Review: Clinical Trial Outcomes

CLINICAL INVESTIGATION

Histone deacetylase inhibitors: recent outcomes from clinical trials and the implications for oncology treatment approaches

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Histone deacetylases play an important role in multiple processes, including gene expression, proliferation, apoptosis, cytoskeletal organization, migration and angiogenesis. Histone deacetylase inhibitors are able to induce cell death and growth arrest as targeted anticancer agents. Whilst only two, vorinostat and romidepsin, are licensed in oncology, several have reached Phase III trials and many more are in Phase I and II. In addition to this, multiple novel drugs, including more targeted agents, are emerging from preclinical studies. This paper examines the outcomes of recent clinical trials in 11 key histone deacetylase inhibitors, both as monotherapy and in combination with other antitumor drugs. An overview of the advantages and disadvantages between the different classes and individual drugs is discussed, as well as a brief outlook on the future developments in the field.

Keywords: antineoplastic agents • clinical trials • epigenetics • histone deacetylases • histone deacetylase inhibitors • neoplasms • oncology

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The histone deacetylases (HDACs) are a diverse family of proteins that have been gaining interest of late through emerging evidence of their role in cancer pathogenesis.

DNA in eukaryotes is packaged into chromatin, which is made up of the combination of DNA and the histone proteins. Acetylation by histone acetyltransferases (HATs) and deacetylation by HDACs activates and represses chromatin, respectively [1]. As such, they play an important role in gene transcription, chromatin formation, DNA repair and replication [2]. More recently, a large number of additional nonhistone substrates to HDACs have been identified as key contributors to the antitumor activity of the histone deacetylase inhibitors (HDIs) (Figure 1).

Currently, 18 HDACs have been identified in humans and are classified into four classes based on their similarity to yeast proteins (Table 1) [3]. Unlike the 'classic' HDACs, class III HDACs or 'sirtuins' do not contain zinc at their functional sight and are NAD+ dependant. Thus they are often considered separately to the other HDAC classes and will not be covered within this paper [1,3,4].

HDIs can be divided into several chemical classes: the hydroxamic acids, aliphatic acids, cyclic peptides and benzamides. Most are 'pan-inhibitors' inhibiting both class I and II HDACs (and variably intravenously [iv.]) There is an emerging interest in creating HDAC-specific inhibitors, such as ACY-1215, a selective inhibitor of HDAC6, with the aim of improving targeting and reducing toxicities.

The HDIs are able to induce growth arrest, cell death and terminal differentiation in transformed cancer cells through many different mechanisms, whilst normal cells remain highly resistant to these changes. Whether cytostasis or cytotoxicity occurs depends on the drug and the dose used (Figure 2) [3,4].

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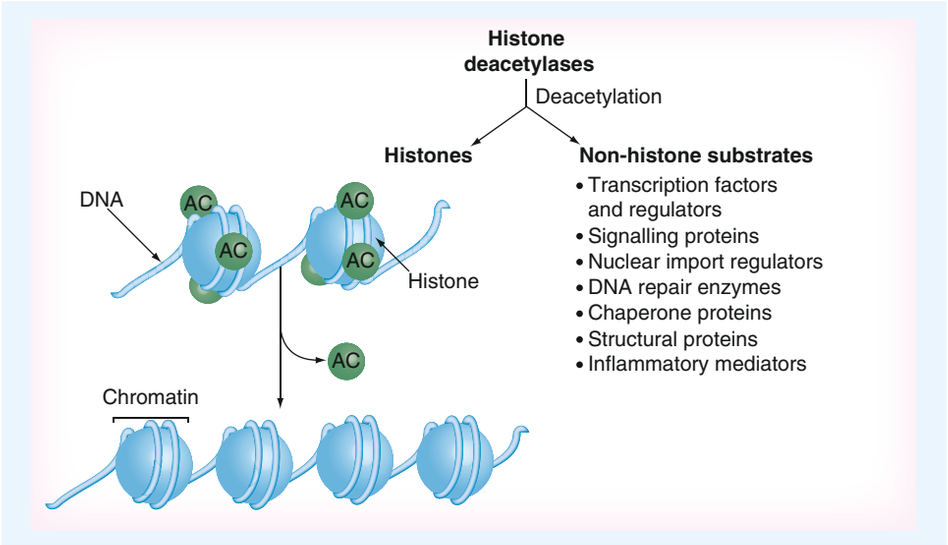


Figure 1. Histone deacetylases cause deacetylation of many substrates. Deacetylation of histones within chromatin causes deactivation and, thus, gene silencing.
AC: Acetyl group.

The first non-short-chain fatty acid HDI to be discovered was trichostatin A in the early 1990s. Since then there have been multiple novel HDIs descovered and several licensed drugs used in other conditions have been found to have HDI capabilities. Currently, the only licensed HDIs used in cancer chemotherapy are vorinostat and romidepsin. However, the HDIs are generating considerable interest in cancer therapy and further drug developments are on the horizon. This review will examine the outcomes of clinical trials for the main HDIs in cancer chemotherapy thus far (Table 2) [4].

Table 1. The classical histone deacetylases.

HDAC	Position in the cell
Class I	
HDAC 1	Nucleus
HDAC 2	Nucleus
HDAC 3	Nucleus
HDAC 8	Nucleus
Class IIa	
HDAC 4	Nucleus and cytoplasm
HDAC5	Nucleus and cytoplasm
HDAC 7	Nucleus and cytoplasm
HDAC 9	Nucleus and cytoplasm
Class IIb	
HDAC 6	Cytoplasm
HDAC 10	Cytoplasm
Class IV	
HDAC 11	Nucleus and cytoplasm

Class III 'sirtuins' are not considered here.
HDAC: Histone deacetylase.

Hydroxamic acid derivatives

Vorinostat

Vorinostat, a hydroxamic acid-based compound, also known as suberoylanilide hydroxamic acid or MK0683, was the first HDI to be US FDA approved in 2006, for the treatment of progressive, persistent or recurrent cutaneous manifestations of cutaneous T-cell lymphoma (CTCL) following two systemic therapies [5]. For this application, it has an FDA-approved oral dose of 400 mg/day. In addition to its use in CTCL, vorinostat has shown *in vitro* and *in vivo* action in other hematological and solid tumors. It has also demonstrated a synergistic effect with several other chemotherapeutic agents, including bortezomib, 5-fluorouracil and platinum-based compounds, and a sensitizing effect to radiotherapy [6].

Hematological malignancy

Following the FDA approval of vorinostat, Dummer *et al.* examined the use of vorinostat combined with another approved treatment for CTCL, bexarotene, in 23 patients with CTCL with the aim of identifying a maximum tolerated dose (MTD) [7]. Ten patients experienced a serious adverse event with three requiring vorinostat dose reduction and two requiring a bexarotene reduction. Four patients experienced an objective response and seven gained symptomatic relief from pruritus. It was concluded that this combination is only feasible if both drugs are given at a lower dose than recommended for monotherapy [7].

Vorinostat monotherapy has been investigated in other non-Hodgkin lymphomas including follicular lymphoma, mantle zone lymphoma or mantle cell lymphoma. A Phase I trial observed an overall response rate (ORR) of 40% (three complete responses [CR] and one partial response [PR]) in ten patients [8], whilst a later Phase II trial in 35 patients, had an ORR of 29% (five CR and five PR) [9]. Both trials recommended further work in follicular lymphoma with inconsistent results in mantle cell lymphoma and mantle zone lymphoma. A Phase II study, in 25 heavily pretreated relapsed and/or refractory Hodgkin's lymphoma patients, found limited single-agent activity

and the second stage was not initiated due to the lack of objective response [10].

In myeloma, vorinostat demonstrated moderate single-agent activity agent efficacy in a ten-patient Phase I trial with one minimal response and nine stable disease (SDs) [11]. Further trials have focused on the efficacy of vorinostat in combination with an established myeloma drug, bortezomib, a proteasome inhibitor. Two Phase I/II trials examining this combination have observed antimyeloma activity. Weber *et al.* recorded a 33% ORR in 34 patients (nine PR, two minimal responses [MR]) with 59% SD [12], whilst Badros *et al.* saw 42% ORR amongst 21 patients (11 PR) and 52% SD [13]. Both studies saw responses in Bortezomib refractory patients with Weber *et al.* finding that response rates between the bortezomib resistant and bortezomib naive patients were similar. The open-label, single-arm Vantage 095 (Phase IIB) trial in 143 Bortezomib refractory patients observed an ORR of 11% according to the European Group for Blood and Marrow Transplant criteria with a median response duration of 6.3 months. The Vantage 088 trial is a Phase III double-blinded, randomized, controlled trial that randomized 637 patients to vorinostat/bortezomib and placebo/bortezomib arms. In terms of the primary end point – progression free survival (PFS) – there was a 23% increase in time to progression (hazard ratio = 0.774; p = 0.01) in the bortezomib/vorinostat group; however, this equated to an increase of 25 days and median survival did not increase. However, there was a significant increase in the European Group for Blood and Marrow Transplant measured ORR of 56% compared with 41% in the bortezomib monotherapy arm [301,302].

The first investigation into the use of vorinostat in leukemia was a Phase I trial that determined an MTD of 200 mg twice daily (b.i.d.) or 250 mg thrice daily, with good biological activity demonstrated by increased histone acetylating at all doses. Of 41 participants, a total of 31 had acute myeloid leukemia (AML) and the seven hematological improvements or responses observed were in these patients treated at or below the MTD [14]. However, a Phase II trial in AML found minimal effect (one response in 37 patients) [15]. Vorinostat and gemtuzumab ozogamicin, also in AML, had moderate efficacy although its activity was confined to those with normal karyotype disease (NPM1-positive/FLT3-ITD-negative normal) with a response rate of 46.2% in this group (six out of 13) [16]. Examination of a vorinostat/idarubicin combination in advanced leukemia was found to be well tolerated and feasible, with a 17% (seven out of 41) clinical response rate [17]. Garcia-Manero *et al.* added cytarabine to this combination in 75 patients with AML and myelodysplastic syndromes (MDS), also demonstrating the combination to be safe and active, with an ORR of 85% (76 and 9% CR with incomplete platelet response, respectively) [18].

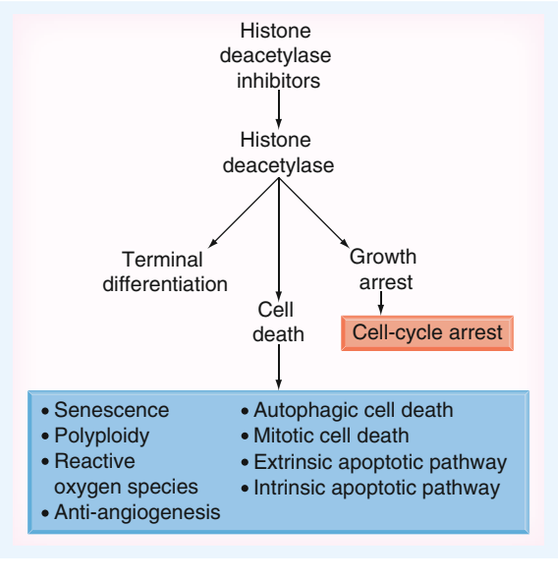


Figure 2. Histone deacetylase inhibitors exert an antitumor effect through multiple pathways.

Solid tumors

Several studies have investigated vorinostat's single-agent activity in a range of advanced solid tumors, demonstrating only moderate clinical benefit, with SD being the most frequent positive outcome (21–50%) [19–22]. The most frequently recommended dose was 200 mg b.i.d. for 14 days, followed by 7 rest days. iv. vorinostat has been investigated and found to be well tolerated at doses of up to 900 mg/m^2/day in solid tumors, but with a MTD of 300 mg/m^2/day with hematological malignancies [23, 24]. The most common grade 3/4 toxicities were thrombocytopenia, with other milder effects including anorexia, fatigue, diarrhea, nausea and anemia. Vorinostat has also been investigated in combination with several other drugs at Phase I (Table 3).

■ Vorinostat combination therapy in nonspecified solid tumors

Brain tumors

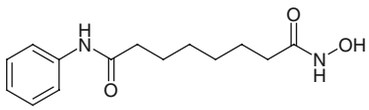
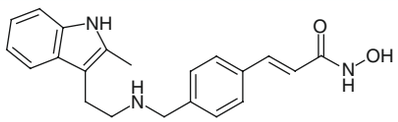
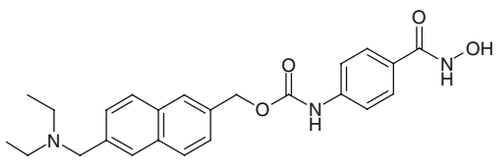
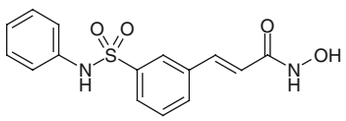
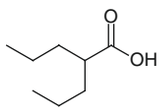
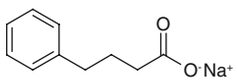
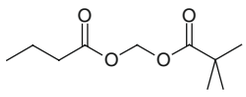
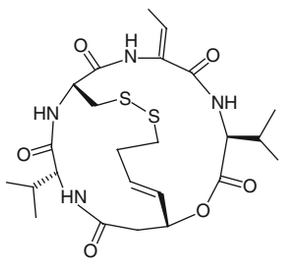
Vorinostat was tested as a single-agent against recurrent glioblastoma in a Phase II trial, with nine of 52 patients progression free at 6 months [34]. Three combination regimens have been assessed. A vorinostat/temozolomide combination was trialed in patients with high-grade glioma and found to be well tolerated with no pharmacokinetic interactions between the two drugs. This combination has now moved to Phase I/

II, with the addition of radiotherapy [35]. The combination of vorinostat and two established glioblastoma drugs, bevacizumab and irinotecan, had positive results, with a median PFS of 3.6 months and overall survival of 7.3 months. However, the combination was poorly tolerated, mostly due to toxicities associated with irinotecan [36]. A Phase II trial investigating the combination of bortezomib and vorinostat was terminated as 0 of the 34 patients exchibited PFS [37].

Non-small-cell lung cancer

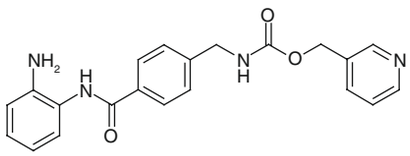
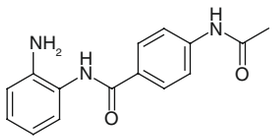
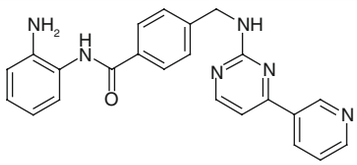
Traynor *et al.* did not observe any objective antitumor activity with single-agent vorinostat in 16 patients with non-small-cell lung cancer (NSCLC) [38]. A Phase I trial investigating a synergistic relationship between vorinostat, paclitaxel and carboplatin observed a 53% ORR [25] and Phase II randomized controlled trial (RCT) in 94 patients the combination had 34% CR rate compared with 12.5% in the placebo/carboplatin/paclitaxel

Table 2. The main histone deacetylase inhibitors in clinical development: structure and phase of development.

Histone deacetylase inhibitors	Structure	Stage in clinical trials	Ref.
Hydroxamic acid derivatives			
Vorinostat/suberolyanilide hydroxamic acid		Licensed for cutaneous T-cell lymphoma	[5]
Panobinostat		Phase II/III (awaiting results)	[57,60–62,64, 65,67,68,303]
Givinostat		Phase I (published) Phase II (underway)	[81,82,303]
Belinostat		Phase II	[92–94,96,304]
Aliphatic acids			
VPA/Valproate		Phase III	[105,135,136]
Sodium phenyl butyrate		Phase I	[137–145]
Pivanex/AN-9		Phase II	[147,149]
Cyclic peptides			
Romidepsin		Licensed for cutaneous T-cell lymphoma	[150–152]

VPA: Valproic acid.

Table 2. The main histone deacetylase inhibitors in clinical development: structure and phase of development (cont.).

Histone deacetylase inhibitors	Structure	Stage in clinical trials	Ref.
Benzamides			
MS-275/entinostat		Phase II	[174,175,178]
Ci-994		Phase II	[183–185]
MGCD0103/mocetinostat		Phase II	[187,188,191]

VPA: Valproic acid.

group (p = 0.02) with nonsignificant trends towards improved PFS and overall survival in the vorinostat group [39]. This combination was tested again in a placebo controlled Phase II/III trial, but was terminated early when interim analysis revealed the end point was not being achieved – overall survival was 11 months in the vorinostat group and 14 months in the placebo group [303]. The combination of bortezomib and vorinostat as induction therapy prior to surgery demonstrated inconsistent biological activity but had a good effect on intratumoral gene changes. Six of 20 patients had more than 60% histological tumor necrosis after surgery and the combination was thought to be feasible [40].

Malignant mesothelioma

The Vantage 014 trial, a Phase III double-blinded RCT in 660 patients, showed that vorinostat did not significantly increase survival, with a median survival of 31 weeks for patients on vorinostat and 27 weeks for those on the placebo (hazard ratio: 0.98; p = 0.858). There was a statistically significant improvement in median PFS in the Vorinostat group, however, this equated to an increase of 0.2 weeks [41].

Breast cancer

The earliest study examining vorinostat as a single agent in metastatic breast cancer was terminated due to a lack of CRs or PRs. However, four of 14 patients received clinical benefit in the form of disease stabilization, and vorinostat was well tolerated [42]. Two studies have investigated vorinostat as a combination therapy. Combination with paclitaxel and bevacizumab had moderate results as a first-line therapy for metastatic breast cancer. The primary objective was to detect an increase in the response rate from 40 to 60%. Of 54 patients with no previous chemotherapy, 55% demonstrated objective responses (24 out of 44) [43].

Following preclinical demonstration that Vorinostat can reverse aromatize inhibition and tamoxifen resistance in hormone receptor positive breast cancer, the combination of vorinostat and tamoxifen was examined in a Phase II trial of 43 patients, 98% of whom had progressed after treatment with one aromatize inhibitor and 58% had previously received tamoxifen adjunctive treatment. ORR measured by the Response Evaluation Criteria In Solid Tumors criteria was 19%, with an overall clinical benefit (response or SD >24 weeks) of 40% [44]. Recently, a Phase I/II trial examining the combination of vorinostat with trastzumab was terminated in its second stage due a low Response Evaluation Criteria In Solid Tumors (RESIST) defined response rate after four cycles [303]. There are currently 13 known active trials investigating vorinostat in breast cancer, in a multitude of stages and combinations.

Colorectal cancer

Preclinical research has shown that Vorinostat is synergistic with fluorouracil, through the down regulation of thymidylate synthase. The Phase I part of Fakih *et al.*'s work with this combination and leucovorin in colorectal cancer (CRC) established an MTD, and 21 of the 43 patients demonstrated a response: 20 SDs and one PR [45]. However, in the Phase II trial both high-and low-dose arms failed to meet the primary efficacy end point

Table 3. Phase I trials of combination therapies.

Treatment	Dose	Patients (n)	Results	Ref.
Carboplatin and Paclitaxel	Vorinostat 400 mg q.d. or 300 mg b.i.d. Carboplatin iv. paclitaxel every 3 weeks	28	Well tolerated with good antitumor activity particularly in NSCLC	[25]
Decitabine	Recommended Phase II dose: Sequential schedule of vorinostat 200 mg b.i.d. on days 6–12 decitabine 10 mg/m^2/day on days 1–5	43	Combination was well tolerated and demonstrated antitumor activity (DS in 29%)	[26]
Marizomib	300 mg vorinostat daily for 16 days in 28 day cycles Marizomib weekly	22	Well tolerated with good antitumor activity (61% stable disease, 39% experienced a decrease in tumor measurement)	[27]
Doxorubicin	MTD: 800 mg/day vorinostat days 1–3 Doxorubicin (20 mg/m^2) on day 3 for 3 of 4 weeks	32	Tolerated: two PR and two DS >6 months	[28]
Radiotherapy	MTD: 300 mg q.d. vorinostat with short-term palliative pelvic radiotherapy (30 Gy in 3 Gy daily fractions over 2 weeks)	16	Safe combination Tumor volume change ranged from 54% reduction to 28% increase (mean 26% reduction)	[29]
Vinorelbine	MDT: 200 mg vorinostat for 7 days 25 mg/m^2 weekly vinorelbine	7	Unexpectedly high mean vorinostat plasma AUC, possibly causing two cases of Grade 3 hyperglycemia No vinorelbine/vorinostat interaction was detected	[30]
Sorafenib	Vorinostat 300 mg daily on days 1–14 of a 21-day cycle. Sorafenib 400 mg PO b.i.d.	17	Poorly tolerated in RCC and NSCLC groups but tolerable in other tumor types. Two unconfirmed PR and five MR	[31]
Docetaxel	Four dose levels: 100, 100, 200 and 200 mg vorinostat and 50, 60, 60, and 75 mg/m^2 docetaxel, respectively	12	Poorly tolerated. Terminated due to DLT. No responses detected	[32]
Flavopiridol with intermittent oral pulse-dose schedule	21-day schedule MTD: vorinostat 600 mg/day Flavopiridol 60 mg/m^2 bolus 28-day schedule MTD: vorinostat 800 mg/day Flavopiridol 30 mg/m^2 over 30 min and 30 mg/m^2 over 4 h	34	Higher serum vorinostat concentrations achieved than reported with oral dosing. Eight patients had DS (average 5.5 months)	[33]

b.i.d.: Twice per day; DLT: Dose-limiting toxicities; DS: Disease stabilization; iv.: Intravenously; MR: Minimal response; MTD: Maximum-tolerated dose; NSCLC: Non-small-cell lung cancer; PO: By mouth; PR: Partial response; q.d.: Once per day; RCC: Renal-cell carcinoma.

of 2 months PFS for 27 out of 43 patients. The authors felt that these results did not recommend the unselective use of a vorinostat and 5-fluorouracil combination [46]. Another Phase II/III trial examined this combination (with vorinostat given on 6 consecutive days) in the same group of patients, using elevated intratumoral thymidylate synthase as a possible marker of success. However, this study did not establish an MTD as dose-limiting toxicities were found at all levels and biological activity was inconsistent [47]. The use of vorinostat in CRC is currently not an active area of research.

Other areas

Vorinostat has been investigated in pediatric oncology. Altered doses of vorinostat alone are well tolerated in children [48,49]. Combinations with both 13-cis retinoic acid and bortezomib were found to be well tolerated in solid tumors [48,50].

Vorinostat has undergone unsuccessful trials as a single agent for persistent or recurrent epithelial ovarian cancer, primary peritoneal carcinoma [51] and recurrent/metastatic head and neck cancers [52]. However, both studies suggested that further research using combination therapies was warranted. A Phase II study investigating vorinostat in castration resistant prostate cancer (CRPC), which found that a high rate of toxicities impeded efficacy assessment. However, it did uncover an interesting association between high IL-6 levels and withdrawal from the trial for toxicity [53]. Vorinostat treatment, assessed in two dosing groups in

GI carcinoma, achieved SD for over 8 weeks in both groups [54].

Summary

In both solid and hematological malignancy, vorinostat appears to have promise in early-phase trials, which does not translate into efficacy at later phases. However, it is becoming clear that its potential lies in combination with other established drugs. Combination therapies have largely been shown to be tolerable with reasonable efficacy and the sensitizing effect of vorinostat on radiotherapy is also an interesting area that is currently under investigation.

Panobinostat

Panobinostat (LBH589) is a hydroxamic acid and pan-HDAC inhibitor. Despite *in vitro* indications that panobinostat has greater potency than vorinostat (tenfold) and other HDIs [55], there has not yet been sufficient evidence for its approval for any clinical application. It is an area of extensive exploration, particularly in hematological malignancies, and the results of several large, multicentertrials are expected in the next few years.

Hematological malignancies

A Phase I trial in refractory hematological malignancies found that iv. panobinostat given as a <11.5 mg/m^2 daily infusion on days 1–7 of a 21-day cycle was well tolerated and had transient antileukemic effect [56]. A Phase II nonrandomized trial to examine the use of oral panobinostat in low or intermediate risk MDS, observed limited clinical efficacy and single-agent panobinostat did not consistently induce histone acetylating. However, at an oral dose of 20 mg, three-times weekly (1 week rest) the safety profile was favorable [57]. Two trials examining the combination of panobinostat with either cytarabine and mitoxatrone [58] or 5-azacitidine (5-AZA) [59] have demonstrated tolerable toxicity profiles, with both combinations now entering an expansion phase.

The first Phase Ia/II trial examining panobinostat in Hodgkin's lymphoma demonstrated a reduction in metabolic activity (seven out of 12 patients) as assessed by positron emission tomography and a computerized tomography PR (>50% reduction of sum of product diameter) was seen in 38% (five out of 12 patients) [60]. A large multicenter Phase II trial assessing panobinostat (40 mg, three-times a week) in patients with relapsed or refractory Hodgkin's lymphoma after autologous stem cell transplant showed good antitumor activity. In 129 heavily pretreated patients, tumor reduction was recorded in 74% of patients and the ORR was 27% (PR 23% and CR 4%). The safety profile was acceptable with manageable toxicities [61]. A combination of everolimus and panobinostat has also been investigated at the Phase I stage. Data suggests that this is safe combination and tumor reduction was seen in 20 of 28 patients, with a 50% ORR. This study has progressed on to its Phase II stage [62].

Following the success of vorinostat and romidepsin, panobinostat is also being investigated as a therapy for CTCL. Preclinical, Phase I and II trials have demonstrated a response to panobinostat [63, 64] and there are currently two Phase III trials in progress. As with vorinostat and romidepsin, a potential combination is with bexarotene. Panobinostat and bexarotene have demonstrated activity with a manageable safety profile in a Phase II trial in 139 patients (79 bexarotene resistant and 60 bexarotene naive), with an ORR of 17.3% (15.2% in bexarotene-exposed and 20.0% in bexarotene-naive patients) [65].

Following preclinical evidence of potent antimyeloma activity, several combination therapies have been investigated [66]. Preliminary data from the PANORAMA 1 trial, a multicenter RCT assessing panobinostat with bortezomib and dexamethasone in 672 patients, suggests that the combination is safe, with a similar safety profile to that of dexamethasone and bortezomib alone [67]. PANORAMA 2 is investigating the same combination in relapsed multiple myeloma (MM) and bortezomib refractory MM patients. Early results show that of 55 patients, 18 achieved ≥PR and 13 MR. This demonstrates evidence that this combination can recapture responses in bortezomib refractory patients [68]. Another combination has focused on the akylating agent melphalan. Early results from one trial have observed, with an ORR of 16% [69] and a recently published study examining the combination of panobinostat and mephalan, with the addition of thalidomide and prednisone, showed ≤PR in 38.5% [70].

Solid tumors

Preclinical work has demonstrated that panobinostat has antitumor activity in a number of solid tumor cell lines; however, this has only translated into clinical trials in a few areas.

Two trials have examined panobinostat as a single-agent therapy and provide two possible well-tolerated regimes of 20 mg three-times a week [71] or 20 mg/m^2 on days 1 and 8 of a 21-day cycle iv. [72]. In both monotherapy trials, the best response was SD (five of 11 and six of 14 patients, respectively). The most frequent dose-limiting toxicities were myelosuppression with nausea, vomiting and fatigue as other common toxicities. Panobinostat has also been investigated as combination treatment in groups of heterogenous solid tumour types (Table 4).

A Phase I, two-armed study investigating panobinostat alone and in combination with docetaxel and

Table 4. Phase I trials of combination therapies.

Treatment	Dose	Patients (n)	Results	Ref.
Gemcitabine	10 mg three times weekly for 1 week Gemcitabine 800 mg/m^2 on days 1 and 8 every 21 days	17	DLTs occurred at all dose levels. The potential of this combination is limited by myelosuppression. One unconfirmed PR and five DS lasting longer than four cycles	[73]
Paclitaxel and Carboplatin	Recommended Phase II dose: 10 mg three-times weekly for 1 week. Paclitaxel 175 mg/m^2 and carboplatin AUC 5 administered intravenously on day 1 of every 21-day cycle	21	Two-thirds of patients experienced myelosupression, three PR and 11 DS	[74]
Bevacizumab and Everolimus	10 mg panobinostat three-times weekly, 5 or 10 mg everolimus q.d., and 10 mg/kg bevacizumab every 2 weeks	12	Unacceptable safety and tolerability profile at the lowest dose of 10 mg of panobinostat three times weekly, 5 mg everolimus daily, and bevacizumab at 10 mg/kg every 2 weeks	[75]

DLT: Dose-limiting toxicities; DS: Disease stabilization; PR: Partial response; q.d.: Once per day.

prednisone in CRPC, found that panobinostat had an acceptable safety profile at doses that inhibited HDAC activity. There was no objective response in the singe-agent panobinostat arm, and a more rapid rise in PSA post-therapy than pretherapy was seen. In the docetaxel arm, five of eight patients had a ≥50% reduction in PSA and two patients achieved a PR by RESIST criteria [76]. Rathkopf *et al.* suggested that further investigation into the use of panobinostat in CRPC should focus on iv. formulations due to its ability to produce a higher peak concentration [76].

A Phase I trial examining panobinostat in combination with bevacizumab for the treatment of recurrent high-grade glioma observed three PR and seven SD amongst 12 patients. A dose of 30 mg, three-times a week every other week, with bevacizumab 10 mg/kg every other week was recommended and this combination has now progressed to a Phase II trial [77].

Despite the evidence of a synergistic interaction between bortezomib and panobinostat, a Phase II trial examining this combination in advanced pancreatic cancer, was suspended due to lack of treatment response and early treatment-related toxicity [78]. Panobinostat as a single agent in refractory renal-cell carcinoma (RCC) demonstrated a similar lack of response [79].

Summary

Panobinostat has had encouraging results in hematological malignancies, particularly in lymphoma, MM and CTCL. It has been active in heavily pretreated populations and in recapturing responses to bortezomib in MM patients previously refractory to it. It is currently registered for 52 trials in hematological malignancy so a more definitive role may be established in the next few years. Positive preclinical results have been achieved in solid tumors but this has not yet translated to clinical efficacy with only a modest response seen in high-grade glioma and CRPC. However, the use of panobinostat is still a current area of investigation, with multiple trials currently examining combination treatments in generic and specific solid tumors.

■ Givinostat

Whilst a number of preclinical trials have demonstrated that Givinostat (ITF2357) shows antileukemic activity, most clinical work has focused on its anti-inflammatory action [80]. It has been granted orphan drug status in the EU for the treatment of systemic juvenile idiopathic arthritis and polycythemia vera. Its safety and efficacy in oncology has been assessed in two trials to date. Rambaldi *et al.* investigated givinostat in JAK2V617F positive chronic myeloproliferative neoplasms, using a dose of 50 mg b.i.d. for 24 weeks. It was well tolerated with one CR and six PR amongst the polycythemia vera and essential thrombocytopenia patients, and amongst the myelofibrosis patients there were three major responses. Many patients had symptomatic improvement, including reduction of pruritus and splenomegaly [81]. Galli *et al.* investigated a higher dose regimen in 19 patients with MM, concluding an MTD of 100 mg b.i.d. [82]. Givinostat demonstrated modest activity and was tolerable alone or in combination with dexamethasone [82]. Givinostat is currently in several Phase II trials for a range of hematological malignancies.

■ Belinostat

Belinostat (PXD101) is another hydroxamic acid derived pan-HDAC inhibitor. It has been given orphan

drug status by the FDA and European Commission for the treatment of peripheral T-cell lymphoma (PTCL). On the basis of the results of the multicenter BELIEF trial, which examined belinostat safety and efficacy in 129 patients with PTCL, and achieved an ORR of over 20%, it is expected that a New Drug Application will be filed with the FDA this year [304].

Two parallel Phase I studies were carried out in 2008, investigating the safety and pharmacokinetics of belinostat in solid and hematological malignancy. The MTD was 1000 mg/m^2/day on days 1–5 in a 21-day cycle. Common toxicities are nausea, diarrhoea, fatigue and flushing. Grade3/4 adverse events included atrial fibrillation and hematological changes including lymphopenia and thrombocytopenia. QTc elongation was thought to be a problem but QTcF increase > 60 ms above baseline was not observed in a study examining this issue [83]. Five of 16 patients with hematological malignancies [84] and 18 out of 46 with solid tumors achieved disease stabilisation [85]. Belinostat was considered to be well tolerated and demonstrated dose dependent effects.

Two combinations have been investigated in advanced solid tumors. 1000 mg/m^2/day belinostat in combination with carboplatin and paclitaxel or 750 mg/m^2/day fluorouracil is well tolerated and clinically active [86,87]. An oral formulation of 250 mg every day, four-times per day and 250 mg b.i.d. is also well tolerated, although further information on pharmacokinetics and activity is yet to be published [88].

Previous studies have examined a single-agent role in B-cell lymphoma and MDS, both closed due to failure to meet primary outcome goals [89,90]. Some clinical activity has been observed with a combination of belinostat with 5-AZA in advanced myeloid malignancies. Of 23 enrolled patients, there were two CR, one PR and four patients with hematological improvement [91].

Three Phase II trials have investigated the use of belinostat in ovarian tumors. Mackay *et al.* observed moderate activity in 11 evaluable patients with micropapillary/borderline ovarian tumors, with one unconfirmed PR and ten SD [92]. In platinum resistant epithelial ovarian tumors, nine of 18 patients achieved SD as best response. The limited activity in epithelial ovarian tumors was also observed by Dizon *et al.*, who tested a belinostat/carboplatin combination, resulting in an ORR of 7.4%, with 12 SD amongst 27 evaluable participants [93]. The study was closed early due to lack of drug activity. When paclitaxel was added to the combination an ORR of 43% was observed, with three CR and 12 PR and, when stratified, demonstrated an ORR of 44% amongst those who were platinum resistant [94].

Two studies have examined the use of belinostat in thymic malignancies. As a single agent it was found that belinostat was active in those with thymoma, with no responses amongst patients with thymic cancer [95]. A Phase I/II trial investigating belinostat combined with cisplatin, doxorubicin and cyclophosphamide in 13 patients observed an ORR of 54% including 33% PR (two out of six) amongst the thymic carcinoma group. This study has now progressed to Phase II [96].

Belinostat monotherapy has undergone isolated trials. One, in mesothelioma, demonstrated no significant activity [97]. Another, in unresectable hepatocellular carcinoma resulted in a 2.4% PR and 45.2% SD [98].

Summary

FDA approval for belinostat appears to be underway for the treatment of PTCL. The use of belinostat in on-going clinical trials appears to be focussed on its use in PTCL and CTCL. However, this success has not been replicated in other areas. It has not yet been tested for the treatment of a wide range of tumor types and combination with the right drugs, is a challenge for the future. A number of trials are currently recruiting, with the hope that its efficacy will be better defined within the next few years.

Aliphatic acids

The aliphatic acids are week HDIs compared with the other classes. Three drugs have entered clinical trials. Valproic acid (VPA) and phenyl butyrate have been previously licensed in nononcologic conditions but have recently been discovered as having HDI capability. AN-9 or pivanex is a novel agent [3]. VPA and pivanex have entered Phase II trials and Phase III trials are underway in VPA.

VPA

VPA is a primarily oral agent, first discovered by chance in the 1960s as an anti-epileptic, which remains its primary role [99]. It has since become well established in several neurological conditions including migraines, bipolar disorder, and schizophrenia [100]. VPA teratogenic studies revealed potential antitumor activity and further studies revealed it to be a potent pan-HDI. In particular, preclinical studies highlighted VPA as being potentially beneficial in several forms of chemoresistant malignancies, including refractory leukemias and androgen resistant prostate cancer [101].

Phase I and II studies have primarily focused on advanced cancers, often persistent, relapsing and those resistant to chemotherapies [101,102]. Generally VPA is well tolerated, the most serious toxicities being neurocognitive; for example, fatigue, delirium, dizziness and vertigo, and so forth, with other common toxicities of mild GI symptoms such as diarrhea, nausea and vomiting and some myelosuppression [103–105].

Hematological malignancies

VPA has been shown *in vitro* to induce differentiation and apoptosis in several hematological cell lines [102] and several studies in leukemia and myelodysplastic syndromes have been performed. There is particular interest in combination therapies with other nontoxic epigenetic agents such as all-trans retinoic acid (ATRA), which has been shown to significantly improve differentiation and apoptosis of myeloid cells *in vitro* and *in vivo*. There is also considerable interest in 5-AZA and its deoxy derivative decitabine, both DNA methyltransferase inhibitors [102,106].

The first trial in 2004 in 18, mostly low grade AML and MDS patients, using VPA monotherapy at doses used for seizure control (median dose 1250 mg) showed interesting results. The drug was well tolerated with only one withdrawal for neurotoxicity. ORR was 40% with one PR, two minor and five major hematological improvements and four SDs. Improved response rate was observed in lower-risk prognostic groups. Subsequent addition of ATRA in four patients produced two new responses suggesting some sensitisation effect by VPA [107].

Four studies have assessed the addition of ATRA to VPA but have not shown any major improvements with this combination. One in 11 older poor risk patients showed a good response rate of 30% but showed high rates of neurotoxicity [108]. Other studies have not reflected this though. Two small studies showed ORR of 10 and 5%, respectively [109,110].

A third larger trial showed 44% ORR but found that this varied considerably between disease groups ranging from nothing to 52% in MDS patients [111]. Several trials, including this one, observed a trend to improved results in lower risk patients. No studies could confirm the addition of ATRA as providing any additional benefit.

5-AZA has already shown activity as a monotherapy in myeloid malignancies where several trials have assessed the addition of VPA to it with more promising results [112]. Three sizeable studies showed ORRs of 22, 44 and 37% with a significant number of CRs in all [113–115]. The previously observed trends were again observed in this third study, with previously untreated patients reaching an ORR of 57% and MDS patients achieving 64% [115]. However, neurotoxicity was cited as problematic in all three studies limiting its use at higher doses. It is suggested that alternative HDIs might provide more effective combinations [113, 114]. The problem of variable bioavailability is also cited, with significant differences in the MTDs found between trials [114].

Two Phase II studies have combined VPA, ATRA, and 5-AZA. Both showed some efficacy with ORRs of 42 and 26% and reflected the trends previously stated. The contribution of individual drugs is hard to quantify but the second study at least supported previous ones suggesting that ATRA was of no additional benefit [116, 117].

More recently, two trials have looked into VPA in combination with standard chemotherapeutic agent cytarabine in elderly patients The first, a Phase I/II trial found treatment was well tolerated and ORR was 35% including eight CRs, comparing favorably to 24% as the highest response rate seen in cytarabine monotherapy. 61% had resistant or relapsed disease suggesting VPA might restore sensitivity to cytarabine [118]. However, the second study in 15 patients of a similar cohort found no clinical responses and concluded limited clinical activity [119].

Solid tumors

Preclinical studies have found VPA to be effective against a variety of tumor cell lines particularly in combination with other chemotherapeutics [120].

Early Phase I studies in VPA monotherapy showed it to be well tolerated in both oral and iv. regimes, with DLTs primarily neurocognitive [121, 122]. Two trials have assessed VPA in pediatric patients with brain and CNS tumors and have found it well tolerated in this group. Response rate was limited in both (one PR seen in each and some disease stabilisation) but results were viewed as good given the patient population. [123, 124]. All four monotherapy trials concluded that future trials should look into combination therapies with VPA with cytotoxic, epigenetic and raditation therapies

A single study in melanoma has combined VPA with standard chemotherapy. One CR and two PRs were observed but the target doses of the study the study concluded VPA produced no improvement compared with standard treatment [125].

Based on a preclinical study that found VPA had synergy to topoisomerase II inhibitors [126], two Phase I/II studies have looked into VPA combined with topoisomerase II inhibitors. The first used the athracycline chemotherapy epirubicin in 41 patients with advanced solid tumors and found ORR of 22% (all PR). Response was seen in heavily pretreated patients thought to be anthracyline resistant and in tumor types thought to be epirubacin resistant, such as melanoma and cervical cancer [127]. Expansion in 15 breast cancer patients showed enhanced activity and a Phase II study is now underway [128]. The second examined doxorubicin in mesothelioma and produced 16% ORR with additional 36% SD comparing well with previous studies using doxorubicin as monotherapy. However, of note, most patients had high-performance status and there were two fatal toxicities in those with lower-performance status suggesting its use

should be restricted in this patient group. The authors suggest a trial is required to see if VPA can improve the efficacy of current platinum-based first-line treatments [129]. Additionally, a Phase I/II trial in 39 patients with melanoma examined addition of the topoisomerase I inhibitor, karenitecin. The best response was SD in 47%; however, this was an improvement on karenitecin monotherapy and as most patients had progressed on multiple previous therapies, this was viewed as a positive outcome [130].

Combination therapy with 5-AZA or its derivative decitabine has been explored in solid tumors. A study in advanced cancer combining VPA with 5-AZA was unable to conclude a significant advantage over single therapy 5-AZA with no clinical responses but did show 25% SD rate; an improvement on previous 5-AZA monotherapy studies [131]. A second, more recent study by Chu *et al.* in NSCLC cancer achieved similar limited results [132]. Both studies suggest that a less neurotoxic, more potent HDI should be considered for further studies where it could be given at higher doses.

Finally, there has been considerable interest in the combination of VPA with demethylating agent hydralazine, based on preclinical studies showing their synergy [133]. Three studies were conducted in tandem combining these two and standard chemotherapy in advanced breast cancer, refractory solid tumors and stage IIIb cervical cancer. The first showed 81% ORR (31% CR). This is only the upper limit of results seen with standard chemotherapy alone, but nonetheless a Phase III study has commenced to further assess the combination [105]. The second study achieved a 27% ORR in patients rechallenged with chemotherapy they had previously progressed on, suggesting VPA may have a role in overcoming chemoresistance. Three of the four PRs, and four of the eight SDs in the study were in cervical cancer [134], reflecting the results of the third trial where all patients were able to achieve a CR [135]. Two Phase III trials have commenced in this triple therapy in ovarian and cervical cancer. Preliminary results from 36 patients in the cervical cancer RCT show four PRs in the VPA hydralazine group compared with one in the placebo group ($p = 0.27$). The differences so far have not reached significant levels ($p = 0.27$) [136].

Summary

Overall, the results of the VPA trials are mixed. VPA has some clear advantages: being an older orally available drug it is cheap, with well-known dosing schedules and side-effect profiles. It is generally well tolerated even when combined with cytotoxic therapies, although the narrowness of its therapeutic window has proved problematic in several trials. Many trials have found they are unable to achieve the serum concentrations indicated by preclinical studies. Some studies seem to advocate longer, lower dose regimes, whilst others recomend shorter intensive revimes that aim to reach maximum serum levels. Antitumor activity has proved variable. Results seem to tend towards greater benefit at the earlier stages of disease, particularly in hematological cancers. Whilst monotherapy seems to have somewhat limited results, particularly in solid tumors, there are some improvements seen in combination therapies; however, none of these combination studies was randomized, limiting the reliability of their conclusions.

Sodium phenylbutyrate

Sodium phenylbutyrate (SPB) is a prodrug to phenylacetate, a drug that previously showed promising antitumor activity but was both poorly tolerated and toxic. It is already a licenced drug for use in children with hyperammonemia due to urea cycle disorders [137]. Multiple Phase I studies have looked into its action against hematological and solid tumors.

Hematological

Two monotherapy trials found SPB to be excellently tolerated at the MTD (375 mg/kg/day), above which neurotoxicity occurred. However, this could not achieve the serum levels that preclinical studies had indicated would be effective and only minor hematological responses were seen in both trials, even when utilizing prolonged iv. infusion in an attempt to overcome this shortcoming [138, 139].

However, a recent pilot study in ten patients combineding SPB and 5-AZA produced three PRs and two SDs; better than results seen in monotherapy of either drug alone and SPB was administered at a lower dose than previously tolerated (200 mg/kg/day) [140].

Solid tumors

In monotherapy, chronic oral administration is well tolerated with some additional gastroenterological toxicities. Response rates were low across the board though, with SD as best response in three trials performed in advanced solid tumors [137,141,142]. Some significant results were seen in certain tumor types. Notably, 12 of 23 rapidly progressing patients with RCC achieved SD [141]. Brain cancers showed comparatively high response [137], which was reflected in a focused trial in glioblastoma that reported one CR and five SDs [143].

There have been two trials looking at combination therapy of SPB in solid tumors. The first combined SPB with 5-AZA in mixed solid tumors and found well-tolerated response rates to be very low [144]. The second combined SPB with cytotoxic drug fluorouracil in advanced CRC and achieved a good response, however the trial was very small and the regime and dosing so

intense that half the patients requested to be removed from the trial [145].

SPB seems to have a limited future. Whilst having the same advantages of VPA, as an older drug with a very attractive toxicity profile it also shares its disadvantages, with a narrow therapeutic range unable to achieve desired serum levels. Most studies seem to reflect this with limited success across the board and there are currently no clinical trials underway.

■ Pivanex

Pivanex or AN-9 is a novel prodrug to butyric acid developed to combat some of the drawbacks of the aliphatic acid class. It has shown promise in preclinical studies, with increased potency observed. The first was a Phase I study of pivanex as iv. monotherapy in 28 patients with advanced solid malignancies. Due to low solubility the maximal formulatable dose for pivanex is 3.3 g/m^2/day, which did not produce any DLTs. Mild-to-moderate nausea, vomiting, fever and fatigue were the most common side effects. One patient achieved a PR and six SD [146]. A Phase II study in NSCLC patients achieved three PRs, 14 SDs and showed improved median survival [147]. Both monotherapy trials suggested future trials should explore combinations with cytotoxic therapies and a Phase I study combining docetaxel and pivanex in NSCLC patients suggested some improvements achieving two PRs and one CR [148]. A Phase IIb trial has since been performed with the same combination and the response rate was reported as 10.6% [149]. Whilst difficult to judge with so few trials performed, the results in pivanex seem to be the most promising of aliphatic acids. The favorable toxicity profile of the class is maintained but lower concentrations are required allowing expansion of therapeutic window.

Cyclic peptides

■ Romidepsin

Romidepsin, also known as FK228 and FR901228, is a bicyclic selective HDI. Romidepsin was approved by the FDA in November 2009 for the treatment of CTCL for patients who have received at least one prior systemic therapy [150–152]. Despite some initial concerns regarding myocardial damage and impaired cardiac function, romidepsin is not thought to cause permanent changes, although reversible ECG changes are regularly observed, particularly ST/T wave changes [153].

Hematological malignancies

Having been FDA approved as a monotherapy for the treatment of CTCL, romidepsin is being investigated in combination with localized electron beam radiation therapy (LEBRT). Four out of five patients experienced a fast and durable response to symptomatic treatment using romidespin in conjunction with LEBRT [154].

Romidepsin was given accelerated approval in 2011, for use in relapsed and refractory PTCL after treatment with at least one prior systemic therapy [155]. This approval was based on the results of two Phase II trials demonstrating ORR of 38 [156] and 25% [157].

For the treatment of MM, romidepsin has been investigated as a single-agent and a combination therapy regimen. A Phase II trial investigating romidepsin monotherapy demonstrated no objective responses and it was concluded that it is unlikely to be associated with a response rate of ≥30%, although some patients experienced symptomatic improvement [158]. Romidepsin, bortezomib and dexamethasone combination has been assessed in Phase I, showing good activity (>MR in 18 out of 25 patients) and manageable toxicity [159]. Several trials are currently looking at a combination with bortezomib.

Romidepsin has been investigated in three trials for the treatment of leukemia. Two studies described problems with tolerability. Bryd *et al.* used 13 mg/m^2 romidepsin iv. on days 1, 8, and 15 of therapy in 20 patients with AML and chronic lymphocytic leukemia. Toxicities including fatigue and nausea, prevented repeated dosing. There were no objective responses but some antileukemic activity was observed [160]. Similar problems were experienced with an 18 mg/m^2 iv. dose on days 1 and 5, every 3 weeks for patients with AML and MDS, where GI symptoms and fatigue were found to limit the number of treatment cycles. Of 11 patients, there was one CR and six SD. This study concluded that romidepsin has limited activity in unselected patients [161]. Another study recruited patients into two cohorts based on the presence or absence of chromosomal abnormalities known to recruit HDACs (including those effecting core binding factor). No antileukemic activity was observed in those without chromosomal aberrations, whereas, in the other group, although there were no clinical responses, antileukemic activity was observed in five of seven patients [162].

Solid tumors

For use in solid tumors, romidepsin has undergone a number of single trials for specific metastatic tumors, including recurrent head and neck cancer [163], RCC, CRC [164], glioma [165] and castration resistance prostate cancer [166]. Phase II trials examining the efficacy of romidepsin in these targets, have all found that it was ineffective at the commonly used dose (13 mg/m^2 on days 1, 5 and 15 of a 28-day cycle), although it was felt that further investigation might be warranted if combination therapy was used. The combination of gemcitabine and romidepsin in advanced solid tumors has been attempted in a Phase I trial, and despite suspected additive hematological toxicities, one MR and 12 SD were observed amongst 33 patients, warranting further examination [167].

Romidepsin has demonstrated an effect on cell growth and apoptosis in lung cancer cells *in vitro*. Two Phase II trials have investigated this effect with discouraging results. Schrump *et al.* did not observe any objective responses in 19 patients but did see transient stabilization in nine patients and significant biological activity [168]. Otterson *et al.* supported this suggestion of minimal clinical efficacy with the observation that a weekly infusion of 13 mg/m^2 romidepsin on 3 weeks out of 4 was inactive in SCLC [169].

Summary

The most promising area for progress seems to be in that of potentiating romidepsin use in CTCL and PTCL, as has been demonstrated by its combination with LEBRT and its use as an injectable formulation. In other areas of hematological malignancy and in solid tumors, romidepsin has not demonstrated significant efficacy, and problems with toxicities have been noted in several studies. As with other HDIs that demonstrate moderate single-agent effect, romidepsin may produce more encouraging results as a combination therapy.

The most promising area for romidepsin's progress seems to be in that of potentiating its use in CTCL and PTCL, as has been demonstrated by its combination with LEBRT. In other areas of hematological malignancy and in solid tumors, romidepsin has not demonstrated significant efficacy and problems with toxicities have been noted in several studies. As with other HDIs that demonstrate moderate single-agent effect, romidepsin may produce more encouraging results as a combination therapy.

Benzamides

■ MS-275/entinostat

Entinostat is a newer HDI and the first major candidate of the class of benzamide-derived HDIs. It is unique in that it inhibits HDAC class I more than class II. The first Phase I study in 30 advanced solid tumor and lymphoma patients revealed some important points. An initial schedule of 2 mg/m^2/day orally for 14 days was commenced but DLTs were immediately observed, primarily in the form of abdominal pain and cardiac arrhythmia. Subsequently, the half-life was found to be over 30-times greater than previously suggested by animal studies. A new schedule of once every 14 days was commenced and tolerated well with DLTs of anorexia, nausea, vomiting diarrhea and hypoalbuminemia. MTD was found to be 10 mg/m^2; however, the majority of patients at the higher doses had to have dose reduction over time suggesting chronic or greater frequency dosing should be lower. Whilst there were no CRs or PRs, 15 patients achieved SD [170].

Based on these rather surprising results, a variety of dosing schedules were explored in solid and hematological cancers for tolerability and efficacy. One trial in solid and lymphoid malignancies found a 6 mg/m^2 once weekly for 4/6 weeks was well tolerated, although only produced one SD out of 19 in a patient with rapidly progressive CRC [171]. Another similar study looked into three different regimes. Fortnightly, up to 6 mg/m^2 could not find an MTD and, promisingly, produced two PRs including one for over 5 years in a patient with refractory metastatic melanoma treated at the lowest dose. Biweekly for 3/4 weeks at 2 mg/m^2 was not tolerated at all, and the weekly for 3/4 weeks at 4 mg/m^2 was well tolerated and although it produced no clinical responses, disease stabilization was seen in all three schedules [172]. A similar trial in acute leukemia (n = 38) found a regimen of 8 mg/m^2 weekly for 4/6 weeks was well tolerated with no DLTs. No PRs were observed but 12 patients achieved SD as defined hematological [173].

These earlier studies have stimulated trials using various combination therapies in some specific tumor types. Two Phase II studies in breast cancer have been performed. The first looked at the role of entinostat in restoring estrogen receptor-positive breast cancers sensitivity to aromatize inhibitors, thus prolonging the time before chemotherapy or surgery. Of 27 patients enrolled, one achieved PR and one SD of >6 months; addition of entinostat was deemed to be of benefit [174]. The second study was a randomized controlled trial in 130 patients with breast cancer adding entinostat to the hormonal drug exemestane. Results were positive, with the entinostat arm tending towards improved overall survival reaching significance (p = 0.06) at median PFS. Response rates were similarly small though [175]. A Phase III study is planned based on this and four other trials in breast cancer are in progress.

A recent Phase I study in 19 solid tumor patients is the first to combine entinostat with 13-cis retinoic acid. The treatment was well tolerated at 4 mg/m^2/week and seven patients achieved SD including one RCC patient who achieved SD for six months and had reduction of lung nodules after 4 months [176].

There has been interest in the combination 5-AZA as well. One study in hematological cancer (n = 30) observed three CRs, four PRs and seven hematological improvements, at a schedule of 8 mg/m^2/week for 2–4 weeks [177]. A second in NSCLC patients (n = 42) on a similar schedule, found one CR for 14 months, one PR and ten SDs. Overall survival times seemed to be improved and, interestingly, on follow up of a subset of 19 patients, who went on to receive further therapies, there seemed to be improved response, including four patients with major responses to chemotherapy [178].

Generally, entinostat is emerging as an attractive option in prolonging disease stabilization and other long-term therapies as it is well tolerated but active at chronic low doses and has the advantage of nonintensive weekly or

fortnightly regimes. This seems to be improved in combination therapy and various combination studies are currently underway with focused interest in hematological, lung, breast and CRCs.

CI-994

CI-994 is another HDI originally investigated as an anticonvulsant but found to have anticancer activity in a number of cancer models, although it tends towards cytostatic rather than cytotoxic effects.

An initial Phase I trial investigated a chronic oral dosing schedule in 53 pretreated patients with advanced solid tumor, over 50% CRCs. The MTD was found at 8 mg/m^2 daily with the main DLT being thrombocytopenia and other common mild toxicities of nausea, diarrhea, vomiting, constipation, mucositis and fatigue. One patient achieved a PR for over 2 years and three others achieved SD [179].

Three Phase I studies in advanced solid tumors have combined CI-994 with standard cytotoxic chemotherapies. The first assessed combination with gemcitadine in advanced solid tumors (n = 20) and found MTD lowered at 6 mg/m^2/day above which dose limiting thrombocytopenia occurred. Two patients achieved a minor response and 12 SD [180]. Another assessing a capecitabine combination (n = 54) also found MTD at 6 mg/m^2/day achieving one PR and 19 SDs [181]. Finally, a carboplatin and paclitaxel combination found MTD lower again at 4 mg/m^2/day, but nonetheless showed promising results with five of 30 patients achieving a PR and two achieving a CR [182].

Several specific-tumor Phase II studies have also been performed with less encouraging results in general. The first in RCC (n = 48) observed only minor responses were along with 26 SDs [183]. An RCT in NSCLC (n = 180) compared gemcitabine combined with CI-994 or placebo and concluded CI-994 did not increase activity of gemcitabine and patients in the treatment arm experienced reduced quality of life [184]. A large placebo controlled study in pancreatic cancer (n = 174) also with gemcitabine came to the same conclusions (p = 0.908) [185].

Generally the results for CI-994 are not encouraging, although it suggested by several of the studies that CI-994s cytostatic as opposed to cytotoxic abilities put it at a disadvantage in studies where patients often have advanced disease. There are no trials currently in progress for CI-994.

MGCD0103/mocetinostat

Mocetinostat is one of the newer members of the benzamide class and is unusual in that it inhibits class I and IV HDACs only. The first Phase I trial was as monotherapy in advanced solid tumors (n = 38). On a three-weekly oral schedule for 2/3 weeks, the MTD was found at 45 mg/m^2. It was well tolerated with DLTs of fatigue, nausea, vomiting, anorexia and dehydration, with no apparent myelosuppression. There were no objective responses but five patients achieved SD [186].

A Phase II monotherapy trial in Hodgkin's lymphoma (n = 51) found MTD at a fixed dose of 85 mg; equivalent to the previous study. Promisingly, there was a 27% ORR. The study authors point out that this is the first HDI shown to have activity in Hodgkin's lymphoma and suggest that mocetinostat might be a good candidate for maintenance therapy in those who achieve a remission on standard therapy [187].

A combination study with gemcitabine in advanced solid tumors (n = 29) produced four PRs and two SDs, of which a total of three were in pancreatic cancer. The Phase II part of this study has now commenced specifically in pancreatic cancer patients [188].

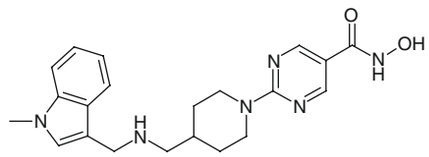
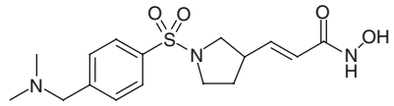
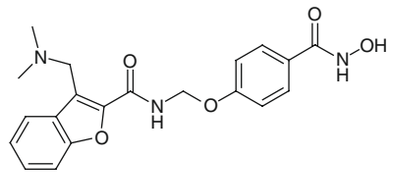
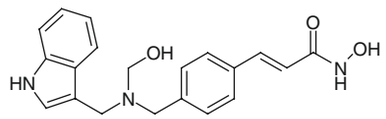
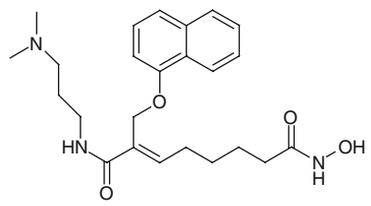
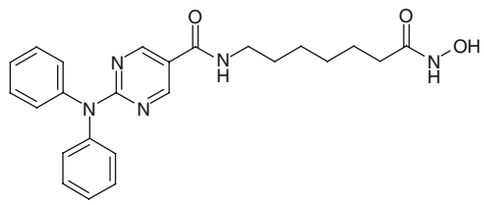
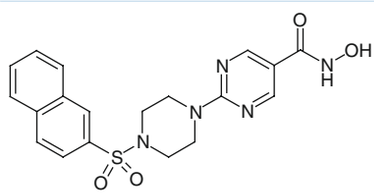
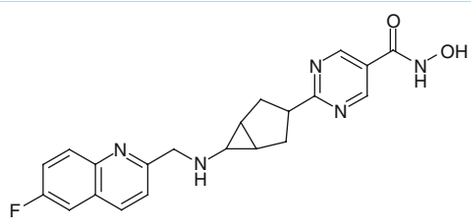
Two Phase I monotherapy trials have been conducted in hematological malignancies. The first assessed a b.i.d. schedule and found MTD of 66 mg/m^2 with no DLTs at this dose and similar toxicities [189]. The second assessed a continuous three-weekly schedule and found similar tolerance with three patients achieving a complete bone marrow response [190]. A Phase II study has since been performed in 21 chronic lymphocytic leukemia patients but showed limited activity. Finally a Phase I/II trial in AML or MDS has been performed combining mocetinostat with 5-AZA. So far in the Phase I portion of the trial, seven of 24 patients have achieved a response including three CRs [191].

Generally, mocetinostat is still in its infancy and it is difficult to draw any clear clinical conclusions. It seems to show limited benefit as monotherapy except where it has shown some promise in pancreatic cancer and Hodgkin's lymphoma. Combination therapy may improve activity, and the results of the 5-AZA trial are encouraging, but it will await future clinical trials to confirm this.

Novel compounds

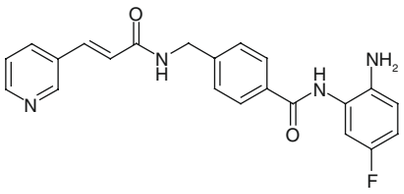
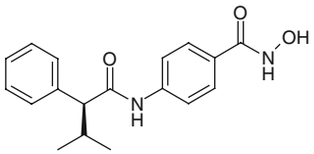
Hydroxamic acid-based compounds appear to be the most numerous and advanced of the novel HDIs, with eight hydroxamate-based HDIs having recently entered Phase I and II clinical trials (Table 5). Despite attempts to design specific HDIs, the majority of the most developed of these drugs are pan-HDIs, similar to predecessors within their class, such as vorinostat and panobinostat. Three selective HDIs have progressed into clinical trials. ACY-1215 (rocilinostat) a selective inhibitor of HDAC6, is the most developed of these, showing some efficacy in combination with bortezomib for the treatment of MM [192]. The benefit of selective HDAC inhibition is theoretically in a reduced side-effect profile. However, these compounds have not developed enough to be compared with pan-HDI in clinical practice. Two non-hydroxamic

Table 5. Histone deacetylase inhibitors in early phase clinical development.

HDI	Structure	Clinical trials/status	Ref.
Hydroxamic acid derivatives (Pan-HDIs)			
Quisinostat JNJ 26481585		Phase II Currently in four trials targeting hematological malignancy	[196]
Resminostat 4SC-201		Phase II Ongoing trials: SAPHIRE (HL) SHORE (colorectal cancer)	[197]
Abextinostat CRA-024781 PCI-24781		Phase II Currently registered in six trials in both hematological and solid tumors Previous promising results in HL and NL	[198–200]
Dacinostat LAQ824		Phase I Previously shown to be tolerable in solid tumors Not currently in trials	[201].
CG200745		Phase I Currently recruiting for first-in-human trial	[202]
Hydroxamic acid derivatives (selective HDIs)			
Rocilinostat ACY-1215		Phase II Previous encouraging results in MM, in combination with bortezemib and dexamethasone Currently being assessed for MM in combination with lenalidomide and dexamethasone	[192,203]
R306465 JNJ-16241199		Phase I Recommended for progression to Phase II, not currently in trials	[204,205]
CHR-3996		Phase I Recommended for progression to Phase II, not currently in trials	[206]

HDI: Histone deacetylase inhibitors; HL: Hodgkin's lymphoma; MM: Multiple myeloma; NL: Non-Hodgkin lymphoma.

Table 5. Histone deacetylase inhibitors in early phase clinical development (cont.).

HDI	Structure	Clinical trials/status	Ref.
Benzamide derivative			
Chidamide HBI-8000 CS055		Phase I Well tolerated in Phase I, not currently in trials	[193,194]
Aliphatic acid			
AR42 OSU-HDAC42		Phase I Currently recruiting for Phase I trial in hematological malignancy	[195]

HDI: Histone deacetylase inhibitors; HL: Hodgkin's lymphoma; MM: Multiple myeloma; NL: Non-Hodgkin lymphoma.

acid-based compounds have been developed and entered into clinical trials, although, neither of these have yet progressed into Phase II [193–195]. Many other novel compounds have also been developed and these are currently in preclinical stages.

In addition to the discovery of novel compounds, a new approach to treatment is also being tried. SHP141 is a topical HDI treatment, designed to have increased efficacy through delivery at high concentration, without the systemic effects common to HDIs. It is currently undergoing a Phase I trial in CTCL. In addition to its antitumor effect, it is hoped that this application can also be used in inflammatory and proliferative skin conditions.

Conclusion

The recent developments regarding belinostat and the prospect of its licensing in the next year, demonstrate that the role of HDIs is still progressing, particularly in hematological malignancy. HDIs also appear to have a developing role in the treatment of MM and other types of lymphoma.

Some interesting trends have emerged, such as the benefit of pivanex in chronic disease stabilization and VPA in the early stages of disease. These factors, in addition to growing knowledge of HDI combination therapies and newly developed formulations, offer a way to potentiate the effects of HDIs, and target patients for the greatest benefits.

HDIs have not met expectations when translating the preclinical effect on solid tumor cancer lines to clinical situations. Only a few HDIs, such as vorinostat and panobinostat, have undergone clinical trials in a really wide range of tumor types. The majority of HDIs are still early in their clinical development. The vast number of clinical trials currently investigating these drugs will yield vital information as to the best use of HDIs in the next few years.

Future perspective

Many published trials have observed that the level of antitumor activity demonstrated *in vitro* does not translate to clinical efficacy. Current trial design benefits from the knowledge that there is a necessity to examine ways of increasing peak concentration and identifying indicators as to which patients might gain most benefit.

Over 500 trials are currently underway to investigate the 11 drugs discussed in this review. These trials are investigating multiple drug combinations in a vast number of different tumors types; with such a quantity and range of trials, the future of clinical HDI development is dependent on the results that will emerge over the next few years.

Preclinical development of new HDI compounds is also an exciting and ongoing area of development, with numerous new compounds demonstrating activity *in vitro*. A clear next step in HDI development is the development of selective HDIs and with ACY-1215 (rocilinostat) already in Phase II trials, it is possible that the next 5 years will see a demonstration of increased efficacy, accompanied by reduced toxicity.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Histone deacetylases (HDACs) are a family of proteins which, through their ability to repress chromatin play a variety of important roles in gene transcription, chromatin formation, and DNA repair and replication. They also have a number of additional nonhistone substrates to contribute to these and other roles.
- HDAC inhibitors (HDIs) inhibit HDACs and thus produce an antitumor effect through growth arrest, terminal differentiation and cell death through mechanisms such as anti-angiogenesis, autophagy and mitotic failure.
- There are four chemical classes of HDIs; the hydroxamic acids, aliphatic acids, cyclic peptides and benzamides.
- The hydroxamic acids are the largest and most prominent family of clinical HDIs. Vorinostat in particular has been licensed for use in cutaneous T-cell lymphoma, and has entered Phase III trials in other areas. Combination with other therapies appears to produce the greatest effect. Newer more potent agents panobinost, givinostat and belinostat are in the earlier stages of development but have shown success in a number of areas, particularly in hematological cancers.
- The aliphatic acid class is much less potent and, whilst many trials have been performed in its main contender, valproic acid's success is limited with only low serum levels achievable and problematic neurotoxity often encountered. Novel agent AN-9 shows more promise with greater potency but is still very much in the early stages of development.
- The cyclic peptide class consists romidepsin only, which has shown promise to the point of recent licensing in the treatment of cutaneous T-cell lymphoma and peripheral T-cell lymphomas but has shown limited activity in other areas.
- The benzamide class is made of primarily of entinostat, CI-994, and mocetinostat. Whilst not particularly potent agents they may play a role in prolonging disease stabilization, as they have been shown to be safe and effective at chronic low oral doses.
- Many novel agents are currently under development, in preclinical studies, or awaiting results of initial clinical trials. This includes some specific HDAC inhibitors, for example, ACY-1215, inhibitor to HDAC6. Most future studies are focussing on the HDIs in combination with a variety of cytotoxic and epigenetic therapies and multiple trials in known and novel agents are due to be published in the next few years.

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