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Marije Slingerland
Henk-Jan Guchelaar
Hans Gelderblom

Histone deacetylase inhibitors: an overview of the clinical studies in solid tumors

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ABSTRACT

The histone deacetylase inhibitors (HDACi) are a group of small molecules that target histone deacetylases (HDACs) by inhibiting their activity. HDACi have a long history of use in neurology and psychiatry as anti-epileptics and mood stabilizers. More recently, they have been investigated as possible treatments for cancer. HDACi have undergone rapid clinical development in recent years, on the basis of their preclinical *in vitro* and *in vivo* antitumor activity in hematological malignancies and solid tumors. Many HDACi have entered phase I-III clinical trials. Among the HDACi, vorinostat and romidepsin are currently the most extensively studied. In 2006 and 2009, respectively, they received approval by the United States Food and Drug Administration for treatment of cutaneous T-cell lymphoma and romidepsin for the treatment of peripheral T-cell lymphoma. Other HDACi, such as panobinostat and valproic acid, also demonstrated activity as therapeutic anticancer agents. In this article we give an overview of the clinical studies of HDACi in solid tumors. We start with a short description of the working mechanism of HDACi in general.

HISTONE DEACETYLASE INHIBITORS

In addition to genetic mutations, epigenetic changes play an important role in the onset and progression of cancer.[1] Epigenetic changes are defined as heritable changes in gene expression that are not accompanied by changes in DNA sequence. Changes to the patterns of epigenetic alterations are common in cancer, and epigenetic dysregulation may be a preliminary transforming event often observed in early-stage tumors and benign neoplasms.[2,3] DNA and histones are the main compounds of nucleosomes, which are the structural units of chromatin that are important for wrapping eukaryotic DNA. Gene expression is affected by changes in the structural configuration of chromatin to a relatively open or more closed form, which alters the accessibility of DNA for transcription.[4] Transcription factor binding to DNA is mainly regulated through changes in chromatin conformation. This in turn is governed by chemical modifications such as the acetylation and deacetylation of lysine residues in the amino tails of the histones. The opposing activities of histone acetyltrans-ferases (HATs) and histone deacetylases (HDACs) tightly regulate gene expression through chromatin modification (Figure 6.1). HATs, by acetylating histones, produce an open chromatin structure, resulting in greater accessibility of regulatory proteins to DNA. HDACs, by contrast, catalyze acyl group removal, leading to a closed chromosomal configuration and transcriptional repression. Histone proteins were traditionally considered to be the primary focus for HDAC and HAT activities. However,

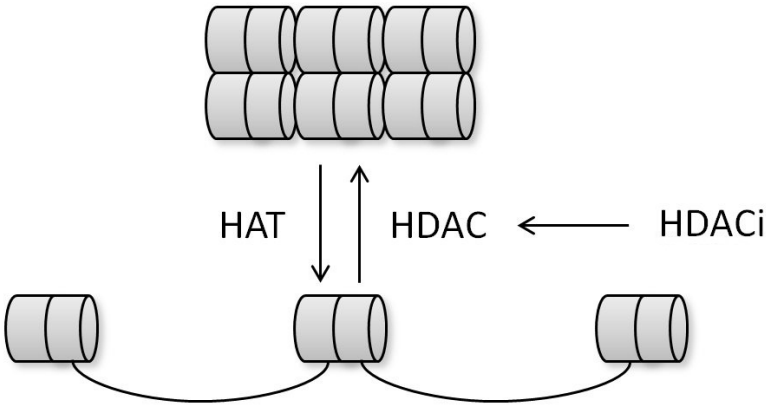


Figure 6.1 Histone acetyltransferase (HAT), histone deacetylase (HDAC), and histone deacetylase inhibitors (HDACi). The opposing activities of HATs and HDACs: HATs, by acetylating histones, produce an open chromatin structure; HDACs catalyze acyl group removal, leading to a closed chromosomal configuration.

acetylation also plays a crucial role in contexts other than histone and DNA-dependent processes. A considerable number of nonhistone proteins that play an important role in cell cycle proliferation and apoptosis are also being regulated by HAT and HDAC, for example transcription factors such as p53, E2F1, and NF-κB, which play important roles in tumor onset and antitumor response, as well as proteins that, instead of regulating gene expression, regulate the cellular cytoskeleton (α-tubulin), DNA repair (Ku70), and protein stabilization (Hsp90).[5] Hsp90, a nonhistone HDAC substrate, plays a major role in the proper wrapping and stability of several oncoproteins. HDAC activity also controls cell protein turnover through the aggresome pathway. Interference of this pathway results in the accumulation of misfolded protein aggregates, finally leading to apoptosis in tumor cells through autophagy.[6] These observations have revealed that the antitumor activity of histone deacetylase inhibitor (HDACi) includes effects on nonhistone proteins, implicated in many oncogenic pathways, in combination with epigenetic changes.

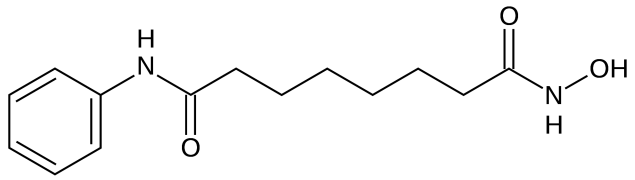
Already in 2001, Lin *et al*. stated that deregulation of HDAC activity in association with chromosomal translocated proteins is closely implicated in blocking differentiation and tumor suppressor genes, resulting in stimulating leukemogenesis.[7] The use of HDACi to reverse aberrant epigenetic changes in cancer cells, because of this important link, has emerged as a potential strategy for the treatment of solid tumors and hematological malignancies. The additional activity of deacetylases on nonhistone proteins provides HDACi with the opportunity to reverse and prevent the effects of aberrant deacetylation through epigenetic modifications and via effects on nonhistone protein targets, which are important in oncogenesis.[8,9]

CLINICAL STUDIES OF HISTONE DEACETYLASE INHIBITORS IN SOLID TUMORS

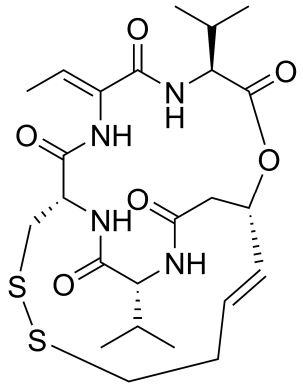
Vorinostat

Vorinostat (suberoylanilide hydroxamic acid; Zolinza®) inhibits HDAC by binding to a zinc ion in the catalytic domain of the enzyme (Figure 6.2).[10] Vorinostat demonstrated activity in murine xenograft models and it was additive or synergistic when combined with chemotherapy drugs in induction of differentiation and apoptosis of various cancer cell lines.[11] In 2006, the US Food and Drug Administration (FDA) granted regular approval to vorinostat for the treatment of cutaneous T-cell lymphoma (CTCL) in patients with progressive, persistent, or recurrent disease on or following two systemic therapies.[12]

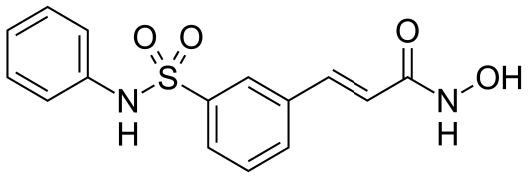
Vorinostat



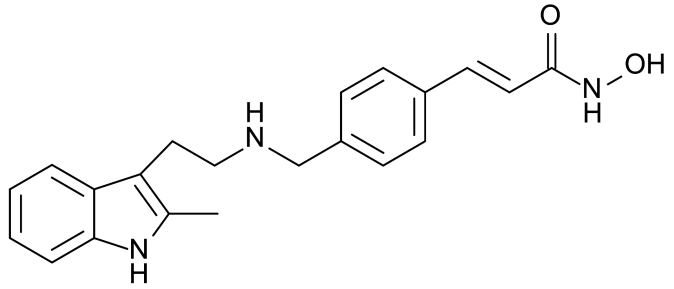
Romidepsin



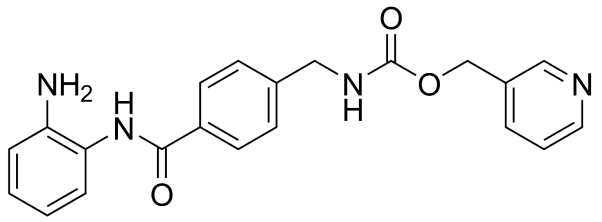
Belinostat



Panobinostat



Entinostat



Valproic acid

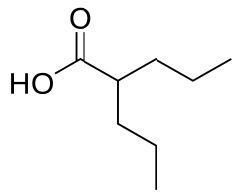


Figure 6.2 Structures of histone deacetylase inhibitors.

The pivotal study supporting approval was a single-arm open-label phase II trial.[13] An additional single-center study enrolled 33 patients with baseline and demographic features similar to the pivotal trial.[14] Despite the demonstrated effect in CTCL and other hematological tumors, unfortunately no such success has been demonstrated in solid tumors, although the phase I trials seemed rather encouraging. In two phase I studies with, respectively, intravenously and orally administered vorinostat Kelly *et al*. concluded that daily intravenous vorinostat was well tolerated, inhibited the biological target *in vivo*, and had antitumor activity in solid tumors. Oral vorinostat had linear pharmacokinetics (PK) and good bioavailability, inhibited HDAC activity in peripheral-blood mononuclear cells, could be safely administered chronically, and had a broad range of antitumor activity.[15,16] In 2007, Ramalingam *et al*. demonstrated in a phase I study that both schedules of vorinostat (400 mg orally daily 14 days or 300 mg twice daily 7 days) were tolerated well in combination with carboplatin (area under the concentration versus time curve = 6 mg/ml·min) and paclitaxel (200 mg/m^2) and that encouraging anticancer activity was noted in patients with previously untreated non-small cell lung cancer (NSCLC).[17] On the basis of these results, Vansteenkiste *et al*. conducted an early phase II trial of oral vorinostat in relapsed or refractory breast cancer, colorectal cancer, and NSCLC.[18] Sixteen patients (median age, 62 years; median 5.5 prior therapies) were enrolled. Six patients received 400 mg twice daily, six received 300 mg twice daily, and four received 200 mg twice daily (14 days/3 weeks). Dose-limiting toxicities (DLTs) at the 400 or 300 mg twice daily level were anorexia, asthenia, nausea, thrombocytopenia, vomiting, and weight loss. No DLTs were observed at the 200 mg twice daily level. Disease stabilization was observed in eight (50%) patients, but there were no confirmed responses. The median time to progression was only 33.5 days. Eleven patients (69%) discontinued because of clinical adverse events (AEs). The most common drug-related AEs were anorexia (81%), fatigue (62%), nausea (62%), diarrhea (56%), vomiting (56%), thrombocytopenia (50%), and weight loss (50%). Drug-related AEs of at least grade 3 included thrombocytopenia (50%), anemia (12%), asthenia (12%), and nausea (12%). They concluded that vorinostat on a daily oral schedule for 14 days/3 weeks was tolerable at 200 mg twice daily only, but that no responses were observed in this study because most patients had limited drug exposure, which did not allow a reliable efficacy analysis. In 2009, Woyach *et al*. could also not demonstrate a therapeutic effect of vorinostat in patients with metastatic radioiodine-refractory thyroid carcinoma.[19] Also in a phase II trial by Luu *et al*. in 2008 in metastatic breast cancer patients, vorinostat did not show adequate single-agent activity.[20] Other phase II trials with vorinostat in patients with recurrent head and/or metastatic head and neck cancer, respectively, by Blumenschein *et al*., recurrent platinum-refractory ovarian or primary peritoneal carcinoma by Modesitt *et*

al., relapsed NSCLC by Traynor *et al*., and recurrent glioblastoma multiforme by Galanis *et al*. all showed limited to no activity.[21-24] Study results with vorinostat in combination with, respectively, 5-fluorouracil/leucovorin in refractory colorectal cancer and bortezomib in recurrent glioblastoma were also disappointing.[25,26] However, in 2009 Ramalingam *et al*. published a phase II randomized, double-blind, placebo-controlled study evaluating the efficacy of vorinostat in combination with carboplatin and paclitaxel in patients with advanced-stage NSCLC.[27] Ninety-four patients initiated protocol therapy. The confirmed response rate was 34% with vorinostat versus 12.5% with placebo ($P = 0.02$). There was a trend toward improvement in median progression-free survival (PFS) (6.0 versus 4.1 months; $P = 0.48$) and overall survival (OS) (13.0 versus 9.7 months; $P = 0.17$) in the vorinostat arm. Grade 4 platelet toxicity was more common with vorinostat (18 versus 3%; $P < 0.05$). Nausea, emesis, fatigue, dehydration, and hyponatremia were also more frequent with vorinostat. In 2011, Munster *et al*. published their phase II trial of vorinostat combined with tamoxifen for the treatment of patients with hormone therapy-resistant breast cancer, which showed that this combination was well tolerated and exhibits encouraging activity in reversing hormone resistance.[28] Forty-three patients (median age 56 years (31-71)) were treated. Twenty-five patients (58%) received prior adjuvant tamoxifen, 29 (67%) failed one prior chemotherapy regimen, 42 (98%) progressed after one, and 23 (54%) after two aromatase inhibitors. The objective response rate by Response Evaluation Criteria In Solid Tumors (RECIST) criteria was 19% and the clinical benefit rate (response or stable disease (SD) > 24 weeks) was 40%. The median response duration was 10.3 months (confidence interval (CI): 8.1-12.4).

Romidepsin

Romidepsin (depsipeptide; Istodax®) acts as a prodrug with the disulfide bond undergoing reduction within the cell to release a zinc-binding thiol (Figure 6.2).[29-31] The thiol reversibly interacts with a zinc atom in the binding pocket of zinc-dependent HDAC to lock its activity. Romidepsin was licensed by the US FDA in 2009 for CTCL on the basis of two phase II trials conducted in a total of 167 patients suffering from relapsed, refractory, or advanced CTCL.[32,33] In 2011, romidepsin was also approved by the US FDA for peripheral T-cell lymphoma (PTCL) on the basis of the results from two studies: a phase II multicenter international open-label single-arm study in patients with PTCL who had failed at least one prior systemic therapy, which was presented at the 2010 American Society of Hematology annual meeting; and a single-arm clinical study in patients with PTCL who had failed prior therapy.[34,35] A series of phase I and phase II trials of romidepsin were conducted in patients with solid tumors, all with disappointing results. In 2002 Sandor *et al*. conducted a

phase I trial in patients with refractory neoplasms.[36] DLT was observed, and the maximum tolerated dose (MTD) exceeded 24.9 mg/m^2. The DLTs included grade 3 fatigue (three patients), grade 3 nausea and vomiting (one patient), grade 4 thrombocytopenia (two patients), and grade 4 cardiac arrhythmia (one patient, atrial fibrillation). The MTD was defined at the seventh dose level (17.8 mg/m^2). Reversible ST/T changes and mild reversible dysrhythmias were observed on the post-treatment electrocardiogram (ECG). There were no clinically significant changes in left ventricular ejection fraction. One patient with renal cell carcinoma (RCC) achieved a partial response (PR). Because of the refractory nature of metastatic human RCC and to follow up on this anecdotal response observed in the phase I studies, a single-arm, phase II, multi-institutional study was conducted to assess the antitumor activity of romidepsin in metastatic RCC.[37] The 29 evaluable patients, who were accrued so that 25 patients who received at least three doses of romidepsin could be observed, were heavily pretreated with a median of two previous systemic therapies and a 2-year median duration of metastatic disease. Twenty-four patients (83%) had clear-cell histology. The most common serious toxicities were fatigue, nausea, vomiting, and anemia. Two patients developed a prolonged QT_c interval, one patient each developed grade 3 atrial fibrillation and tachycardia, and there was one sudden death. Two patients experienced an objective response (one complete response (CR)) for an overall response rate (ORR) of 7% (95% CI: 0.8-23%). Schrump *et al*. could also not observe any objective responses in their phase II trial of romidepsin in lung cancer patients.[38] In this trial 19 patients were evaluable for toxicity assessment; 18 were evaluable for treatment response. Myelosuppression was dose-limiting in one individual. No significant cardiac toxicities were observed. In colorectal cancer patients romidepsin also seemed not to be effective. Whitehead *et al*. included 28 patients with previously treated colorectal cancer with advanced disease in a phase II trial of romidepsin, two of whom were ineligible.[39] One eligible patient refused all treatment and was not analyzed. For the 25 remaining patients, performance status was 0 in 16 patients and 1 in nine patients. Ten patients had received one prior chemotherapy regimen and 15 two prior regimens. Out of the 25 eligible and analyzable patients accrued in the first stage of the protocol, no objective responses were observed and the study was permanently closed. Four patients had SD as the best response. Twenty-five patients were assessed for toxicity. No grade 4 or greater toxicities were seen. Fourteen of the 25 patients experienced grade 3 toxicities, the most common of which were fatigue and anorexia. Molife *et al*. found minimal antitumor activity in chemotherapy-naive patients with castration-resistant prostate cancer in their phase II trial with romidepsin.[40] Thirty-five patients were enrolled in this study. Two patients achieved a confirmed radiological PR (RECIST) lasting for at least 6 months, along with a confirmed prostate-specific antigen decline of at least 50%. Eleven

patients experienced toxicity necessitating early discontinuation. The commonest AEs were nausea (30 patients; 85.7%), fatigue (28 patients; 80.0%), vomiting (23 patients; 65.7%), and anorexia (20 patients; 57.1%). There was no significant cardiac toxicity. In 2010 Otterson *et al*. published the results of their phase II trial of romidepsin in chemosensitive recurrent small cell lung cancer (SCLC).[41] Sixteen patients (10 male, six female) were accrued to the first stage of this study. Most (11 patients, 69%) presented with extensive-stage SCLC, and all had received prior chemotherapy, with 11 having received prior radiation. Eastern Cooperative Oncology Group performance status was excellent with 0 in six patients (38%) and 1 in 10 patients. No objective responses were seen, and SD was the best response seen in three patients (19%). Toxicity was modest with three patients suffering grade 3 toxicity (lymphopenia, insomnia, nausea, vomiting, and hyponatremia) and one patient suffering grade 4 thrombocytopenia. Median PFS was 1.8 months, and median OS was 6 months. They concluded that romidepsin given on a weekly schedule in patients with chemosensitive, recurrent SCLC was inactive. Iwamoto *et al*. found in their phase I/II trial that romidepsin was also ineffective for patients with recurrent glioblastomas.[42] Two dose cohorts were studied in the phase I component of the trial (13.3 and 17.7 mg/m^2/day). Patients in the phase II component were treated with intravenous romidepsin at a dosage of 13.3 mg/m^2/day on days 1, 8, and 15 of each 28-day cycle. Eight patients were treated in the phase I component. A similar romidepsin PK profile was demonstrated between patients receiving enzyme-inducing anti-epileptic drugs and those not receiving enzyme-inducing anti-epileptic drugs. Thirty-five patients with glioblastoma were accrued to the phase II component. There was no objective radiographic response. The median PFS was 8 weeks and only one patient had a PFS time of at least 6 months (PFS6 = 3%). At publication, 34 patients (97%) had died, with a median survival duration of 34 weeks. In 2012 Jones *et al*. published the results of their phase I trial that was conducted to determine the MTD for two schedules of romidepsin plus gemcitabine in patients with advanced solid tumors in which gemcitabine had previously demonstrated clinical activity.[43] The recommended phase II dose was 12 mg/m^2 romidepsin plus 800 mg/m^2 gemcitabine on days 1 and 15 every 28 days. They concluded that the results suggested additive hematologic toxicities of romidepsin plus gemcitabine, but the level of antitumor activity observed warranted more formal trials of this combination to further assess safety and efficacy. Also in 2012, Sherman *et al*. published their single-institution Simon two-stage phase II clinical study to evaluate the clinical activity of romidepsin and radioactive iodine (RAI) re-uptake in RAI-refractory thyroid carcinoma.[44] They observed preliminary signs of *in vivo* reversal of RAI resistance after treatment with romidepsin. However, no major responses were observed and accrual was poor after a grade 5 AE. Haigentz *et al*. conducted a phase II

trial in patients with advanced squamous cell carcinoma of the head and neck.[45] Objective responses were not observed, although two heavily pretreated patients had brief clinical disease stabilization. Observed toxicities were expected, including frequent severe fatigue.

Belinostat

Belinostat (PXD101) is a hydroxamic acid HDACi with anti-proliferative and HDAC inhibitory activities *in vitro* (Figure 6.2).[46] Belinostat has growth inhibitory and pro-apoptotic activities in a variety of human tumor cell lines at nanomolar concentrations. *In vivo*, belinostat inhibits growth in human tumor xenografts without apparent toxicity to the host mice.[46] Growth inhibition *in vitro* and *in vivo* is associated with a marked increase in the level of acetylation of histone proteins.[46]

In 2008, Steele *et al*. conducted a phase I study to determine the safety, DLT, MTD dose, and PK and pharmacodynamic profiles of belinostat in patients with advanced refractory solid tumors. Forty-six patients received belinostat at one of six dose levels (150-1200 mg/m^2/day). DLTs were grade 3 fatigue (one patient at 600 mg/m^2; one patient at 1200 mg/m^2), grade 3 diarrhea combined with fatigue (one patient at 1200 g/m^2), grade 3 atrial fibrillation (one patient at 1200 mg/m^2; one patient at 1000 mg/m^2), and grade 2 nausea/ vomiting leading to inability to complete a full 5-day cycle (two patients at 1000 mg/m^2). The MTD was 1000 mg/m^2/day. SD was observed in a total of 18 (39%) patients, including 15 treated for at least four cycles. Of the 24 patients treated at the MTD (1000 mg/m^2/day), 50% achieved SD.[47] Lassen *et al*. showed in their phase I trial that the combination of belinostat and carboplatin and/or paclitaxel in patients with solid tumors was well tolerated, with no evidence of PK interaction. The MTD of belinostat was 1000 mg/m^2/day for days 1-5, with paclitaxel 175 mg/m^2 and carboplatin area under the curve (AUC) 5 administered on day 3. Grade 3/4 AEs were (*n*; %): leucopenia (5; 22%), neutropenia (7; 30%), thrombocytopenia (3; 13%) anemia (1; 4%), peripheral sensory neuropathy (2; 9%), fatigue (1; 4%), vomiting (1; 4%), and myalgia (1; 4%). The PK of belinostat, paclitaxel, and carboplatin were unaltered by the concurrent administration. There were two PRs (one rectal cancer and one pancreatic cancer). A third patient (mixed mullerian tumor of ovarian origin) showed a complete cancer antigen-125 response. In addition, six patients showed an SD lasting for at least 6 months.[48] In 2009, Ramalingam *et al*. concluded in a phase II study that belinostat was not active as monotherapy against recurrent malignant pleural mesothelioma.[49] Other phase II trials could only demonstrate limited activity.[50-52] However, in 2012 Dizon *et al*. demonstrated that belinostat, carboplatin, and paclitaxel combined (BelCaP) was reasonably well tolerated and

demonstrated clinical benefit in heavily pretreated patients with epithelial ovarian cancer. Thirty-five women were treated. The median age was 60 years (range, 39-80 years), and patients had received a median of three prior regimens (range, 1-4). Fifty-four percent had received more than two prior platinum-based combinations; 16 patients (46%) had primary platinum-resistant disease, whereas 19 patients (54%) recurred within 6 months of their most recent platinum treatment. The median number of cycles of BelCaP administered was 6 (range, 1-23). Three patients had a CR, and 12 had a PR, for an ORR of 43% (95% CI: 26-61%). When stratified by primary platinum status, the ORR was 44% among resistant patients and 63% among sensitive patients. The most common drug-related AEs related to BelCaP were nausea (83%), fatigue (74%), vomiting (63%), alopecia (57%), and diarrhea (37%). With a median follow-up of 4 months (range, 0-23.3 months), 6-month PFS is 48% (95% CI: 31-66%). Median OS was not reached during study follow-up.[53]

Panobinostat

Panobinostat (LBH589) is a hydroxamic acid and acts as a non-selective HDACi (Figure 6.2). In 2010 the first phase I trial was published by Rathkopf *et al*. In this phase I trial 16 patients with castration-resistant prostate cancer were included. In arm I, oral panobinostat (20 mg) was administered on days 1, 3, and 5 for 2 consecutive weeks followed by a 1-week break. In arm II, oral panobinostat (15 mg) was administered on the same schedule in combination with docetaxel 75 mg/m^2 every 21 days. DLTs were grade 3 dyspnea (arm I) and grade 3 neutropenia greater than 7 days (arm II). In arm I, all patients developed progressive disease despite accumulation of acetylated histones in peripheral-blood mononuclear cells. In arm II, five of eight patients (63%) had at least a 50% decline in prostate-specific antigen, including one patient whose disease had previously progressed on docetaxel.[54] In 2011 Jones *et al*. showed in their phase I trial that the combination of panobinostat and gemcitabine was limited by myelosuppression. The recommended doses for further study were intermittent oral panobinostat administered at a dose of 10 mg three times weekly for 2 weeks in combination with gemcitabine 800 mg/m^2 administered intravenously on days 1 and 8 every 21 days.[55] Fukutomi *et al*. concluded in 2012, in their phase I trial, that panobinostat administered orally once daily on Monday, Wednesday, and Friday of each week was well tolerated at doses up to 20 mg in Japanese patients. Dose escalation did not proceed after exploration of the 20 mg dose due to emerging global clinical data at that time.[56] Morita *et al*. reported a phase I study to evaluate intravenous panobinostat given on days 1 and 8 of a 21-day cycle in Japanese patients with solid tumors. They concluded that the MTD was 20 mg/m^2.[57] Drappatz *et al*. concluded in their phase I study of panobinostat

in combination with bevacizumab for recurrent high-grade glioma that the recommended doses for further study are oral panobinostat 30 mg three times per week, every other week, in combination with bevacizumab 10 mg/kg every other week.[58] However, in 2012 Strickler *et al*. concluded in their phase I trial that adding everolimus to panobinostat and bevacizumab did not have an acceptable safety and tolerability profile.[59] DLTs in cohort 1 included grade 2 esophagitis and grade 3 oral mucositis; DLTs in cohort 1 were grade 2 ventricular arrhythmia and grade 2 intolerable skin rash. Common AEs were diarrhea (50%), headache (33%), mucositis/stomatitis (25%), hyperlipidemia (25%), and thrombocytopenia (25%). In a phase I trial Jones *et al*. investigated panobinostat in combination with paclitaxel and carboplatin in patients with solid tumors. They concluded that the recommended phase II dose is panobinostat 10 mg orally three times weekly in combination with paclitaxel 175 mg/m^2 and carboplatin AUC 5 administered intravenously on day 1 of every 21-day cycle.[60] Unfortunately, the phase II results of panobinostat were very disappointing. Hainsworth *et al*. concluded that panobinostat had no activity in patients with refractory renal carcinoma and Wang *et al*. could not support the treatment of advanced pancreatic cancer with bortezomib in combination with panobinostat in their clinical study.[61-62]

Entinostat

Entinostat (MS-275) is a benzamide derivative with potent HDAC inhibitory and antitumor activity in preclinical models (Figure 6.2). Several phase I trials have been performed since 2005. Ryan *et al*. conducted a phase I study that demonstrated that the entinostat oral formulation on the daily schedule (once daily 28 every 6 weeks (daily), starting dose 2 mg/m^2) was intolerable at the dose and schedule explored. The q14-day schedule was reasonably well tolerated. DLTs were nausea, vomiting, anorexia, and fatigue.[63] In 2007, Kummar *et al*. showed that entinostat was well tolerated at a dose of 6 mg/m^2 administered weekly with food for 4 weeks every 6 weeks. No grade 4 toxicities were observed. Grade 3 toxicities were reversible and consisted of hypophosphatemia, hyponatremia, and hypoalbuminemia.[64] Gore *et al*. showed that entinostat was well tolerated at doses up to 6 mg/m^2 every other week or 4 mg/m^2 weekly for 3 weeks followed by 1 week of rest and resulted in biologically relevant plasma concentrations and antitumor activity. Twice-weekly dosing was not tolerable due to asthenia, and further evaluation of this schedule was halted. The recommended dose for further disease-focused studies is 4 mg/m^2 given weekly for 3 weeks every 28 days or 2-6 mg/m^2 given once every other week.[65] Another phase I trial showed that the combination of entinostat and 13-cis retinoic acid was reasonably well tolerated. The recommended phase II doses are entinostat 4 mg/m^2 once weekly and 13-cis retinoic acid

1 mg/kg/day. Grade 3 toxicity included hyponatremia, neutropenia, and anemia. Fatigue grade 1 and 2 was a common side effect.[66] Unfortunately, the limited phase II results were rather disappointing: no objective responses in pretreated metastatic melanoma and no improvement in the outcomes of patients with advanced NSCLC treated with erlotinib combined with entinostat when compared with erlotinib monotherapy.[67,68] However, in 2011 Juergens *et al*. published their phase I/II trial of combined epigenetic therapy with azacitidine, inhibitors of DNA methylation, and entinostat in extensively pretreated patients with recurrent metastatic NSCLC. This therapy was well tolerated and objective responses were observed, including a CR and a PR in a patient who remains alive and without disease progression approximately 2 years after completing protocol therapy. Median survival in the entire cohort was 6.4 months (95% CI: 3.8-9.2), comparing favorably with existing therapeutic options. Demethylation of a set of four epigenetically silenced genes known to be associated with lung cancer was detectable in serial blood samples in these patients and was associated with improved PFS ($P = 0.034$) and OS ($P = 0.035$). Four of 19 patients had major objective responses to subsequent anticancer therapies given immediately after epigenetic therapy.[69]

Valproic acid

Valproic acid (divalproex sodium; Depakote®) relieves repression of transcription factors that recruit HDACs and activates transcription from diverse promoters (Figure 6.2). Valproic acid causes hyperacetylation of the N-terminal tails of histones H3 and H4 *in vitro* and *in vivo* and it inhibits HDAC activity, most probably by binding to the catalytic center and thereby blocking substrate access.[70,71] In 2005, Chavez-Blanco *et al*. published their phase I study titled 'Histone acetylation and histone deacetylase activity of magnesium valproate in tumor and peripheral blood of patients with cervical cancer'. Twelve newly diagnosed patients with cervical cancer were treated with magnesium valproate after a baseline tumor biopsy and blood sampling at the following dose levels (four patients each): 20, 30, or 40 mg/kg for 5 days through the oral route. On day 6, tumor and blood sampling were repeated and the study protocol ended. Tumor acetylation of H3 and H4 histones and HDAC activity were evaluated by western blot and colorimetric HDAC assay, respectively. Plasma levels of valproic acid were determined on day 6 once the steady state was reached. Toxicity of treatment was evaluated at the end of the study period. All patients completed the study medication. Mean daily dose for all patients was 1890 mg. Corresponding means for the doses 20, 30, and 40 mg/kg were 1245, 2000, and 2425 mg, respectively. Depressed level of consciousness grade 2 was registered in nine patients. Ten patients were evaluated for

H3 and H4 acetylation and HDAC activity. After treatment, we observed hyperacetylation of H3 and H4 in the tumors of nine and seven patients, respectively, whereas six patients demonstrated hyperacetylation of both histones. Plasma levels of valproic acid ranged from 73.6 to 170.49 mg/ml. Tumor deacetylase activity decreased in eight patients (80%), whereas two had either no change or a mild increase. There was a statistically significant difference between pretreatment and post-treatment values of HDAC activity (mean, 0.36 versus 0.21, two-tailed *T*-test $P < 0.0264$). There was no correlation between H3 and H4 tumor hyperacetylation with plasma levels of valproic acid. It was concluded that magnesium valproate at a dose between 20 and 40 mg/kg inhibits deacetylase activity and hyperacetylates histones in tumor tissues.[72] Arce *et al*. demonstrated in their proof-of-principle study that treatment with hydralazine and magnesium valproate exerts its proposed molecular effects of DNA demethylation, HDAC inhibition, and gene reactivation in primary tumors of patients with breast cancer. Importantly, this doxorubicin-associated and cyclophosphamide-associated treatment was safe and well tolerated, and appeared to increase the efficacy of chemotherapy.[73] Several phase I studies of valproic acid alone or in combination with another agent were performed: valproic acid followed by the topoisomerase II inhibitor epirubicin in advanced solid tumors, alone in patients with refractory advanced cancer, in combination with 5-azacytidine in patients with advanced cancers, in combination with all-trans-retinoic acid intravenously in patients with advanced solid tumor malignancies, and in combination with 5-aza-20-deoxycytidine (decitabine) in patients with advanced-stage NSCLC.[74-78] Some phase II trials were also performed. Candelaria *et al*. conducted a phase II study in 17 patients who were evaluable for toxicity and 15 for response. Primary sites included were cervix (three), breast (three), lung (one), testis (one), and ovarian (seven) carcinomas. A clinical benefit was observed in 12 (80%) patients: four PR and eight SD. The most significant toxicity was hematologic. Reductions in global DNA methylation, HDAC activity, and promoter demethylation were observed.[79] The combination of valproic acid and chemoimmunotherapy did not produce results overtly superior to standard therapy in patients with advanced melanoma.[80] In combination with karenitecin, a topoisomerase I inhibitor, valproic acid was associated with disease stabilization in 47% of patients with metastatic poor prognosis melanoma.[81] Scherpereel *et al*. demonstrated that valproic acid plus doxorubicin appeared to be an effective chemotherapy regimen in good performance score (80-100) patients with refractory or recurrent mesothelioma, for which no standard therapy was available.[82] The pilot phase II study by Mohammed *et al*. showed that valproic acid may have a role in treating low-grade neuroendocrine carcinoma.[83] However, in 2011 Coronel *et al*. published their randomized phase III, placebo-controlled study of hydralazine and valproate (HV) added to cisplatin-

topotecan in advanced cervical cancer. This study represents the first randomized clinical trial to demonstrate a significant advantage in PFS for epigenetic therapy over one of the current standard combination chemotherapies in cervical cancer. Patients received hydralazine at 182 mg for rapid or 83 mg for slow acetylators, and valproate at 30 mg/kg, beginning a week before chemotherapy and continuing until disease progression. Response, toxicity, and PFS were evaluated, and 36 patients (17 cisplatin topotecan (CT) plus HV and 19 CT plus placebo (PLA)) were included. The median number of cycles was 6. There were four PRs to CT + HV and one in CT + PLA. There was SD in five (29%) and six (32%) patients, respectively, whereas eight (47%) and 12 (63%) showed progression (P = 0.27). At a median follow-up time of 7 months (1-22), the median PFS is 6 months for CT + PLA and 10 months for CT + HV (P = 0.0384, two tailed).[84]

Mocetinostat, chidamide, SB939, and LAQ824

Some other HDACi were only studied in single phase I studies, for example mocetinostat (MGCD0103), chidamide (CS055/HBI-8000), SB939, and LAQ824.[85-89] The recommended phase II dose of mocetinostat was 45 mg/m^2/day. DLTs consisting of fatigue, nausea, vomiting, anorexia, and dehydration were observed in three (27%) of 11 and two (67%) of three patients treated at the 45 and 56 mg/m^2/day dose levels, respectively.[85] With chidamide no DLTs were identified in the two times per week for 4 consecutive weeks every 6-week cohorts up to 50 mg. DLTs were grade 3 diarrhea and vomiting in two patients in the three times per week for 4 consecutive weeks every 6-week cohort at 50 mg, respectively.[86] In a phase I study by Yong *et al*. the MTD of SB939 was 80 mg/day. DLTs were fatigue, hypokalemia, troponin T elevation, and QT_c prolongation.[87] Razak *et al*. demonstrated that the recommended phase II dose of SB939 was 60 mg given for 5 consecutive days every 2 weeks. The most frequent non-hematologic AEs of at least possible attribution to SB939 were fatigue, nausea, vomiting, anorexia, and diarrhea.[88] DLTs of LAQ824 were transaminitis, fatigue, atrial fibrillation, raised serum creatinine, and hyperbilirubinemia. On the basis of these data in the phase I trial, De Bono *et al*. concluded that future efficacy trials with LAQ824 should evaluate doses ranging from 24 to 72 mg/m^2.[89]

DISCUSSION

Despite promising results in the treatment of CTCL, HDACi have generally not been effective in clinical trials involving solid tumors. Many clinical trials have assessed the efficacy of vorinostat against different solid tumors, including refractory breast, colorectal, non-small cell lung, and thyroid cancers. Disappointingly, almost none of the patients in these trials showed PR or CR to treatment, but the prevalence of drug-induced side effects was very high.[18,19] Romidepsin has also been evaluated as a monotherapy against solid tumors. Similarly to vorinostat, romidepsin has also been ineffective against solid tumors and also induced serious side effects. Before its approval by the FDA, there were six cases of unexpected death in patients treated with romidepsin, one attributed to pulmonary embolus and the other five cases attributed to sudden cardiac arrest.[90,91]

The same disappointing results were found with studies of belinostat, panobinostat, and entinostat in solid tumors. Valproic acid is the only HDACi that completed a phase III trial in solid tumors, which demonstrated a significant advantage in PFS over one of the current standard combination chemotherapies in cervical cancer; however, the results were preliminary and should be taken as such. Current published studies indicate that so far HDACi have serious limitations, including ineffectively low concentrations in solid tumors and cardiac toxicity, including T-wave flattening, ST segment depression, and QT interval prolongation, which is hindering their progress in the clinic.[92] Although it is not completely understood why HDACi seem more effective in hematological malignancies than in solid tumors, it is suggested that in hematological malignancies, such as CTCL and multiple myeloma, the short PK half-life of HDACi compounds may not preclude their effectiveness, compared with less permeable solid tumors, in which their instability is a problem.[57] It is also possible that HDACi are not selective enough for solid tumors, which means that they are not target specific and are not delivered selectively. An interesting question is whether HDAC expression in a given tumor might predict the therapeutic response to HDACi. As in other targeted therapies, it is probable that treatment response is greater in those patients who strongly express HDACs in their cancer cells. Translational studies including this topic should be attached to clinical trials on HDACi to find adequate biomarkers for the future. The hope of up-and-coming cancer treatments of all kinds is to deliver high potency at the site of action, while eliminating the toxicities that result from off-target effects. Gryder *et al*. recently suggested that designing and developing HDACi with extremely high potency and selectivity for a unique molecular entity and not others and directing the medicine to the location of interest would help to overcome the problems

Table 6.1 Open clinical trials (with histone deacetylase inhibitors in solid tumors) recruiting patients

Title	Phase	ClinicalTrials.gov Identifier
Safety and tolerability study of RAD001 and LBH589 in all solid tumors with enrichment for EBV driven tumors	1	NCT01341834
Belinostat for solid tumors and lymphomas in patients with varying degrees of hepatic dysfunction	1	NCT01273155
Azacitidine and MS-275 in treating patients with recurrent advanced non-small cell lung cancer	1/2	NCT00387465
High-dose vorinostat and fractionated stereotactic body radiation therapy in treating patients with recurrent glioma	1	NCT01378481
A phase I study of belinostat in combination with cisplatin and etoposide in adults with small cell lung carcinoma and other advanced cancers	1	NCT00926640
Vorinostat in children	1/2	NCT01422499
High-dose or low-dose vorinostat in combination with carboplatin or paclitaxel in treating patients with advanced solid tumors	1	NCT01281176
Clinical study of vorinostat in combination with etoposide in pediatric patients < 21 years at diagnosis with refractory solid tumors	1/2	NCT01294670
Vorinostat and lapatinib in advanced solid tumors and advanced breast cancer to evaluate response and biomarkers	2	NCT01118975
Adjuvant valproate for high grade sarcomas	1	NCT01010958
Sorafenib and LBH589 in hepatocellular carcinoma (HCC)	1	NCT00823290
Study to evaluate panobinostat (DACi) pharmacokinetics and safety in solid tumors and varying renal function	1	NCT00997399

of HDACi in solid tumors.[92] While searching for 'HDAC inhibitor solid tumor' we found only 12 open clinical trials on http://www.clinicaltrials.gov recruiting patients (Table 6.1). But to fulfill the high expectations in solid tumors and to overcome the existing problems, a great deal of research is still necessary.

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