Fojo, T. Low concentrations of the histone deacetylase inhibitor, depsipeptide (FR901228), increase expression of the Na(+)/I(−) symporter and iodine accumulation in poorly differentiated thyroid carcinoma cells. *J. Clin. Endocrinol. Metab.* **2001**, *86* (7), 3430–5.

(124) Bruzzese, F.; Rocco, M.; Castelli, S.; Di Gennaro, E.; Desideri, A.; Budillon, A. Synergistic antitumor effect between vorinostat and topotecan in small cell lung cancer cells is mediated by generation of reactive oxygen species and DNA damage-induced apoptosis. *Mol. Cancer Ther.* **2009**, *8* (11), 3075–87.

(125) Nguyen, T.; Dai, Y.; Attkisson, E.; Kramer, L.; Jordan, N.; Nguyen, N.; Kolluri, N.; Muschen, M.; Grant, S. HDAC inhibitors potentiate the activity of the BCR/ABL kinase inhibitor KW-2449 in imatinib-sensitive or -resistant BCR/ABL+ leukemia cells in vitro and in vivo. *Clin. Cancer Res.* **2011**, *17* (10), 3219–32.

(126) Luchenko, V. L.; Salcido, C. D.; Zhang, Y.; Agama, K.; Komlodi-Pasztor, E.; Murphy, R. F.; Giaccone, G.; Pommier, Y.; Bates, S. E.; Vartikovski, L. Schedule-dependent synergy of histone deacetylase inhibitors with DNA damaging agents in small cell lung cancer. *Cell Cycle* **2011**, *10*, 18.