Interpreting clinical assays for histone deacetylase inhibitors

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Abstract: As opposed to genetics, dealing with gene expressions by direct DNA sequence modifications, the term epigenetics applies to all the external influences that target the chromatin structure of cells with impact on gene expression unrelated to the sequence coding of DNA itself. In normal cells, epigenetics modulates gene expression through all development steps. When “imprinted” early by the environment, epigenetic changes influence the organism at an early stage and can be transmitted to the progeny. Together with DNA sequence alterations, DNA aberrant cytosine methylation and microRNA deregulation, epigenetic modifications participate in the malignant transformation of cells. Their reversible nature has led to the emergence of the promising field of epigenetic therapy. The efforts made to inhibit in particular the epigenetic enzyme family called histone deacetylases (HDACs) are described. HDAC inhibitors (HDACi) have been proposed as a viable clinical therapeutic approach for the treatment of leukemia and solid tumors, but also to a lesser degree for noncancerous diseases. Three epigenetic drugs are already arriving at the patient’s bedside, and more than 100 clinical assays for HDACi are registered on the National Cancer Institute website. They explore the eventual additive benefits of combined therapies. In the context of the pleiotropic effects of HDAC isoforms, more specific HDACi and more informative screening tests are being developed for the benefit of the patients.

Keywords: histone deacetylase inhibitors, epigenetic, clinical trials interpretation

Introduction

The transcriptional state of a eukaryotic gene is determined by the surrounding chromatin architecture, the state of DNA cytosine methylation in the promoter/first exons and the associated regulating microRNAs (miRNAs).

In the cell nucleus, the genome is packaged into a superstructure, the chromatin, whose elementary dynamic units are the nucleosomes. Each is made up of four associated dimers of core histones (H2A, H2B, H3, and H4) around which 147 base pairs of DNA are wrapped, the nucleosomes being finally linked together via the linker histone H1 (Figure 1). Chromatin is compacted around the DNA into a so-called “closed state” when cells are resting. It is opened into an “active state” to allow for gene transcription by adenosine triphosphate (ATP)-dependent protein complexes, which remodel the chromatin architecture. Theses complexes modify the accessibility of DNA regulatory sites through both repositioning (sliding) and ejecting nucleosomes. Modeling complexes include transcription co-activators, transcription factors, and epi-enzymes.¹ Histone acetyltransferases (HATs), for instance, acylate specific lysine residues of histones and convert them into an amide form, loosening histone contacts with DNA, resulting in exposed binding sites for the transcription
machinery. On the other hand, other complexes function as gene silencers and deny the same machinery access to DNA. Repressive complexes include histone deacetylases (HDACs), which deacetylate specific lysine residues of the histones tails to induce tighter interactions between the now positively charged lysine (Nε protonated form) and the negatively-charged DNA phosphate groups. Beside acetylation, several other post-translational lysine modifications in histones have been described: methylation, phosphorylation, SUMOylation, and ATP-ribosylation.

All the modifications incurred by histones form the “histone code”. For example, K9 in H3 turns the chromatin into inactivity when methylated. Phosphorylation of serine 10 in the same H3 is required for methylation of K4 and acetylation of K9 and K14. Similar enzyme crosstalk has also been described, for ubiquitination of K120 in H2B prior to methylation of K79 in H3. Several epi-enzyme families are involved in the histones modifications. HATs and HDACs have balancing actions for histones acetylation. Methylation of histones is controlled by histones methyltransferases (HMTs) and histones demethylases (HDMs), while histone arginine methylation is catalyzed by the protein arginine N-methyltransferases family of enzymes. One, two, or three methylations are possible, with impact on gene expression/repression. The analysis of epigenetic marks at the genome-wide scale has shown that monomethylated H3K4 is associated with transcription factors binding to enhancers, trimethylated H3K4 with transcription start sites, and dimethylated H3K4 with both transcription start sites and enhancers.

DNA methyltransferases (DNMTs) repress gene expression via DNA cytosine methylation, unfavorable to transcription factor binding. DNMTs are recruited and stabilized, on DNA, by HMTs and HDMs. Both are also able to recruit HDACs, methyl-binding proteins like methyl CpG binding protein 2 (MECP2), and several co-factors to further tune gene expression. Since DNMT co-factors are lacking in normal tissues, gene re-expression induced by DNMT inhibitors could be limited to tumor tissues to reduce “off-target” effects. DNA methylation in the epigenomes of human embryonic stem cells is an important field of research. The roles of DNA methylation in cancer genesis have also been extensively studied.

Catalyzed by the ten-eleven translocation 1 (TET) family of enzymes, DNA hydroxymethylcytosine has been recently described as a step towards cytosine demethylation. Mutations and translocations of TET are present in myeloid malignancies. The role of hydroxymethylcytosine, if any, is not yet understood, but its existence questions all the results obtained so far when determining the cytosine methylation status.

In a further step of complexity, specialized miRNAs read the epi-code and target effectors genes to modulate their expression. MiRNAs are small non-coding RNAs of 20–22 nucleotides that inhibit gene expression when they engage either in imperfect base-pairing with their target mRNA 3′-untranslated region or affect its stability. MiRNA 29-a, -b and -c target DNMT3a and b directly and cell-specifically. HDAC4 is targeted by both miR-1 and miR-140, while miR-449-a targets HDAC1 in prostate malignant cells. Onco-proteins like promyelocyte leukemia retinoic acid receptor-α (PML-RARα) in promyelocytic leukemia and B-cell lymphoma 6 in non-Hodgkin’s lymphoma result from translocations. It is near the premiR-223 region that the t(8;21) translocation juxtaposes the Runt-related transcription
factor 1 gene on chromosome 21 with the Cytochrome B Termination 1 gene on chromosome 8, generating the acute myeloid leukemia (AML)-Eight Twenty One fusion gene.17 The recruitment, on this chimerical site, of DNMT, MeCP2, and HDAC1 repressor complexes, promotes leukemogenesis. Epi-miRNAs write their own epi-code when their cytosines are methylated. Downregulation of miR-124a induces an up regulation of its target, cyclin-dependent kinase 6 (CDK6), as well as phosphorylation of retinoblastoma, and contributes to the abnormal proliferation of acute lymphoblastic leukemia (ALL) cells both in vitro and in vivo.

Most epigenetic changes translate into either up regulation or silencing of gene expression.18 When inappropriate, they predispose the organism to more mutational events via increased genomic instability and aberrant cellular signaling. The field of epigenetic being extremely prolific, we have restricted our reference list to the essentials.

HDACs
HDACs remove the acetyl group from an N-ε-acetyl lysine located near the amino terminus of a core histone, cleaving an amide bond and increasing the positive charge of the histone. The removal of acetyl groups from the histones tails stabilizes nucleosomal DNA-histones interactions by its subsequent change in electrostatic charges. It is the basis for HDAC-mediated transcriptional repression via chromatin condensation.19 HDACs have been categorized into four classes. Class I HDACs (HDAC1, 2, 3, and 8) are nuclear proteins with ubiquitous expression involved in regulating cell proliferation.20 HDAC2 has been shown to suppress apoptosis in tumor cells not only by the intrinsic/ mitochondrial and the extrinsic/death-receptor pathways, but also via mitotic failure and autophagic cell death, while HDAC3 is involved in bone structure and S-phase check point.21,22 Class II HDACs have a tissue-specific expression and can shuttle between the nucleus and the cytoplasm. They are divided into two subclasses: IIa with HDAC4, 5, 7, and 9. HDAC4 represses condrocyte hypertrophy. HDAC7 functions in the down regulation and apoptosis of T-cells.20 HDAC9 is involved in cardiomyocyte differentiation.21 Class IIb includes HDAC10 and HDAC6. The latter contains two tandem catalytic domains: one is for histones deacetylation and the other for deacetylation of α-tubulin. HDAC6 has also the capacity to bind directly to ubiquitinated proteins through an ubiquitin-binding domain, to target cargo proteins for subsequent processing. HDAC6-specific effects on cell motility and the proteasome are thought to be responsible for much of the toxicity of HDAC6 inhibitors (HDACi). HDAC 10 and 9 are required for chromosome homologous recombination.24 Class III HDACs include 7 different members of the sirtuin (SIRT) family. They are dependent on nicotinamide adenine dinucleotide (NAD+) to remove the acetyl group from lysine residues in histones and nonhistone substrates. Resveratrol from grapes and red wine is a SIRT1 activator.25 HDAC11 is the only member of Class IV.

Thus, it appears that HDAC activity depends on isoform types, sub cellular localization, association into multi-protein complexes and even post-translational modifications.

HDACs are also able to deacetylate nonhistone proteins such as transcription factors, chaperone proteins and effectors of DNA repair, cell-signaling and metabolism. The ongoing concept is that deacetylation stabilizes these proteins. HDACs have different developmental functions, as shown by the different phenotypes obtained in knockout mice.26 Disruption of HDAC1 causes early embryonic lethality. HDAC2 knockout mice are viable but present fatal multiple cardiac defects. Germline HDAC3-deficiency causes embryonic lethality. HDAC3 conditional knockout mice gave severe deficits in membranous and endochondral bone formation. Germline deletion of HDAC4 causes premature ossification of the developing bones. HDAC6-deficiency slightly enhances trabecular bone formation. HDAC7 knockout gave vascular defects. HDAC8 is essential for neural crest progenitor cell differentiation and skull bone formation. HDAC9 knockout mice are viable at birth but have a myocardial hypertrophy.

HDACs and their inhibitors
In tumor cells, deletion of a single HDAC is not sufficient to induce cell death but leads to nuclear bridging and fragmentation, and ‘in fine’ to cell mitotic catastrophe. This suggests that inhibition of HDAC may be sufficient for anticancer activity and provides a rational incentive for the development of HDACi.27 In the 1970s, seminal experiments showed that treatment of cells with the short-chain fatty acid NaB (sodium butyrate) caused hyperacetylation of histone octamers and led to the discovery of HDACs.28 The zinc-dependent HDACs of classes I, II, and IV are now known to have a common active site made of a tubular hydrophobic channel with a zinc atom (Zn2+) at its end, forming the enzyme catalytic pocket.29 The acetyl part of the lysine substrate in histones/ proteins bind to the zinc atom while the protein four carbon lysine chains fits into the catalytic pocket, and deacetylation then follows. An HDACi is designed to block the HDAC catalytic activity. Several possibilities exist: irreversible or reversible binding to the enzyme catalytic site, competition...
with the enzymatic substrate, and deformation of the enzyme. Accordingly and as shown in Figure 2, the pharmacophore model for HDACi includes a zinc-binding group, competing somehow with the natural acetyl lysine substrate, a hydrophobic cap interacting with the external surface of the active site (generally aromatic) generating specificity, and a short linker connecting these two elements, which fits in the catalytic pocket. The zinc-binding groups can be a carboxylic acid in valproic acid (VPA), a hydroxamic acid in Vorinostat, benzamide in Entinostat, sulfhydryl in Romidepsin, and a ketone in Trapoxin. The linkers can be simple carbon chains, like in Vorinostat, or aromatic groups, like in Entinostat. HDACi in clinical trials are reported in Figure 3. Trichostatin (TSA) from Streptomyces hygroscopius was isolated as an antifungal antibiotic and was incidentally shown to have an anti-proliferative activity on murine leukemia cells. Further studies demonstrated that it was a pan-HDACi. The hydroxamate portion at the end of the molecule acts as a zinc-binding group. Because of toxic side effects, it is not used clinically but participates in the rational conception of HDACi via molecular modeling, as shown in Figure 2.

Besides its HDAC inhibition activity, Vorinostat (Figure 2, left), deriving from TSA, has a complex and not yet fully characterized activity leading to the accumulation of acetylated histones and non histone proteins. First generation HDACi are not selective except for a partial selectivity achieved in rare cases using bulk chemical groups to generate specific interactions with the external surface of the active site of the enzyme, like in tubacin. Sulfur-based zinc-binding groups also showed some selectivity in compounds like Largazole, a potent and selective HDACi for HDAC1 and 2. It is a densely functionalized macrocyclic peptide isolated from the cyanobacterium symploca sp. by Luesch and coworkers. Entinostat and Mocetinostat have selectivity for HDAC1-3 and also against HDAC11 for the latter. Valproate and sodium butyrate (NaB) better target HDAC I and IIa. For sirtuins inhibition is based on NAD+ competitive binding with attempts to propose a pharmacophore, according to the various inhibitor structures described.

SIRT1 activation is the novel therapeutic approach to treat chronic inflammatory diseases, and enzyme activators are therefore sought. Many screening tests to search for HDACi use short histone peptides, capturing baits and engineered cells. All have their limitations because, in vivo, HDAC are parts of mega Daltons modeling chromatin complexes that may change within each cell type.

**HDAC inhibitors metabolism**

The metabolism of HDACi is an important concern during clinical assays. It is studied to determine the correlation between HDACi blood concentration, effective biological effects and eventual drug interactions. The known metabolisms of some HDACi are reported in Figure 4. TSA is metabolized as the inactive trichostatic acid, which is further demethylated for rapid clearance (Figure 4). Phenyl butyrate (PB) metabolism has been described in several contexts. PB is β-oxidized to phenylacetate, and cleared out upon glutamine addition. Vorinostat is also oxidized to 4-anilino-4-oxobutanoic acid and glucuronylated. Romidepsin is a disulfide prodrug. The real active form corresponds to the free thiol metabolite, produced in vivo; the butenthiol part being thought to be the zinc-binding group. A glutathione conjugate has also been described, which is metabolized in vivo by the cytochrome P450s with slow and high acetylating subjects. Other HDACi stabilities have been investigated.

**In-vitro effects of HDAC inhibitors**

DNA chips studied the transcriptome of cells treated with Vorinostat and Romidepsin, revealing that the expression of 40% of all genes was affected over a period of 16 hours. A Belinostat mRNA signature of 25 genes was sufficient to assess the overall gene modulation. Panobinostat modulated cell cycles and angiogenic genes. Tumor antigen expression modulation and major histo-compatibility antigen (MHC) molecule induction have been observed with Dacinostat. Mice bearing human tumor xenografts treated with Belinostat showed a modulation of the expression of genes active in the cellular G2/M phase. This was different from what was seen with 5-fluorouracil (5-FU), Cisplatin, Paclitaxel, or...
Thiotepa. Synergistic effects were obtained when combining HDACi and DNA demethylating agents, or HDACi and all-trans retinoic acid (ATRA), a cell-differentiating agent used to treat acute promyelocytic leukemia (APL). The influence of epigenetic modulators to modify stem cell fate and its relevance for curing diseases has been reviewed. Successful therapeutic use of HDACi may thus depend on the cellular environment, the specific HDAC targeted, and the relative dependence of the tumor on the unique set of pathways influenced by a specific HDAC. Results are summarized in Table 1.

Clinical trials with zinc-dependent HDACi
This part of the review describes the HDACi that have been or are being investigated in clinical trials. In Table 2, all current trials are recapitulated. In Table 3, and for each molecule, some data related to epigenetic measurements are summarized.

PB or its sodium salt
PB or its corresponding sodium salt (NaPB) is a short chain fatty acid approved by the Food and Drug Administration (FDA) for the treatment of hyperammonemia. It stops the
cell cycle in its G1–G0 phase. PB is an efficient HDACi at about 0.5 mM.54,55 PB induces apoptosis – probably via c-jun N-terminal kinase (JNK) – in lung carcinoma cells,56 p21waf1-mediated growth arrest in MCF-7 cells,57 tumor necrosis factor (TNF)-α58 or peroxisome proliferator-activated receptor (PPAR)γ-mediated59 cell differentiation, and is more potent than phenylacetate in prostate cancer cells,60 while increasing MHC class I expression. PB is converted in vivo into the active metabolite phenylacetate (PA) by β-oxidation in the liver and kidney mitochondria.61 Most dose-limiting toxicities (DLTs) are fatigue, nausea, and somnolence. Preliminary studies have been conducted in patients with recurrent glioblastoma multiform (GBM)62 (Table 3, A). Phase I studies have been conducted in patients with hormone refractory prostate cancers,63 refractory solid tumor malignancies64 like colon carcinoma, non small cell lung cancer (NSCLC), anaplastic astrocytoma, GBM, bladder carcinoma, sarcoma, ovarian carcinoma, rectal hemangiopericytoma, and pancreatic carcinoma,65 mainly as intravenous infusions but also in AML and myelodysplastic syndrome (MDS).66 DLTs were neuro-cortical with milder fatigue and nausea/vomiting, light-headedness, short-term memory loss, sedation, confusion, and hypocalcemia. Although central nervous system (CNS) toxicity was observed, infusions were well tolerated (Table 3, B). The active metabolite PA accumulated.

In the AML/MDS study,67 with sequential administration of 5-aza-cytidine (5-aza) (Table 3, C), partial remissions or stable diseases were obtained. Targeting different biological mechanisms is feasible with acceptable toxicity. Phase I trials in combination with several drugs have been reported. Prostate, colorectal, leiomyosarcoma, and esophageal cancers were treated in combination with 5-aza (Table 3, C),68 metastatic colorectal cancer with fluorouracil 5-FU as a 24-hour continuous intravenous infusion (CIV).69 With 5-aza, no re-expression of E-cadherin, endothelin B, and glutathione S transferase (GST) pi was observed, a result explained by the lack of dose effect or by the fact that DNA methylation is an S-phase-dependent process while in-vivo prostatic cells may be in S-phase at any given time. Stable disease was the best response. Combining 5-FU appeared also feasible.

**Pivaloyloxymethyl butyrate**

Pivaloyloxymethyl butyrate (AN)-9, is an ester prodrug of butyric acid (BA)70 but with a greater potency at inducing malignant cell differentiation and tumor growth inhibition. It showed more favorable toxicological, pharmacological,
and pharmacological properties than BA in preclinical studies. BA itself induces p16 expression and growth arrest of colon cancer cells, and modifies caspase distribution during apoptosis. AN-9 down regulates c-jun and c-myc and induces differentiation in leukemia cells. It is decomposed by esterases in vivo to yield butyric and pivaloyl acids and converts into BA itself through esterases in vivo to yield butyric and pivaloyl acids. BA is reprogrammed to induce differentiation in neural progenitor cells, and inhibits HDAC activity in the mM range (preferentially HDAC1, 2). The antiproliferative activity was associated with aberrant cyclin D3 functionality during the C6 glioma G1 phase. Activation of PPARδ was present in F9 cells. VPA induces caspase-dependent and -independent apoptosis in leukemia cells, and in AML cells expressing P-gp and multidrug resistance protein 1 (MRP1), inhibits production of TNF-α and interleukin (IL)-6 and activates nuclear factor kappa B (NF-κB). VPA has been evaluated in combination with other anticancer compounds. For AML, increased 5-aza cytotoxicity was associated to cyclin D1 and p27 (Kip1) expression, while sequential VPA/ATRA treatment reprograms differentiation. VPA induces p16INK4A upregulation and apoptosis and sensitizes melanoma cells to chemotherapy. Interestingly, most of the clinical trials reported are for combination therapies.

A Phase I was conducted for refractory advanced cancer (colorectal, melanoma, NSCLC, and others) (Table 3, G). VPA/ATRA combination was evaluated for several diseases. Poor risk AML (Table 3, H), MDS and relapsed or refractory AML (Table 3, I) have also been investigated. A 52% response rate was observed in MDS patients. ATRA exerted no additional effect in patients receiving the combination, but could be used to induce a second response in relapsing VPA-treated patients. In recurrent or refractory AML or MDS in a Phase II protocol, ATRA was administered when VPA reached the target serum concentration. The differentiation therapy with VPA was effective in 30% of patients. In 11 elderly patients, de novo AML (Table 3, K) was also treated with theophyllin to increase cAMP levels and major cell differentiation. Complete marrow response was observed in three patients, including one complete remission. Two additional patients had hematologic improvement. Patients with AML-M6 were found particularly responsive, probably due to T-cell acute lymphocytic leukemia 1
Table 2 Clinical trials for epigenetic drugs

Safety study of CHR-3996, in patients with advanced solid tumours
Safety and Tolerability of CHR-2845 to treat hematological diseases or lymphoid malignancies
A safety and dose-finding study of JNJ-26481585 for patients with advanced refractory leukemia or myelodysplastic syndrome
Phase II study of Givinostat in very high-risk relapsed/refractory Hodgkin’s lymphoma patients
Phase II study of Givinostat followed by Mechloretamine in relapsed/refractory Hodgkin’s lymphoma patients
Phase II study of Givinostat in refractory/relapsed lymphocytic leukemia
Phase II study of Givinostat in combination with hydroxyurea in polycythemia vera
Clinical trial of Belinostat in patients with advanced multiple myeloma
Belinostat to treat tumors of the thymus at an advanced stage
Belinostat in relapsed or refractory peripheral T-cell lymphoma
Belinostat in treating patients with MSD
Safety and efficacy of Belinostat when used with standard of care chemotherapy for untreated NSCLC
A Phase I study of Belinostat in combination with Cisplatin and Etoposide in adults with SCLC and other advanced cancers
Vorinostat for locally advanced NSCLC
Vorinostat in treating patients with metastatic and/or locally advanced or locally recurrent thyroid cancer
A Study of the efficacy of Vorinostat in patients with polycythemia verae and essential thrombocythemia
Study of Vorinostat Plus Capecitabine and Cisplatin for 1st Line Treatment of metastatic or recurrent gastric cancer
Study of Vorinostat combination with Bortezomib in patients with multiple myeloma
Study of Vorinostat and Gefitinib in relapsed/refractory patients with advanced NSCLC
Proteasome Inhibitor NPI-0052 (marizomib, salinosporamide A) and Vorinostat in patients with NSCLC, pancreatic cancer, melanoma or lymphoma
Vorinostat combined with Gemtuzumab Ozogamicin, Idarubicin and Cytoxan in acute myeloid leukemia
Trial for locally advanced Her2 positive breast cancer using Vorinostat and Paclitaxel, Trastuzumab, Doxorubicin and Cyclophamide on a weekly basis
Sorafenib and Vorinostat in advanced cancer
Temsiorolimus and Vorinostat in treating patients with metastatic prostate cancer
Vorinostat, Carboplatin and Gemcitabine plus Vorinostat maintenance in women with recurrent, Platinum-sensitive epithelial ovarian, Fallopian tube, or peritoneal cancer
Vorinostat and Gemcitabine in treating patients with metastatic or unresectable solid tumors
Vorinostat and Lenalidomide in treating patients with relapsed or refractory Hodgkin lymphoma or non-Hodgkin lymphoma
Hydroxychloroquine + Vorinostat in advanced solid tumors
Vorinostat in combination with palliative radiotherapy for patients with NSCLC
Vorinostat in combination with radiation therapy and infusional Fluorouracil (5-FU) in patients with locally advanced adenocarcinoma of the pancreas
Study of 5-azacytidine in combination with Vorinostat in patients with relapsed or refractory diffuse large B cell lymphoma
An Investigational Study of Vorinostat Plus Targretin (Bexaroten) in cutaneous T-cell lymphoma patients
Phase II Trial of Vorinostat and Tamoxifen for patients with breast cancer
Oral Panobinostat in relapsed or refractory CLL and MCL (non-Hodgkin’s lymphoma)
Panobinostat in Phase II in SCLC
Panobinostat in treating patients with relapsed or refractory acute lymphoblastic leukemia or acute myeloid leukemia
Study of Oral Panobinostat in adult patients with refractory/resistant cutaneous T-cell lymphoma
Study of Bortezomib and Panobinostat in treating patients with relapsed/refractory peripheral T-cell lymphoma or NK/T-cell Lymphoma
Study of Panobinostat to treat malignant brain tumors
Panobinostat in adult patients with advanced solid tumors or cutaneous T-cell lymphoma
A study of Panobinostat as second-line therapy in patients with chronic graft-versus-host disease
Panobinostat treatment for refractory clear cell renal carcinoma
A Study to investigate the effect of food on oral Panobinostat absorption in patients with advanced solid tumors
ERB-B4 after treatment with Panobinostat in ER+ Tamoxifen refractory breast cancer
Panobinostat in addition to corticosteroids in patients with acute graft versus host disease
Panobinostat and Imitabim Mesylate in treating patients with previously treated chronic phase chronic myelogenous leukemia
Study of Imitabim, a Platelet-derived Growth Factor Receptor Inhibitor, and Panobinostat, in the treatment of newly diagnosed and recurrent chordoma
Oral Panobinostat in combination with Carboplatin and Paclitaxel in advanced solid tumors
Safety and efficacy studies of Panobinostat and Bicalutamide in patients with recurrent prostate cancer after castration
Panobinostat and Everolimus in treating patients with recurrent multiple myeloma, non-Hodgkin lymphoma, or Hodgkin lymphoma
Panobinostat and Fluorouracil followed by Leucovorin Calcium in treating patients with stage IV colorectal cancer who did not respond to previous Fluorouracil-based chemotherapy
Sorafenib and Panobinostat in hepatocellular carcinoma
A Safety study of Panobinostat and Everolimus to stabilize kidney cancer
Phase II/III Study of Panobinostat and Erlotinib for advanced aerodigestive tract cancers

(Continued)
(TAL1) and GATA1 interactions with HDACi, inducing differentiation in murine erythroleukemia (MEL) cells. Siitonen et al. reported a negative study trying VP A, in differentiation in murine erythroleukemia (MEL) cells.

Entinostat in treating patients with hematologic cancer

Safety and efficacy study of Entinostat in combination with 5-aza for advanced cancers (Table 3, O, colon, skin melanoma, breast, other) gave stable diseases. A Phase I/II study with 5-aza and ATRA for AML and MDS (Table 3, P) gave 42% positive overall responses.

Other combinations were investigated: a Phase I dose escalation combination trial with epirubicin, 5-FU, and cyclophosphamide in breast cancer (Table 3, Q), and a Phase I trial with epirubicin for solid tumors (Table 3, R). The rationale for the combination was to facilitate epirubicin access to DNA to potentiate its strand breaks activity as a topoisomerase II inhibitor. Intrinsic epirubicin toxicity was not exacerbated. Reverse combination was found inadequate by the same group. The same group investigated combination with the topoisomerase I inhibitor karenitecin (KTN)
Table 3  In vivo HDACi effects from clinical data

<table>
<thead>
<tr>
<th>HDACi (metabolism, half-life, bioavailability) and combinations</th>
<th>MTD, cancer target, DLTs</th>
<th>Biological analyses (source)</th>
<th>Remarks or recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB converted in vivo to the active metabolite PA, not indicated, 78% at 0.5 mM</td>
<td>Phase II, 27 g/day, GBM, common DLTs 300–410 mg/kg/day, various cancers, CNS</td>
<td>H4 acetylation increased (not correlated with response)</td>
<td>Contradictory with P450-inducing anticonvulsants. One complete response for 5 years</td>
</tr>
<tr>
<td>PB + 5-aza</td>
<td>AML/MDS, skin reaction (5-aza) 25 mg/m²/d SQ d. I–14, Several cancers, common DLTs + confusion, hearing loss, triglyceridemia and hyperuricema 3.3 g/m²/day for the solubility limits, advanced solid malignancies, common DLTs + visual complaints</td>
<td>H4 acetylation increased (not correlated with response)</td>
<td>Low DNMT activity</td>
</tr>
<tr>
<td>AN-9</td>
<td>Is converted in vivo to the active metabolite BA, half-life &lt;2 minutes</td>
<td>Phase II refractory NSCLC, common DLTs and dysgeusia</td>
<td>Well tolerated, active alone and usable for NSCLC with chemotherapy, 1-year survival around 30%</td>
</tr>
<tr>
<td>VPA</td>
<td>Glucuronylation, glutamination, half-life: 9–18 hours</td>
<td>60 mg/kg/d, refractory advanced cancer, neurological side effects (Grade 3/4)</td>
<td>H3, H4 acetylation increased, HDAC2 decreased (PMBC)</td>
</tr>
<tr>
<td>VPA+ATRA</td>
<td>AML, neurologic and cardiovascular toxicity</td>
<td>MDS and relapsed or refractory AML</td>
<td>Bone marrow blast count correlated with response</td>
</tr>
<tr>
<td>VPA+13-cis RA or vitamin D3</td>
<td>Recurrent or refractory AML or MDS, neurocortical, severe bone pain (Grade 3/4)</td>
<td>No significant blast count reduction, cytogenetic analysis of patients is described</td>
<td>VPA should be used alone for low risk MDS and with other chemotherapeutics for high risk MDS Platelet transfusion independence should reduce palliative care and improve the quality of life Particular response from patients with AML-M6 Near 50% patients had to end the treatment</td>
</tr>
<tr>
<td>VPA + 5-azaDc</td>
<td>Phase I/II leukemia</td>
<td>DNA demethylation decreased, H3 and H4 acetylation increased, p15 reactivation, p21 cip1 not stimulated</td>
<td>Objective responses rate: 22%, complete remissions: 19%, safety and efficacy correlated with reversal of epigenetics marks</td>
</tr>
<tr>
<td>N</td>
<td>AML, limited non hematologic toxicity (5-azaDc), encephalopathy (VPA)</td>
<td>Correlation with re-expression of ER mRNA and clinical response, p15 promoter methylation decreased DNA methylation decreased, DNMT1 decreased, histone acetylation increased</td>
<td></td>
</tr>
</tbody>
</table>

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Table 3 (Continued)

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<tr>
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</thead>
<tbody>
<tr>
<td>VPA + 5-aza</td>
<td>O Advanced cancers</td>
<td>DNA methylation decreased (not significant), H3 acetylation increased (PMBC)</td>
<td>Safe at doses up to 75 mg/m² for 5-aza</td>
</tr>
<tr>
<td>VPA + 5-aza + ATRA</td>
<td>P 50 mg/kg/d for 7 days, AML and MDS, neurotoxicity</td>
<td>DNA methylation decreased, H3 and H4 deacetylation increased, p21 cip1 and p15 mRNA expression not associated with clinical response</td>
<td>Combination safe with significant clinical activity</td>
</tr>
<tr>
<td>VPA + epirubicin or 5-FU or cyclophosphamide</td>
<td>Q Breast cancers</td>
<td>H3 and H4 acetylation increased (PMBC), strong correlation for HDAC2 decreased in MCF-7 cells, no correlation for HDAC6</td>
<td>Objective responses for 64% (9/15) of the patients</td>
</tr>
<tr>
<td>VPA + epirubicin</td>
<td>R Solid tumors, confusion, hallucinations, hearing loss and dizziness (due to VPA half-life)</td>
<td>48 hours exposure VPA for chromatin decondensation prior to epirubicin exposure. Histone acetylation increased (PBMN)</td>
<td>Responses were obtained for anthracycline-resistant cancers (breast, cervical and NSCLC). Potential use in randomized trials where topoisomerase I inhibitors are involved</td>
</tr>
<tr>
<td>VPA + KTN</td>
<td>S Melanoma, no VPA/KTN synergistic toxicity</td>
<td>H3 and H4 acetylation increased (PMBC, apparent plateau for 60 mg/kg/day VPA)</td>
<td>Combination safe with significant clinical activity</td>
</tr>
<tr>
<td>VPA + dazacarbine + interferon-α</td>
<td>T Advanced inoperable or metastatic melanoma, high doses VPA side effects</td>
<td>Histone acetylation increased (PMBC) with adjusted VPA doses.</td>
<td>Modification of the schedule for further evaluation of VPA with chemotherapeutic agents</td>
</tr>
<tr>
<td>VPA Mg salt</td>
<td>U Cervical cancer, depressed level of consciousness</td>
<td>H3 and H4 acetylation increased (PMBC and tumors).</td>
<td>Patients from cisplatin, carboplatin, paclitaxel, vinorelbine, gemcitabine, pemetrexed, topotecan, doxorubicin, cyclophosphamide, and anastrozole treatments. Supports epigenetic-driven tumor-cell chemoresistance hypothesis.</td>
</tr>
<tr>
<td>VPA Mg salt + hydralazine</td>
<td>V Chemotherapy resistant refractory solid tumors, hematologic toxicity</td>
<td>DNA methylation decreased, histone deacetylase activity decreased, promoter methylation decreased for RAR-α and DPK. 1091 genes upregulated, 89 genes downregulated.</td>
<td>A randomized Phase III study can be proposed for patients already treated unsuccessfully with pemetrexed</td>
</tr>
<tr>
<td>Vorinostat, glucuronylated, β-oxidized, half-life &lt;2 hours, 43% oral bioavailability</td>
<td>W Solid tumors and hematological malignancies, leukopenia, thrombocytopenia, respiratory distress (Grade 3/4)</td>
<td>Histones acetylation increased (v)</td>
<td>(Continued)</td>
</tr>
<tr>
<td>HDACi (metabolism, half-life, bioavailability) and combinations</td>
<td>MTD, cancer target, DLTs</td>
<td>Biological analyses (source)</td>
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</tr>
<tr>
<td>Y</td>
<td>Hematologic malignancies and solid, common DLTs + anorexia</td>
<td>Histone acetylation (PMBC, 200 to 600 mg).</td>
<td>Safe when administered chronically, broad range of antitumor activity. None of the responding patients have a specific mRNA signature for antioxidant genes to be used as a biomarker for further studies.</td>
</tr>
<tr>
<td>Z</td>
<td>Advanced leukemias and MDS (AML, CLL, MDS, ALL, CML), common DLTs (Grade 3/4)</td>
<td>Incomplete blood count recovery (AML), histone acetylation increased at all doses</td>
<td>None of the responding patients have a specific mRNA signature for antioxidant genes to be used as a biomarker for further studies.</td>
</tr>
<tr>
<td>AA</td>
<td>Recurrent or persistent epithelial ovarian or primary peritoneal carcinoma platinum-resistant/refractory, common Grade 3 DLTs + leukopenia and neutropenia (Grade 4).</td>
<td></td>
<td>Enzyme-inducing anticonvulsants gave lower SAHA concentrations, well tolerated, modest activity.</td>
</tr>
<tr>
<td>AB</td>
<td>Measurable, relapsed or refractory breast cancer or NSCLC or colorectal cancer, common DLTs (300–400 mg) No DLTs at 200 mg.</td>
<td></td>
<td>The limited patient exposure was not sufficient to assess SAHA efficacy.</td>
</tr>
<tr>
<td>AC</td>
<td>Recurrent and/or metastatic head and neck tumors, thrombocytopenia, anorexia, and dehydration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>GBM, nonhematologic, and hematologic toxicities (Grade 3).</td>
<td>H2B, H4 and H3 acetylation increased. Upregulation of E-cadherin.</td>
<td></td>
</tr>
<tr>
<td>AE</td>
<td>Metastatic radiiodine-refractory thyroid carcinoma, common DLTs (Grade 3), pneumonia, severe thrombocytopenia</td>
<td>Tg (DTC) and calcitonin (MTC) are not convenient biomarkers.</td>
<td>Lack of therapeutic effect</td>
</tr>
<tr>
<td>Vorinostat + carboplatin and paclitaxel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AF</td>
<td>Advanced solid malignancies, common DLTs + emesis (Grade 3), neutropenia (Grade 4)</td>
<td>SAHA metabolite 4-anilino-4-oxobutanoic acid used as a marker to monitor for adherence to SAHA therapy.</td>
<td>Combinations well tolerated and increased SAHA half-life, paclitaxel PKs not altered</td>
</tr>
<tr>
<td>Belinostat, half-life 1–2 hours</td>
<td>1000 mg/m²/day, refractory solid tumors, common DLTs + atrial fibrillation</td>
<td>H4 acetylation (PMBC), IL-6 expression levels proposed as a marker for HDACi toxicity.</td>
<td>50% of the patients achieve stable disease</td>
</tr>
<tr>
<td>AG</td>
<td>1000 mg/m²/day, heavily pre-treated patients with advanced hematological neoplasia, common DLTs</td>
<td>Histone acetylation increased (PMBC) up to 24 hours post injection.</td>
<td>No bone marrow toxicity as a parameter for combination therapies. No objective responses. One death from cardiac arrhythmia, possibly related to therapy.</td>
</tr>
<tr>
<td>AH</td>
<td>Relapsed malignant pleural mesothelioma, common DLTs</td>
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<tr>
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<tbody>
<tr>
<td>Givinostat</td>
<td>AJ</td>
<td>Resistant micro papillary ovarian tumors (LMP) and epithelial ovarian cancer, common DLTs + thrombosis (Grade 3)</td>
<td>H3 and H4 acetylation increased (PMBC and tumor tissue). Disease.</td>
</tr>
<tr>
<td>Givinostat, alone or + dexamethasone</td>
<td>AK</td>
<td>Relapsed/refractory HL, thrombocytopenia and prolongation of QTc interval</td>
<td>QTc interval in some cases prompting drug discontinuation</td>
</tr>
<tr>
<td>Panobinostat, half-life 11–16 hours</td>
<td>AN</td>
<td>&lt;1.5 mg/m², AML, ALL, MDS, common minor DLTs, and cardiac toxicity (Grade 3)</td>
<td>H3 acetylation increased (B-cells (CD19+) and blasts (CD34+)), H2B acetylation increased (CD19+ and CD34+ cells), apoptosis increased for CD34+.</td>
</tr>
<tr>
<td>Panobinostat + docetaxel</td>
<td>AP</td>
<td>Castration resistant prostate cancer, neutropenia and dyspnea (Grade 3)</td>
<td>Progressive disease despite histone acetylation increased (PMBC) in first regimen, PSA decreased in second regimen</td>
</tr>
<tr>
<td>Dacinostat, half-life 9–18 hours</td>
<td>AQ</td>
<td>Advanced solid tumors, common DLTs and transaminisits, fibrillation, raised serum creatinine, and hyperbilirubinemia.</td>
<td>Histones acetylation increased (PMBC), HSP90 inhibition measured by HSP72 levels increased; H3 and H4 acetylation increased for &gt;24 hours, Hsp70 increased and c-Raf decreased.</td>
</tr>
<tr>
<td>PCI-24781 half-life 5.9 hours, oral bioavailability 34%</td>
<td>AS</td>
<td>Refractory advanced solid tumors, common and cardiac DLTs</td>
<td>Acetylation levels increased at 1.5 hours post dose sustained ≥24 hours (oral).</td>
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**Table 3 (Continued)**

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<thead>
<tr>
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</tr>
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<tbody>
<tr>
<td><strong>Entinostat</strong>&lt;br&gt;34–50 hours, highly protein bound, apparent linear PKs</td>
<td>AT&lt;br&gt;10 mg/m², advanced solid tumors or lymphoma, common DLTs</td>
<td>HDAC inhibition (PMBC)</td>
<td>More frequent dosing proposed for evaluation from linear PKs data, elimination half-life dose-independent clearance</td>
</tr>
<tr>
<td></td>
<td>AU&lt;br&gt;Refractory solid tumors and human lymphoid malignancies, reversible DLTs (Grade 3, hypophosphatemia, hyponatremia, and hypoalbuminemia)</td>
<td>Protein acetylation increased (by multivariable flow cytometry in PMBC (T cells (most robust response), B cells, and monocytes))</td>
<td>Well tolerated administered weekly with food</td>
</tr>
<tr>
<td></td>
<td>AV&lt;br&gt;8 mg/m² weekly for 4 weeks every 6 weeks, AML, infections and neurologic toxicity</td>
<td>Protein and histone H3/H4 acetylation increased (PMBC, BMMC), p21 expression increased, and caspase-3 activation (BMMC)</td>
<td>Detailed cytogenetic analysis performed on patients, inherent resistance to MS-275 for advanced leukemia with complex karyotype</td>
</tr>
<tr>
<td></td>
<td>AW&lt;br&gt;Advanced solid malignancies and lymphomas, hypophosphatemia, and asthenia</td>
<td>High degree of interpatient variations in H3 and H4 acetylation increased (PMBC)</td>
<td>Patients with pretreated metastatic melanoma, treatment well tolerated, no objective responses, median time-to-progression was 51–56 days</td>
</tr>
<tr>
<td></td>
<td>AX&lt;br&gt;Metastatic melanoma, toxicity mild to moderate</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mocetinostat</strong>&lt;br&gt;6.7–12.2 hours</td>
<td>AY&lt;br&gt;45 mg/m²/d, advanced solid tumors, rare common DLTs</td>
<td>H3 acetylation increased (PWBC, measured with the BOC-Lys(ε-Ac)-AMC fluorophore), IL-6 induction</td>
<td>Interpatient variability improved with low pH beverages</td>
</tr>
<tr>
<td></td>
<td>AZ&lt;br&gt;60 mg/m², AML, MDS, ALL, and CML, common DLTs (Grade 3)</td>
<td>Histone acetylation increased (PWBC, measured with the BOC-Lys(ε-Ac)-AMC fluorophore).</td>
<td>Three complete bone marrow response, cytogenetic analysis of patients not correlated with responses, safe and anti-leukemia activity for advanced leukemia</td>
</tr>
<tr>
<td></td>
<td>BA&lt;br&gt;Advanced leukemias or MDS, common DLTs</td>
<td>HDAC inhibition (PMBC)</td>
<td>Four patients with stable disease</td>
</tr>
<tr>
<td><strong>Mocetinostat continued</strong>&lt;br&gt;</td>
<td>BB&lt;br&gt;85 mg dose exhibited meaningful activity, HL</td>
<td>TARC levels correlated with clinical response</td>
<td>Two complete responses (10%), six partial responses (29%)</td>
</tr>
<tr>
<td></td>
<td>BC&lt;br&gt;FL, common Grade 3 DLTs + anorexia, thrombocytopenia, pericardial serious adverse event</td>
<td></td>
<td>No clear relationships with schedules, cardiac diseases, pathologies, and biomarkers such as HDAC activity.</td>
</tr>
<tr>
<td>Tacedinaline</td>
<td>BD&lt;br&gt;8 mg/m²/day for 8 weeks, repeated after a 2-week drug-free interval, solid tumors, common DLTs + thrombocytopenia, anemia, mucositis</td>
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Table 3 (Continued)

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<tbody>
<tr>
<td>Tacedinaline + capecitabine</td>
<td>BE 6 mg/m² Tacedinaline and 2000 mg/m²/day capecitabine, for 2 weeks of a 3-week cycle, advanced solid malignancy, DLT was thrombocytopenia</td>
<td>Histone acetylation increased (MNBP), PC3 cell cycle arrest induction, MDR-1 induced, functional PgP.</td>
<td>No overlapping toxicity</td>
</tr>
<tr>
<td>Tacedinaline + gemcitabine</td>
<td>BF Advanced pancreatic cancers, neutropenia, and thrombocytopenia</td>
<td>Histone acetylation increased (PMBC), MDR-1 induced.</td>
<td>Combination does not improve treatment 4-hour infusion safe</td>
</tr>
<tr>
<td>Depsipeptide, natural disulfide prodrg, half-life 8 hours</td>
<td>BG 13.3 mg/m³, incurable cancers, common DLTs + thrombocytopenia, and fatigue.</td>
<td>Histone acetylation increased (PMBC), MDR-1 induced.</td>
<td>Continuous cardiac monitoring, one partial response, 472.6 ng/mL mean maximum plasma concentration at MTD</td>
</tr>
<tr>
<td></td>
<td>BH 17.8 mg/m³/4 h, advanced or refractory neoplasms, common DLTs + grade-4 thrombocytopenia and cardiac arrhythmia.</td>
<td>Histone acetylation increased (PMBC)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BI CLL, AML, common DLTs</td>
<td>HDAC inhibition increased, histone and p21 promoter H4 acetylation increased, p21 protein and ID10 antigen expression increased, acetylation increased for H4 K5, K12, K8, K16, and H3 K9, K14</td>
<td>Depsipeptide is well tolerated but no objective responses</td>
</tr>
<tr>
<td></td>
<td>BJ 17 mg/m², refractory or recurrent solid tumors, reversible, asymptomatic T-wave inversions, transient asymptomatic sick sinus syndrome, and hypocalcemia</td>
<td>HDAC inhibition increased (PMBC)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BK MDS and AML, common DLTs (Grade 3/4 asymptomatic hypophosphatemia)</td>
<td>Apoptosis increased and changes in myeloid maturation marker expression. No changes in H3 and H4 acetylation, CD34/C13 stimulation.</td>
<td>One complete remission (AML) acceptable toxicity, limited activity in unselected AML/MD patients</td>
</tr>
<tr>
<td></td>
<td>BL Refractory renal cell cancer, cardiac side effects</td>
<td>HDAC inhibition increased (PMBC)</td>
<td>One complete response but not active enough in this population, one sudden death</td>
</tr>
<tr>
<td></td>
<td>BM Metastatic neuroendocrine tumors, common DLTs, serious cardiac adverse events</td>
<td>HDAC inhibition increased, p21 expression increased. 16 gene expressions stimulated $2$-fold, 1000 genes repressed $2$-fold.</td>
<td>One complete response but not active enough in this population, one sudden death</td>
</tr>
<tr>
<td></td>
<td>BN Lung cancers</td>
<td>HDAC inhibition increased, p21 expression increased. 16 gene expressions stimulated $2$-fold, 1000 genes repressed $2$-fold.</td>
<td>Depsipeptide not appropriate but renormalize lung cancer cells to normal bronchial epithelia</td>
</tr>
</tbody>
</table>

**Notes:**

*Resulted from formaldehyde released after AN-9 metabolism; Common DLTs are considered to be fatigue, nausea, vomiting.

**Abbreviations:** 5-aza, 5-aza-cytidine; 5-azaDc, 5-aza-2′-deoxycytidine; 5-FU, 5-fluorouracil; 13-cis-RA, 13-cis-retinoic acid; ALL, acute lymphoblastic leukemia; AMC, 7-amino-4-methylcoumarin; AML, acute myeloid leukemia; AN-9, pivaloyloxymethyl butyrate 9; ATRA, all-trans retinoic acid; BA, butyric acid; BMMC, bone marrow mononuclear cell; CLL, chronic lymphocytic leukemia; CML, chronic myelocytic leukemia; CMML, chronic myelomonocytic leukemia; CNS, central nervous system; CTCL, cutaneous T-cell lymphoma; DLT, dose-limiting toxicity; DNMT, DNA methyltransferases; DTC, differentiated thyroid carcinoma; ER, estrogen receptor; FL, follicular lymphoma; GBM, glioblastoma multiforme; HDAC, histone deacetylase; HDACi, HDAC inhibitor; HL, Hodgkin lymphoma; IL-6, interleukin-6; KTN, karenitecin; LMP, low malignant potential; MDS, myelodysplastic syndrome; MTC, medullary thyroid cancer; MTD, maximum tolerated dose; NSCLC, nonsmall cell lung cancer; PA, phenylacetate; PB, phenylbutyrate; PGp, P-glycoprotein; PK, pharmacokinetic; PMBC, peripheral mononuclear blood cell; PSA, prostate specific antigen; PWBC, peripheral white blood cell; QTc, QT interval corrected for heart rate; RAR-α, retinoic acid receptor-α; SAHA, suberoylanilide hydroxamic acid; TARC, thymus and activation regulated chemokine; Tg, thyroglobulin; VPA, valproic acid.
(Table 3, S) for treating melanoma with both Phase I/II trials. No VPA/KTN synergistic toxicity was observed. The best response was disease stabilization. VPA plus chemotherapy was investigated in a Phase II study for advanced inoperable or metastatic melanoma (Table 3, T). HDACi having been previously found to have a tumorigenic potential in melanoma. Some patients then received dacarbazine plus interferon-α with VPA.

The magnesium salt of VPA has been tested in phase I for cervical cancer (squamous and in adenocarcinoma) and Phase II clinical trials. In the Phase I study (Table 3, U), VPA was given per os, and the authors emphasized the requirement for new endpoint trials based on biomarker analysis with, in this particular case, H3 and H4 acetylation and in vivo HDAC inhibition detection. The Phase II study was conducted with hydralazine, a demethylating agent, to overcome chemotherapy resistance in refractory solid tumors (cervix, breast, ovarian, and others). Partial responses and disease stabilization were the best responses.

**Vorinostat**

Vorinostat (suberoylanilide hydroxamic acid [SAHA], Zolinza®) has probably been the most studied compound in clinical trials on several cancer types. SAHA induces differentiation, growth arrest, or apoptosis at micromolar concentrations. Vorinostat is an unselective zinc-binding hydroxamic-acid-type inhibitor of HDAC1, 2, 3, 6, and 8. In glioma cells, SAHA induced expression of DR5, TNFα, p21Waf1, and p27Kip1 and reduced expression of CDK2, CDK4, cyclin D1, and cyclin D2. SAHA can induce thyroid carcinoma in a Phase II study (Table 3, AE). A Phase II clinical trial was mainly proposed with oral formulations. A multi-institutional trial in women with recurrent or persistent epithelial ovarian (Table 3, AA) or primary peritoneal carcinoma platinum-resistant/refractory breast cancer, NSCLC, or colorectal cancer (Table 3, AB). Disease stabilization was observed in eight patients. SAHA is tolerated at 200 mg only, in a daily oral schedule for 14 days–3 weeks. In recurrent and/or metastatic head and neck cancer (400 mg every day) (Table AC) no confirmed responses have been observed. In patients with metastatic breast cancer, there were no complete or partial responses, and the heterogeneity of the recruited patients did not allow production of significant statistical results. Eight patients were positive for estrogen and/or progesterone receptors, four had amplified CerB-2. Fatigue, nausea, diarrhea, and lymphopenia were the most frequent clinically significant adverse effects. In GBM (Table 3 AD), an oral dose of 200 mg followed by a 7-day rest period showed that SAHA monotherapy was well tolerated with modest single-agent activity. Although HDACi were shown to induce cell death and sensitize cells to cytotoxic chemotherapy in thyroid cancer cell lines, Woyach et al described the lack of therapeutic effect of SAHA in patients with metastatic radiiodine-refractory thyroid carcinoma in a Phase II study (Table 3, AE). A Phase II oral combination therapy was proposed with carboplatin (I.V.) and Paclitaxel (I.V.) for advanced solid malignancies (Table 3, AF). Eleven partial responses occurred and seven disease stabilizations. The regimen requires drug–drug interaction to be determined. Encouraging results were obtained in patients with previously untreated NSCLC.

**Belinostat**

Belinostat (PXD101) is a recent hydroxamic acid HDACi that has growth-inhibitory and pro-apoptotic activity in several cancer types at submicromolar concentrations, and that has been investigated in ovarian cancers. It down regulates thymidilate synthase, vascular endothelial growth factor (VEGF), aurora kinase, and epidermal growth factor receptor (EGFR), and up regulates cyclin A. It has been used in combination. A gene expression-signature profiling has been reported for Belinostat. According to publications PKs gave a general 1–2-hour half-life. In early trials, DNA fragmentation increased with a combination of 5-FU in
ovarian tumors (low malignant potential [LMP]) and epithelial ovarian cancers (EOC) (Table 3, AJ). A Phase II trial with Belinostat gave partial responses or stable diseases for LMP, and stabilized diseases for EOC.

Givinostat
Givinostat (ITF2357) belongs to the hydroxamic acid family of HDACi which is very similar to SAHA. It inhibits IL-6 and VEGF production in stromal cells.

Two Phase II studies were described for relapsed/refractory Hodgkin lymphoma (HL) (Table 3, AK). A first oral one, gave stable diseases by computed tomography (CT) scan that have been associated with a significant reduction in fluorodeoxyglucose-positron emission tomography scan uptake. Galli et al.150 developed a Phase II multicenter trial in 19 heavily treated patients that were relapsing from progressive multiple myeloma (MM) (Table 3, AL). The best responses were disease stabilization. This regimen appears as unlikely to play a significant role for advanced MM, and other combinations with currently used drugs should be investigated. A combination with the alkylating agent mechlorethamine151 (Table 3, AM) was investigated in relapsed/refractory HL.

Panobinostat
Panobinostat (LBH589) is a hydroxamic acid HDACi, which has demonstrated anti-angiogenic and anti-proliferative activities in human prostate carcinoma cell PC-3 xenografts in vivo, inducing H3 and tubulin acetylation152 in human umbilical vascular endothelial cells (HUVEC), which corresponded to G2-M cell cycle arrest and inhibition of HUVEC cell proliferation and viability. Non cytotoxic concentrations of Panobinostat inhibited endothelial tube formation, matrix gel invasion, AKT, extracellular signal-regulated kinase 1/2 phosphorylation, and chemokine receptor CXCR4 expression. Association with anti-VEGF therapies should be considered. Prince et al. have discussed preclinical data on Panobinostat and emerging data from Phase I and II studies in cancer patients.153

A Phase I study in refractory hematologic malignancies (AML, ALL, and MDS) (Table 3, AN)154 with I.V. administration appeared convenient to obtain anti-leukemic and biological effects. In cutaneous T-cell lymphoma (CTCL)47 with oral formulation (Table 3, AO), the responses ranged from disease stabilization to complete remission, showing the potential of this molecule in CTCL. In combination with Docetaxel155 (Table 3, AP), a microtubule interacting agent for castration-resistant prostate cancer, Panobinostat inhibited LnCAP androgen receptor positive prostate cancer cell proliferation, potentiated by Docetaxel. Single or combined treatments were administered with oral Panobinostat.

Dacinostat
Dacinostat (NVPLAQ824, LAQ) is a hydroxamic acid derivative similar to Panobinostat. It showed anti-neoplastic activity and can activate genes that produce cell cycle arrest. It acetylates hsp90, inducing proteosomal degradation of Bcr-Abl and HER-2. Combination of Dacinostat with 5-azaDc157 in human MDA-MB-231 and MCF-7 breast carcinoma cells showed a synergic anti-neoplastic activity for the MDA-MB-231. For the MCF-7 tumor cells, simultaneous 5-azaDc and Dacinostat administration were antagonistic, unseen when used in a sequential schedule (5-azaDc first). This is probably due to interference in the S-phase of Dacinostat since 5-azaDc is a S-phase specific interfering molecule. Dacinostat appeared to be well tolerated in clinical trials. Phase I investigations158 in advanced solid tumors included measure of HSP72 levels and was consistent with HSP90 inhibition (Table 3, AQ). Another group159 reported the same results with increased expression of Hsp70 and decreased c-Raf levels. The biological importance of these non histones mediated effects requires further study. I.V. administration for ALL, AML, CLL, CML160 (Table 3, AR) in blast crisis or advanced MDS gave some stable diseases.

PCI-24781
PCI-24781 is a broad-spectrum hydroxamic acid-based HDACi. PCI-24781 reverses drug resistance in four multi-drug resistant sarcoma cell lines and synergizes with chemotherapeutic agents to enhance caspase-3/7 activity.
In refractory advanced solid tumors\textsuperscript{162} (Table 3, AS), I.V. administration followed by dose escalation was well tolerated. Electrocardiac monitoring revealed grade $\leq$1 QTcF (QT interval corrected for heart rate using Fridericia’s formula) prolongation and asymptomatic nonspecific ST and T wave changes leading to discontinuation.

**Entinostat**

Entinostat (MS-275, SNX-275) is a benzamide HDACi, which promotes expression of genes involved in growth arrest and differentiation, like p21 and the maturation marker: gelsolin,\textsuperscript{163} inducing caspase-dependant apoptosis in CLL B-cells,\textsuperscript{164} p21CIP1/WAF1 differentiation or apoptosis in human leukemia cells,\textsuperscript{165} and also tissue growth factor (TGF)$\beta$III receptor expression in human breast cancer.\textsuperscript{166} Reported half-life in animals is about 1 hour, and species-variable protein binding was reported.\textsuperscript{167} Half-life in human plasma was higher than in animals, which is supposedly to be linked to protein binding, as Entinostat was found to be 80% bound.\textsuperscript{167}

Phase I study in advanced solid tumors or lymphoma by oral route\textsuperscript{168} (Table 3, AT) was reasonably well tolerated. In refractory solid tumors and human lymphoid malignancies\textsuperscript{169} (Table 3, AU), drug exposure increases linearly with dose. In AML\textsuperscript{170} (Table 3, AV), results showed that Entinostat effectively inhibits HDAC in vivo in patients with AML and should be further tested, preferably in patients with less-advanced disease. Several protocols were designed for patients with advanced solid malignancies and lymphomas\textsuperscript{171} (Table 3, AW). PKs revealed dose-dependent and dose-proportional increases. Responses were partial remissions and prolonged disease stabilization. In a Phase II study for metastatic melanoma (Table 3, AX) with low efficacy treatment,\textsuperscript{172} long-term tumor stabilizations have been observed, but no objective responses was assessed.

**Mocetinostat**

Mocetinostat (MGCD0103)\textsuperscript{173} is a benzamide selective class I/IV HDACi (1, 2, 3, and 11). It inhibits neoplastic growth in multiple human tumor xenograft models including colon (HCT116, SW48, and Colo205), NSCLC (A549), prostate (DU145), pancreatic (PANC1), and vulval epidermal (A431) cancer models and does not interact with the potassium voltage-gated channel, subfamily H ( eag-related), member 2 (HERG) channel. Gene expression induced by Mocetinostat is modest compared with other hydroxamic HDACi.\textsuperscript{174}

In patients with advanced solid tumors\textsuperscript{175} (Table 3, AV), a phase I study gave disease stabilization as the best response.

IL-6 induction related to HDACi activity has been postulated but not confirmed. At the tested doses, Mocetinostat appeared tolerable and exhibited favorable PK and PD profiles, as well as evidence of target inhibition in surrogate tissues. Cytogenetically analyzed patients with AML, MDS, ALL, and CML\textsuperscript{176} (Table 3, AZ) were treated orally. A total of 18 of the 29 patients had abnormal cytogenetics. PK analyses indicated rapid absorption of Mocetinostat. Several administration schedules have been proposed for advanced leukemias or MDS\textsuperscript{177} (Table 3, BA). A Phase II study for HL\textsuperscript{178} (Table 3, BB) demonstrated significant anti-tumor activity in relapsed/refractory post-transplant HL. For 437 patients\textsuperscript{179} (Table 3, BC), partial responses were obtained. Extended studies are ongoing.

**Tacedinaline**

The long-known molecule Tacedinaline (CI-994) is a benzamide HDACi similar to MS-275\textsuperscript{180} with anti-tumor activities in HCT-8 colon carcinoma.\textsuperscript{181} Following Tacedinaline administration, inhibition of both histone deacetylation and cellular proliferation at the G1 to S transition phase of the cell cycle are observed.

Oral administration with food intake for solid tumors\textsuperscript{182} (Table 3, BD) did not affect the rate or extent of the drug absorption. Best responses were partial or stable diseases. Advanced solid malignancies (mainly colorectal, pancreatic and mesothelioma) were treated in combination with Capecitabine\textsuperscript{183} (Table 3, BE), an FDA-approved compound used to treat a variety of cancer. Three treatment protocols were implemented. A combination Phase II study with Gemcitabine\textsuperscript{184} for advanced pancreatic carcinoma (Table 3, BF) gave no improvement.

**Depsipептид**

Depsipептид (Romidepsin, FK228, FR901228) is a cyclic tetrapeptide isolated from *Chromobacterium violaceum* which has demonstrated anti tumor activities (A549 lung adenocarcinoma, MCF-7 and ZR-75-1 breast adenocarcinoma, and LOX IMVI melanoma cell lines) and is postulated as a Pg-p substrate.\textsuperscript{185,186} It is considered as a natural HDACi prodrug, as its disulfide bond is reduced in vivo to give the active species\textsuperscript{40} and is the only reported sulfur-based HDACi used in clinical trials. It received FDA approval for cutaneous T-cell lymphoma in 2009. Romidepsin induces growth arrest and apoptosis in lung cancer cells.\textsuperscript{187} Romidepsin induces p21-dependent G1 arrest and p21-independent G2 arrest\textsuperscript{188} by downregulating cyclin D1 and upregulating cyclin E.\textsuperscript{189} It inhibits c-Myc and Fas ligand expression,\textsuperscript{190} modulates p53,
ErbB1, HER2, and Raf-1 expression in lung cancer cells, increases p21, phosphorylation of Bcl2, and apoptosis in human breast cancer cells, increases expression of a NaI symporter in thyroid carcinoma cells for possible resensitization of radio resistant thyroid cancer, and activates the caspase 8-mediated apoptosis and down regulates the c-FLIP protein. Sequential treatments with 5-azaDc facilitates cancer cell recognition by T lymphocytes specific for cancer/testis antigen 1B (NY-ESO-1) as a possible option for immunotherapy or induces tissue factor pathway inhibitor (TFPI)-2 expression in cancer cells. Initial cardiac toxicity was resolved by convenient administration schedules but cardiac monitoring is most of the time implemented during clinical investigations. Concentrations studies in CLL and AML have correlated apoptosis induction and HDAC inhibition. Combination of Romidepsin and DNA demethylating agents is potentiated in ETO positive leukemia cells. A gene signature specific for Romidepsin sensitivity has been reported. Due to the recent approval of Romidepsin in CTCL, all early published clinical trials for this disease are not discussed. Romidepsin induced MDR-1 gene expression in several cancer cell lines.

A Phase I study in advanced, incurable cancers (Table 3, BG) indicated that further clinical trials are warranted. Used for advanced or refractory neoplasms (Table 3, BH), elimination half-life was 8.1 hours. In CCL and AML (Table 3, BI), intravenous treatment gave no responses. Romidepsin effectively inhibits HDAC in vivo in patients with CLL and AML, but future studies should examine alternative administration routes. In refractory or recurrent solid tumors (Table 3, BJ), DLTs were not associated with changes in troponin levels or evidence of ventricular dysfunction, transient asymptomatic sick sinus syndrome and hypocalcemia. For MDS and AML (Table 3, BK), intravenous administration gave no significant cardiac toxicity. Romidepsin therapy can be administered with acceptable short-term toxicity. Gastrointestinal symptoms and fatigue seemed to be treatment-limiting after multiple cycles. Phase II performed on refractory renal cell cancer (Table 3, BL), I.V., gave classical but serious toxicities. Two patients developed a prolonged QT interval, one patient developed grade 3 atrial fibrillation and tachycardia, and there was one sudden death. In metastatic neuroendocrine tumors (Table 3, BM), adverse events were ventricular tachycardia and prolonged QT, possibly resulting in a sudden death, terminating the study prematurely. Romidepsin has serious cardiac adverse events, and risks need to be comprehensively evaluated. In lung cancers (NSCLC and SCLC) (Table 3, BN), Romidepsin was not appropriate. This study presented an in depth gene expression profiling.

Conclusion
A number of clinical trials have been completed and many others are ongoing using HDACi as single agents and in combination with radiotherapy and/or chemotherapy for the treatment of various hematological and solid malignancies with some promising early results. Vorinostat is the most established HDACi, and was approved in October 2006 by the FDA for the treatment of advanced forms of cutaneous T-cell lymphoma that have failed multiple other systemic treatment options. Significant single agent activity for Romidepsin has also been demonstrated in peripheral cutaneous T-cell lymphoma, and encouraging results have also been seen in HL with Mocetinostat. From the trials conducted, it is also clear that a major clinical advantage is that HDACi are well tolerated in the majority of patients. The future of HDACi lies in designing rational combination therapies. The sequence of drug administration may be of paramount importance to avoid antagonistic effects. The possibility of drug–drug interactions and enhanced toxicities is to be considered. HDACi are also evaluated in non cancerous pathologies like AIDS, graft versus host diseases, and polycythemia verae. Very soon, SIRT activators could find therapeutic applications in lung interstitial diseases. Like for the kinase inhibitors, more selective third generation HDACi are sought, yet specific tests remain to be designed to screen for bioactivity in vitro and in vivo.

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Disclosure
The authors report no conflicts of interest in this work.

References


Interpreting clinical assays for histone deacetylase inhibitors


