Clinical Studies of Histone Deacetylase Inhibitors

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Abstract Over the last 5 years, a plethora of histone deacetylase inhibitors (HDACi) have been evaluated in clinical trials. These drugs have in common the ability to hyperacetylate both histone and nonhistone targets, resulting in a variety of effects on cancer cells, their microenvironment, and immune responses. To date, responses with single agent HDACi have been predominantly observed in advanced hematologic malignancies including T-cell lymphoma, Hodgkin lymphoma, and myeloid malignancies. Therefore, in this review we focus upon hematologic malignancies. Generally HDACi are well tolerated with the most common acute toxicities being fatigue, gastrointestinal, and transient cytopenias. Of note, few patients have been treated for prolonged periods of time and little is known about long-term toxicities. The use of the biomarker of histone hyperacetylation has been useful as a guide to target specificity, but generally does not predict for response and the search for more clinically relevant biomarkers must continue.

It is well recognized that chromatin modification plays an important role in the control of gene transcription regulation and the acetylation status of histones plays a critical role (1). Hyperacetylated histones tend to result in transcriptionally active genes, whereas hypoacetylation typically results in repressed transcription. The degree of acetylation is mediated by histone acetyltransferases and deacetylases (2, 3). Currently a total of 18 histone deacetylases (HDACs) have been described, and they have been divided into four general classes. Class I includes HDACs 1, 2, 3, and 8, located within the cell nucleus; class II includes the HDACs 4, 5, 6, 7, 9, and 10; and class IV HDAC 11. In contrast to the other HDACs, class III HDACs, consisting of the NAD^+-dependant Sirtuin family 1 to 7, are not targeted by the currently available HDAC inhibitors (HDACi).

In the clinical setting, relatively weak HDAC inhibition was initially recognized with sodium butyrate, the prototype of the small chain fatty acid group that was later found to include sodium valproate and phenylbutyrate, and their efficacy as HDACi has been evaluated, both as single agents and in combination with other therapies, with modest results (4–8). Since then, a range of much more potent, structurally diverse HDACi have been purified as natural products or have been synthetically produced. These later HDACi are generally subdivided into six groups on the basis of their chemical structure (9). Pan-HDACi agents include vorinostat, panobinostat, and belinostat, whereas the more isotype-selective (class or specific HDAC) agents include romidepsin, MGCD0103, and entinostat. It should be noted however that this is based largely on preclinical *in vitro* studies and few clinical studies report on drug effect on specific HDAC isotype targets (10).

The various mechanisms of action of the HDACi are beyond the scope of this review but it is important to recognize that there are likely to be substantial differences between the various HDACi drugs based not only on the targets for hyperacetylation (i.e., the different classes of HDACs) but also the capacity to hyperacetylate lysine residues on histones and nonhistone targets, and individual pharmacokinetic properties (11–13). For example it remains unclear whether pan-HDACi, which inhibit both class I and II HDACs, are superior to class- or isotype-specific HDACi (e.g., class I inhibitors alone). Along with their similar modes of action HDACi seem to have a general class toxicity profile, which includes gastrointestinal disturbance, myelosuppression, and QTcF (QT interval corrected with Fridericia's formula) prolongation, although idiosyncratic side effects of particular HDACi have been noted and may relate to differences in chemical structure. Hopefully, as we learn more about the specific attributes of each individual HDACi we may eventually be able to "match" individual HDACi to particular tumors or genetic profiles to improve clinical responses (14).

To date, the responses observed in studies using HDACi as a single agent have predominantly been seen in advanced hematologic malignancies, with few seen in solid tumors (Table 1). The focus of this review is to critically appraise clinical studies of HDACi in hematologic malignancies, and examine the various correlative studies linking drug dose, histone acetylation, pharmacology, and biomarker studies. For a review of clinical studies of HDACi in solid tumors, we refer readers to Marsoni and colleagues (15).

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Table 1. Key trials of single agent HDACi in hematologic malignancies

Agent	Study	Disease	Phase	No.	Response
Vorinostat (SAHA)	O'Connor et al (17)	HL/DLBCL/CTCL/other	I	35	CR 1, PR 4, SD 3
	Duvic et al (19)	CTCL	II	33	PR 8 (SS 4)
	Olsen et al (20)	CTCL	IIb	74	CR 1, PR 21
	Garcia-Manero et al (22)	Advanced leukemia/MDS	I	41	CR 2, CRi 2, HI 3
Romidepsin (depsipeptide)	Byrd et al (32)	AML/CLL	I	20	Nil
	Bates et al (35)	CTCL	II	71	CR 6, PR 24, SD 19
	Kim et al (39)	CTCL	II	92	CR 7, PR 10
	Piekarz et al (40)	PTCL	II	43	PR 10, CR 7
MGCD0103	Garcia-Manero et al (45)	Advanced leukemia/MDS	I	29	CR 3
	Lancet et al (46)	Advanced leukemia/MDS	I	19	SD 4
	Bociek et al (47)	HL	II	33	CR 2, PR 6
	Crump et al (48)	DLBCL/FL	II	50	CR 1, PR 4, SD 22
Panobinostat (LBH589)	Giles et al (52)	Advanced leukemia/MDS	I	15	HI 1
	Duvic et al (56)	CTCL	II	95	CR 4, Skin CR 2, PR
	Ottmann et al (51)	AML/MDS/MF/MM/HL/NHL	I/II	146*	CR 3, CRi 1, PR 17, SD 14
	Prince et al (54)	NHL/solid tumors	I	19*	CR 2 (CTCL), PR 2 (CTCL)
Belinostat (PXD-101)	Gimseng et al (59)	NHL/CLL/MM	I	16	SD 5
Entinostat (MS-275)	Gojo et al (61)	Advanced leukemia/MDS	I	38	Nil

Abbreviations: MF, myelofibrosis; HI, hematologic response.
*Ongoing.

Vorinostat

Vorinostat (suberoylanilide hydroxamic acid, SAHA) is a hydroxamic acid derivative that inhibits both class I and II HDACs, and has been approved in the United States by the Food and Drug Administration (FDA) for the treatment of relapsed and refractory cutaneous T-cell lymphoma (CTCL; ref. 16). Initial clinical experience of both the intravenous and oral formulations of vorinostat in 35 patients with advanced hematologic malignancies was reported as an extension of separate phase I studies in advanced cancer (17). Intravenous administration resulted in a fourfold higher Cmax, whereas oral administration produced a significantly higher (22-fold) area under the curve (AUC) value. It is yet to be fully determined if such differences in pharmacokinetics (PK) impact on toxicities or response.

The ability to hyperacetylate target histones (both in blood lymphocytes and tumor cells) has been a useful biomarker in early studies of HDACi. With vorinostat, peripheral blood mononuclear cells (PBMCs) underwent transient histone hyperacetylation 2 hours posttreatment, which returned to the baseline level 8 hours posttreatment (Fig. 1). Although higher doses did not produce an increase in the level of histone acetylation, a longer duration of hyperacetylation was observed. Moreover, hyperacetylation was shown in tumor biopsies posttreatment, although no correlation between acetylation status and tumor response was reported. Indeed, this and numerous other studies have to date failed to show a correlation between the level of hyperacetylation and response, and although hyperacetylation of blood lymphocytes is a useful biomarker to show that HDACi "hit their target," it is likely that there are numerous other targets and mechanisms of response and or resistance that impact on antitumor effect.

In these studies, tumor reduction was observed in five patients with Hodgkin lymphoma (HL), diffuse large B-cell lymphoma (DLBCL), and CTCL (17). Despite this encouraging response in DLBCL, a subsequent study focusing on B-cell malignancies showed only a few responders at the dose administered, with one complete remission (CR), and one sustained stable disease (SD; Fig. 2; ref. 18). Conversely, given the response of a patient with CTCL to oral vorinostat, a successful single center phase II dose finding study was initiated (19). A total of 33 patients with relapsed/refractory CTCL were enrolled and treated with three oral dosing schedules. There was an overall response rate (ORR) of 24%, with a reduction in pruritus seen in 58% of patients. The 400-mg/day dose was considered optimum in terms of response-toxicity profile for evaluation in a phase IIb multicenter trial. This nonblinded single arm pivotal trial in CTCL assessed responses by changes in overall skin disease score using a modified severity-weighted assessment tool (mSWAT; ref. 20). The objective response rate in 74 patients with stage IIB or higher disease was 30%. As previously reported, the most common toxicities were related to gastrointestinal or constitutional symptoms, hematologic abnormalities, or taste disorders, and were mostly mild to moderate in severity.

Correlative studies that included skin biopsies collected prior to treatment from 51 patients, attempted to identify biomarkers predictive of vorinostat response (21). Immunohistochemical analysis showed that nuclear accumulation of signal transduction and activators of transcription (STAT) 1 (STAT1) and high levels of nuclear phosphorylated STAT3 in malignant T cells correlated with a lack of clinical response, implying deregulation of STAT activity may play a role in vorinostat resistance in CTCL, and may be of prognostic value in predicting response to vorinostat.

A dose escalating study of vorinostat in 41 patients with acute myeloid leukemia (AML) was recently reported (22). Grade 3/4 adverse events were predominantly fatigue, diarrhea, and thrombocytopenia. Four patients (17%) with AML responded, including two patients who achieved a CR. Rapid two- to

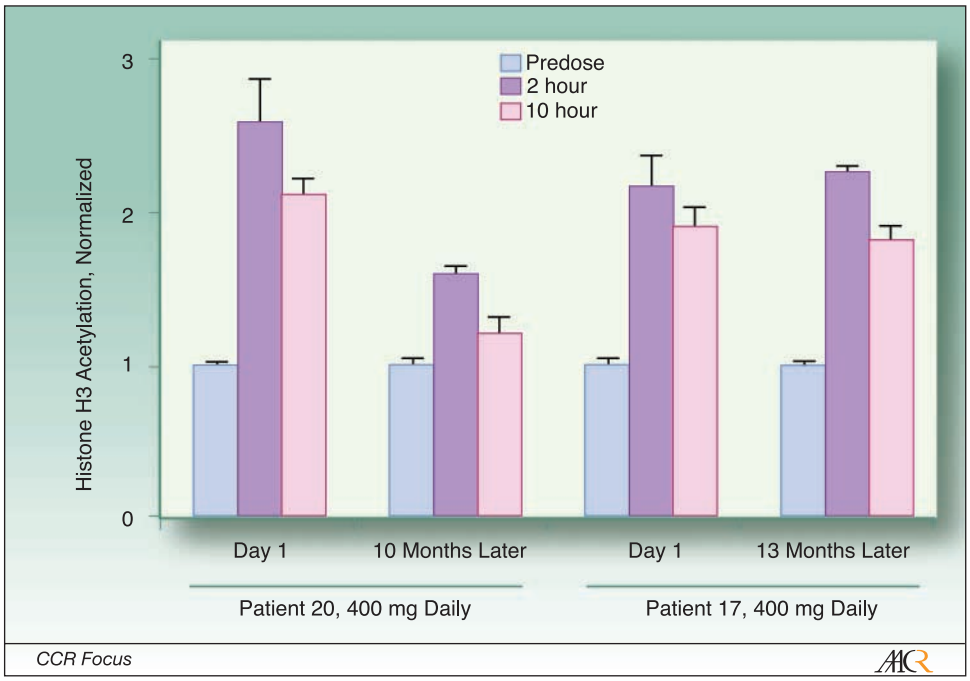

Fig. 1. Long-term evaluation of PBMC histone H3 acetylation in a patient receiving vorinostat. Reprinted with permission, ©2009 American Society of Clinical Oncology. All rights reserved. From Kelly WK, et al., "Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer," J Clin Oncol 2005;23:3923-31.

threefold histone H3 hyperacetylation was observed in blood and bone marrow in all patients at all dose levels, with levels returning to baseline during breaks in treatment. The question remains about whether *sustained* hyperacetylation would result in better clinical responses, and raises the larger question about whether we should be aiming to dose patients according to maximally tolerated dose (MTD) or using biomarkers such as hyperacetylation.

As mentioned, there may be other biomarkers that predict for response. Correlative studies on PBMC confirmed previous *in vitro* data indicating that vorinostat-induced cytotoxicity is dependant on reactive oxygen species (ROS) generation (23). Gene expression of known ROS scavengers was up-regulated in nonresponders (relative to responders) and predicted resistance, supporting the hypothesis that oxidative stress pathways are important in disease growth. Overexpression of p21- and p53-responsive genes have also been associated with resistance to vorinostat (24). Indeed, the gene expression profile along with STAT phosphorylation and ROS scavenger level may in due course help to predict for responders to vorinostat.

A phase I study of oral vorinostat in multiple myeloma (MM) was prematurely terminated because of sponsor withdrawal with the MTD not determined (25). Despite this, there is great interest in the combination of the proteasome inhibitor bortezomib and HDACi, due to preclinical studies demonstrating marked synergy in induction of plasma cell apoptosis (26). Following proteasome inhibition, misfolded proteins are directed to a single perinuclear area along α tubulin, forming an aggresome-a key "escape mechanism" or malignant plasma cells. Hyperacetylation of α tubulin by HDACi, prevents aggresome formation, resulting in an increase in apoptosis (27). Early reports suggest the combination of bortezomib and vorinostat *in vivo* seems tolerable, with near maximum single-agent doses of both drugs deliverable, along with promising response rates (28–30).

Romidepsin

Romidepsin (depsipeptide, FR901228, FK228, NSC 630176) is a relatively unique HDACi as it is a prodrug. Upon entering cells romidepsin is reduced to an active compound, capable of preferentially interacting with the zinc in the active site of the HDAC class I enzymes, however, it is still generally classified as a broad-spectrum inhibitor as it does inhibit class II enzymes (31).

A dose escalation study in chronic lymphocytic leukemia (CLL) and AML patients was done by Byrd and colleagues, with the aim of achieving an *in vivo* dose that increased acetylation of histone proteins H3 and H4 by 100% *in vitro* (32). Although no formal CR or partial responses (PR) were seen in 10 CLL patients, antitumor activity was noted. Of 10 patients with AML none achieved CR or PR although one patient experienced tumor lysis syndrome. Another study with 11 AML/myelodysplasia (MDS) patients had one CR with SD in six patients (33). Correlative studies showed a modest but rapid increase in apoptosis, and changes in myeloid maturation marker expression, although no consistent changes were observed in histone H3 and H4 acetylation levels.

In 2001, responses in four patients with T-cell lymphoma were reported in a phase I trial conducted at the National Cancer Institute (NCI; ref. 34). Subsequently, two groups on both sides of the Atlantic have been investigating romidepsin in phase II trials. Bates and colleagues have reported the final results of 71 patients with CTCL treated on the multicenter NCI study of romidepsin administered as a 4-hour infusion on days 1, 8, and 15 of a 28-day cycle with a starting dose of 14 mg/m^2

(35). The ORR was 34%, with a CR observed in four patients, a PR in 20, and SD in 26. The duration of responses improved with increased depth of response and the median time to progression for patients with a major response (CR or PR) was 15.1 months. CR was achieved even in patients with Sézary syndrome. One of the striking features of romidepsin is the very long duration of response extending beyond 3 years in some patients, some after drug discontinuation. Indeed, one patient remains in an ongoing CR off therapy after 63 months.

There was a significant correlation between global H3 histone acetylation at 24 hours and Cmax, AUC, and clearance, and furthermore patients with major responses were more likely to have higher levels of acetylated histones at this timepoint. RNA analysis of both normal and malignant circulating PBMCs in this trial showed increased histone acetylation. Of interest, microarray analysis revealed specific CTCL signature genes (which have been recognized to differentiate between Sézary syndrome and aleukemic forms of CTCL; ref. 36) were reversed following romidepsin treatment (37), suggesting either effective peripheral blood tumor elimination or differentiation. Because of early concerns about cardiac toxicity, intensive electrocardiogram and ejection fraction monitoring of patients receiving romidepsin has been undertaken along with careful patient selection excluding those with a significant cardiac history, and the use of aggressive electrolyte replacement to reduce risks of QTc prolongation (38). Although minor electrocardiogram changes are relatively frequent there have been no reports of raised cardiac enzymes or altered left ventricular function due to drug administration, even with prolonged treatment.

Favorable responses in CTCL have been confirmed in a European-U.S. study of 96 patients, with remarkably similar outcomes to that of Bates and colleagues (39). The ORR was 32%, as measured by SWAT, with CR observed in six patients.

Very encouraging responses have also been reported in patients with peripheral T-cell lymphoma (PTCL). Piekarz and colleagues reported an ORR of 31%, as a single agent in 48 patients, including four CR and 11 PR (40). The overall median duration of response for all patients was 9 months (range 2-61+ months). Responses were observed independent of prior therapy, with some patients having undergone prior stem-cell transplant, and were observed in a variety of subtypes of PTCL. An example of response to romidepsin is given in Fig. 3.

Single-agent romidepsin has been trialed in myeloma, with no clinical responses, although some patients obtained SD with demonstrable decreases in monoclonal protein (41). An ongoing Australian phase I/II trial is examining the combination of romidepsin with bortezomib in heavily pretreated myeloma patients, including six patients who had received prior bortezomib, and the study has shown good tolerability in the 22 patients treated to date, with four CR, two very good partial responses (VGPR), six PR, five minimal responses (MR), and one SD (42).

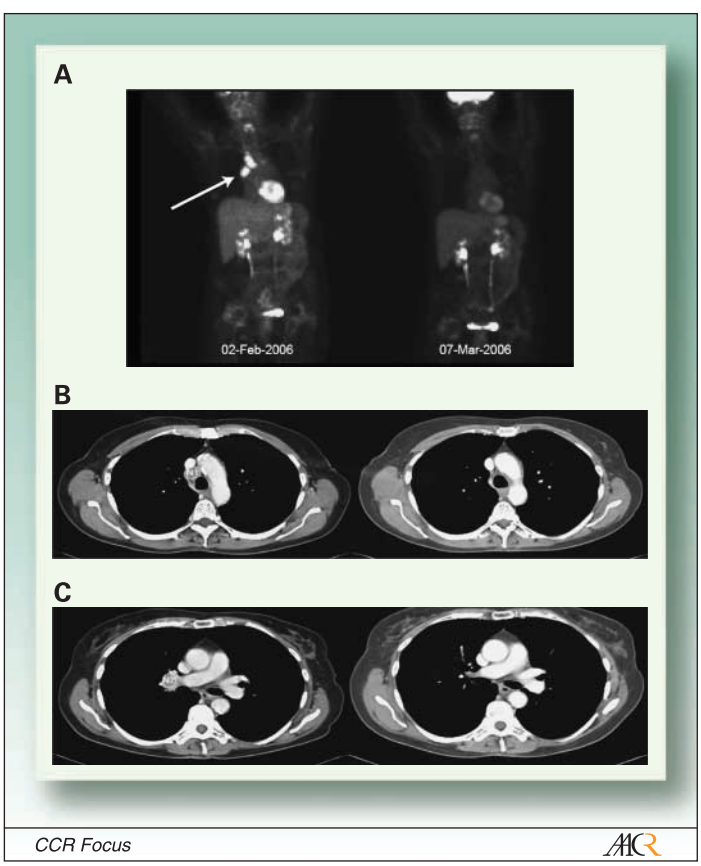

Fig. 2. FDG-PET and CT scans of a 59-year-old patient with relapsed DLBCL who achieved complete remission on vorinostat. *A,* FDG-PET scans at baseline and on day 29 are shown. Corresponding CT scans are shown in *B* and *C*. Reproduced from Crump M, et al., "Phase II trial of oral vorinostat (suberoylanilide hydroxamic acid) in relapsed diffuse large-B-cell lymphoma," Ann Oncol 2008;19;964-9 by permission of Oxford University Press.

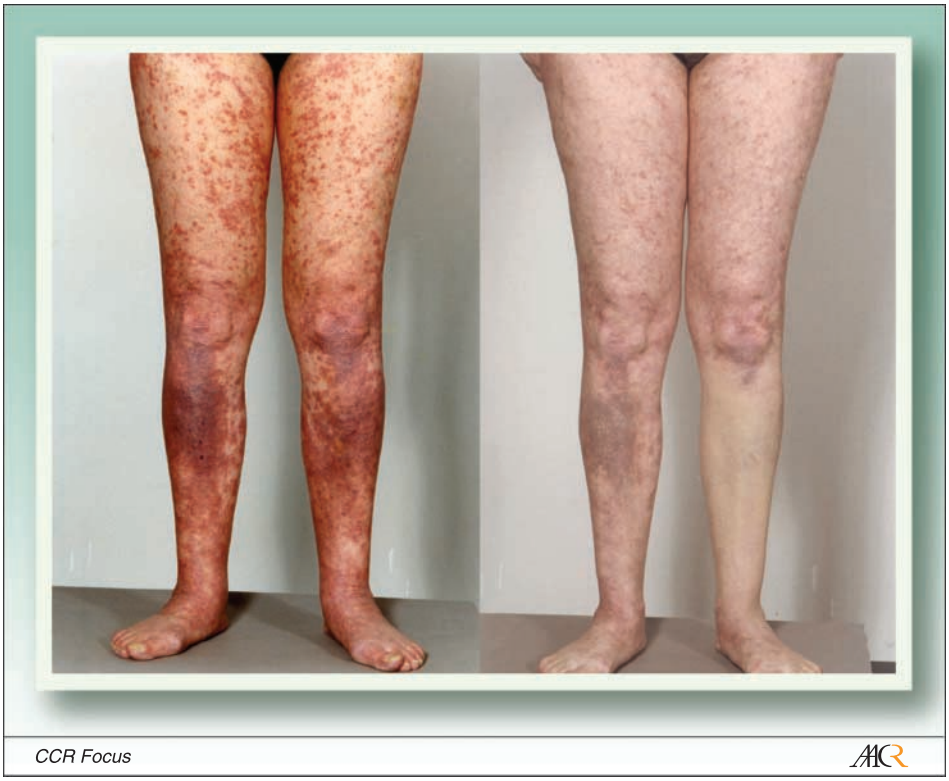

Fig. 3. A patient with PTCL (NOS) with skin involvement pre- and post-romidepsin.

MGCD0103

MGCD0103 is an isotype-specific aminophenylbenzamide that inhibits HDAC classes I and IV, with almost no class II effect. It is yet to be determined if targeting HDAC isotypes results in reduced toxicity while achieving responses equivalent to pan-HDACi (41, 43). Novel whole cell HDAC enzyme assays, using cell permeable deacetylase substrates show a dose-dependent inhibitory activity of MGCD0103 sustained for greater than 48 hours in PBMCs in patients with solid tumors. This prolonged pharmacodynamic (PD) effect may allow less frequent dosing (44).

Recently clinical development of this compound has been suspended because of reports[3] of pericarditis or pericardial effusion. Patients currently enrolled in MGCD0103 trials with no signs or symptoms suggestive of pericardial disease are continuing in their respective studies. Other reported toxicities of MGCD0103 include fatigue and gastrointestinal symptoms, with apparently less hematologic toxicity than other HDACi reported thus far (45–48).

A multicenter phase 1 trial of oral MGCD0103 in patients with leukemia and myelodysplastic syndromes was recently published (45). The majority had AML and responses were observed in three of 23 patients treated at or near the MTD. PK data showed dose-dependant acetylation of histone H3, and whole-cell enzyme assays showing HDAC inhibition increasing in a dose-dependent manner. A second trial in advanced leukemia/MDS utilized a less intensive regimen of twice weekly with no rest week (46). Four of 19 patients had SD, and inhibition of whole cell total HDAC activity was seen in PBMCs, although was not reported to predict for response.

MGCD0103 was evaluated in a phase II trial in relapsed and or refractory classical HL at a dose of either 85 mg or 110 mg thrice weekly (47). Of 33 patients enrolled, 29 (88%) had received a prior transplant. Among 21 evaluable patients in the 110-mg cohort, two had a CR and six PR. The patients in CR had progression free survivals (PFS) of 270+ and 420+ days. Another patient had SD for more than 6 months. Of five evaluable patients in the 85-mg cohort, all showed tumor reduction, with one formal PR and two SD. All grade 3 toxicities seemed to be significantly less in the 85-mg cohort. In this study they assayed thymus and activation regulated chemokine (TARC) levels. TARC is able to attract activated TH2 T cells and is highly expressed by Hodgkin Reed-Sternberg (HRS) cells in HL and dendritic cells in the HRS environment (49, 50). Indeed, the antitumor activity of vorinostat in *in vitro* studies of HL is associated with a decrease in TARC production (50). These observations are mirrored in the MGCD0103 clinical trial in which a decrease in plasma TARC levels measured by ELISA from baseline to day 8 correlated with clinical responses (47). It remains to be proven whether these *in vivo* observations are related to "on target" antitumor or "off target" antiinflammatory mechanisms.

The same group has reported initial results in a phase II trial in adults with relapsed or refractory DLBCL or follicular lymphoma (FL), nearly all of whom had received prior rituximab (48). Responses in 17 patients with DLBCL included

[3] Unpublished data.

one CR and three PR, with PFS for these responders ranging from 168 to greater than 336 days. Interestingly, five patients with DLBCL with SD had PFS ranging from 112 to greater than 336 days. It is of interest that like this study, several HDACi have been reported to prevent disease progression for prolonged periods of time despite the lack of conventional radiological responses (40, 51). One of 10 patients with FL achieved a PR. Inhibition of HDAC activity in PBMCs was seen in 13 out of 18 patients and seemed similar between the 85-mg or 110-mg treated groups.

Panobinostat

Panobinostat (LBH589) is a structurally novel cinnamic hydroxamic acid analog, and both intravenous and oral formulations are being investigated. Based on the hypothesis that leukemic cells might require an extended dosing period for disease control, a two-arm, dose-escalation phase IA/II study in patients with advanced hematologic malignancies was initiated with a 7-consecutive-day dose schedule of intravenous panobinostat (52). Fifteen patients were treated but asymptomatic grade 3 QTcF prolongation was reported in four patients, resulting in premature discontinuation of the study, and all subsequent studies have utilized an intermittent dosing schedule with minimal cardiac effects observed (53).

A phase I dose-escalation study of oral panobinostat on a Monday, Wednesday, and Friday (MWF) schedule on weekly and alternate weekly treatments in advanced solid tumors or non-Hodgkin lymphoma (NHL) enrolled 10 CTCL patients, with two achieving CR, four PR, two SD, and two patients PD (54). Dose-limiting diarrhea and thrombocytopenia was seen, and 20 mg was determined to be the MTD dose level for the MWF every week schedule. PD analysis of histones from normal PBMCs reveal increased acetylation at doses of 15 mg

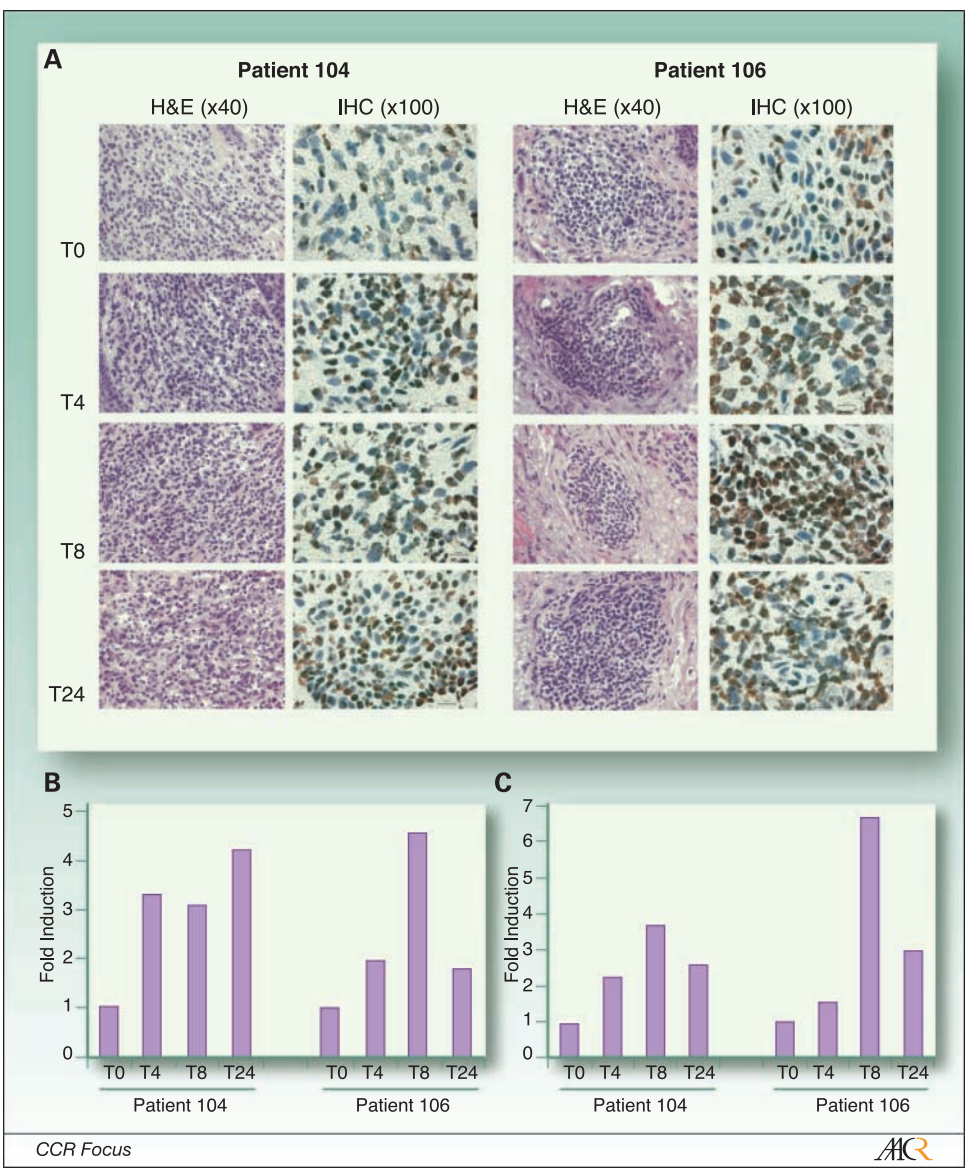

Fig. 4. Effects of panobinostat on histone acetylation. *A,* tumor biopsies from patients obtained directly before their first dose of panobinostat and at 4, 8, and 24 h following therapy. Immunohistochemistry shows an increase in acetylated histone H3 in mononuclear cells over time following treatment. *B,* the increase in acetyl histone H3 staining over background in tumor biopsy samples, and *C,* PBMCs. Adapted with permission from Ellis et al. (55).

and above, and duration of effect for at least 72 hours in 50% of patients post last dose at doses of 20 mg and 30 mg in both arms 1 and 3 (Fig. 4). Correlative skin biopsies were taken from six patients at 0, 4, 8, and 24 hours after drug administration, and the gene expression profiling showed that the large majority of affected genes were down-regulated, although only 23 genes were commonly up- or down-regulated in all patients tested. These genes had wide ranging functions including cell cycle, cell proliferation, angiogenesis, and immune regulation (55). An example of a sustained clinical response in a patient with transformed chemotherapy-refractory CTCL is shown in Fig. 5. On the basis of these results, a phase II study of oral panobinostat MWF is enrolling patients with refractory CTCL (56).

A large, ongoing, two-arm phase IA/II dose-escalation study, with one arm receiving weekly MWF dosing and the other alternate weekly MWF dosing, has enrolled 146 patients thus far with doses ranging from 20 mg to 80 mg (51). At doses ≥20 mg, panobinostat increased histone acetylation in PBMCs and bone marrow core biopsies relative to baseline, however as for other HDACi, the relationship between acetylation status and tumor response remains unclear. Clinical activity in AML seems dose- and schedule-dependant. No antileukemic activity was observed in patients evaluable for response treated alternative weekly, or those treated weekly at doses <40 mg, however, antileukemic activity was seen in seven of 36 evaluable patients treated at dose levels ≥40 mg, including two CR, one CR incomplete (CRi), and four patients with ≥50% reduction in bone marrow and/or peripheral blood blasts. Moreover, three patients achieved maximum responses several weeks after discontinuation of treatment. With regards to HL, in which response was assessed by both ^{18}fluorodeoxyglucose (FDG)-positron emission tomography (PET) and computed tomography (CT), one out of 28 patients showed a metabolic CR, 16 PR, and eight SD, whereas one achieved an anatomical CR, nine PR, and 12 SD (Fig. 6). Furthermore, seven patients had resolution of their constitutional symptoms, whereas two patients have been on therapy for more than 18 months. On the basis of these results an international multicenter trial for relapsed HL has been initiated.

Preliminary results have been reported from a phase II study in which 38 patients with MM were treated with oral panobinostat 20 mg MWF with modest results (57). The combination of oral panobinostat with bortezomib in patients with relapsed disease is under evaluation (58).

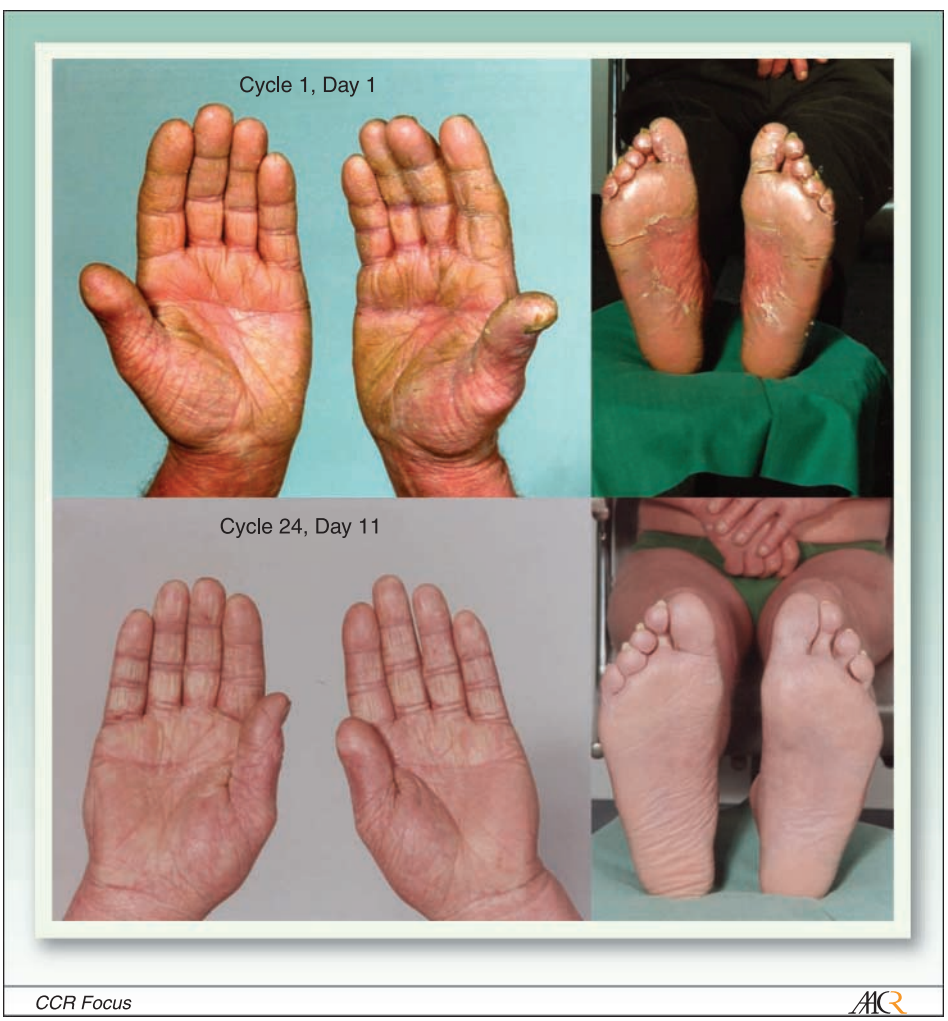

Fig. 5. Photographs of a patient with Sézary syndrome taken at baseline (top) and after 24 mo of treatment with panobinostat (bottom) with ongoing partial remission. Adapted with permission from Ellis et al. (55).

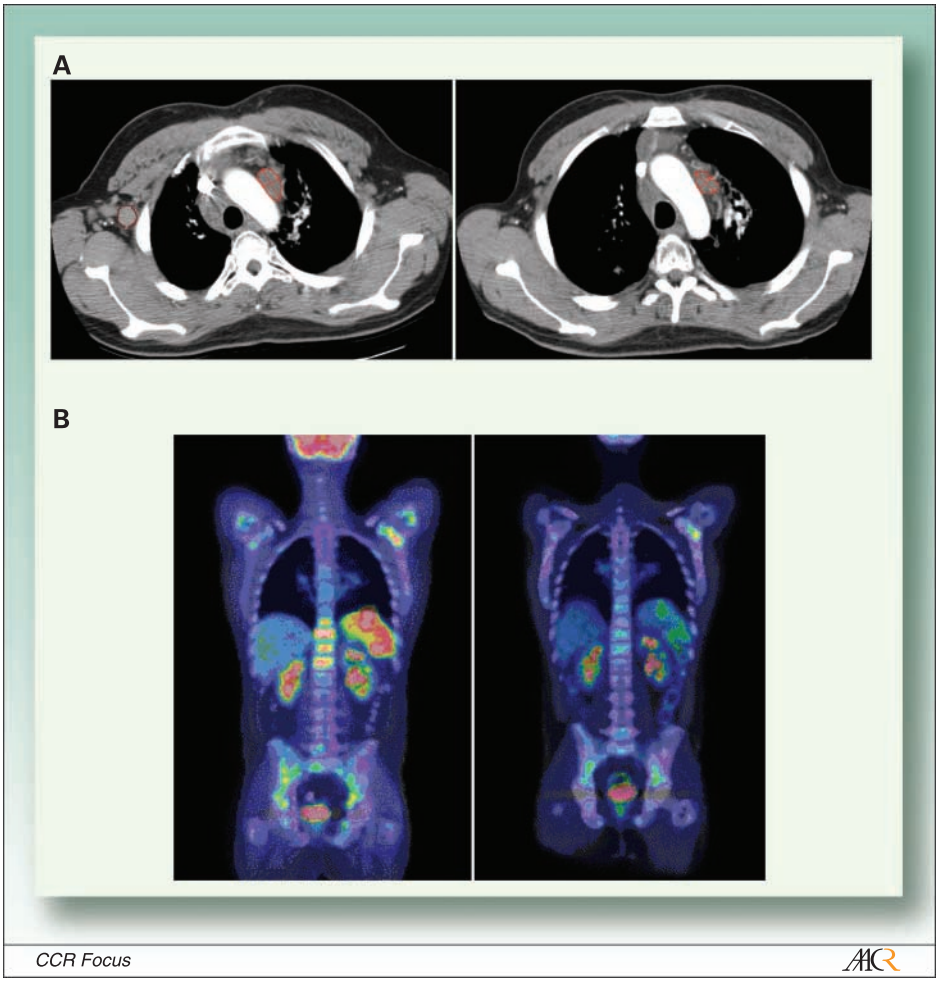

Fig. 6. Radiological responses to panobinostat in Hodgkin lymphoma. 29-year-old male with widespread extra nodal disease. *A,* CT at baseline and after cycle 8 demonstrating stable disease; *B,* PET imaging at baseline and after 2 mo of treatment (left) and 4 mo of treatment (right) with reduced FDG-avidity in all bone sites and spleen.

Belinostat (PXD101)

Belinostat (PXD101) is a hydroxamic acid derivative, which has been administered as an infusion on days 1 to 5 of a 21-day cycle in a phase I study in patients with advanced B-cell malignancies refractory to standard therapy (59). Sixteen patients received a total of 37 cycles (median treatment duration two cycles; range 1-9) of belinostat with common adverse events being nausea, vomiting, and fatigue, and myelotoxicity was relatively spared. At the MTD, cardiac arrhythmia occurred in one patient. This type of cardiac toxicity seems to occur more commonly when HDAC regimens are delivered intravenously for consecutive days, as was seen in the early panobinostat trials (52). PBMC histone hyperacetylation was rapid, modest, and relatively short with the duration related to dose. The equivalent solid tumor study also reported dose-related increase in H4 acetylation (60). The same study showed a significant increase in interleukin-6 (IL-6) levels. Of note, IL-6 has been implicated as a putative mediator of HDACi-induced fatigue (60). A trial in PTCL and CTCL is underway.

Entinostat

Entinostat (MS-275) is an isotype-selective synthetic benzamide derivative HDACi with predominant class I inhibition. Entinostat has been investigated in patients with advanced refractory acute leukemias, mainly AML (61). Fatigue and gastrointestinal symptoms were reported, however no CR or PR was seen, despite 12 patients having a transient reduction in peripheral blood blasts. Histone and protein hyperacetylation was shown at all dose levels on multiparameter flow cytometry but this was difficult to correlate with patient drug exposure as there was substantial interindividual PK variation observed at all dose levels studied. Increases in p21 expression and caspase-3 activity were shown. The MTD of entinostat administered weekly was reported as oral 8 mg/m^2, but given the biomarker data, this may be higher than the biologically effective dose. Furthermore, the half life was 36 hours, far longer than predicted in the animal studies, whereas the AUC did not increase proportionally with dose. Given these issues, a phase I study examining PK and MTD with entinostat in both fasting and nonfasting patients is underway (62).

Combination Therapy with Demethylating Agents

There is particular interest in the sequential administration of the epigenetically active demethylating agents 5-azacytidine or decitabine, followed by a potent HDACi (5, 7, 63–66). Ongoing studies have explored the combination of standard

Table 2. Combination studies involving new generation HDACi

New generation HDACi	Study	Combination	Disease	Phase	Number	Response
Demethylation/HDACi	Garcia-Manero et al (67)	Aza/MGCD0103	AML/MDS	I	24	CR 3, CRi 3, PR 1
	Silverman et al (68)	Aza/vorinostat	AML/MDS	I	20	CR 5, CRi 1, HI 3,
	Gore et al (69)	Aza/MS-275	AML/MDS	I	31	CR 2, PR 4, HI 6
	Odenike et al (70)	Aza/belinostat	AML/MDS/MF	I	21	CR 2, PR 1, HI 4
Bortezomib/HDACi	Weber et al (30)	Bort/vorinostat	MM	I	34	PR 9, MR 7, SD 18
	Badros et al (29)	Bort/vorinostat	MM	I	23	VGPR 2, PR 7, SD 10
	Siegel et al (58)	Bort/panobinostat	MM	I	14	PR 3
	Harrison et al (42)	Bort/romidepsin	MM	I	22	CR 3, VGPR 3, PR 6, MR 5

Abbreviations: MF, myelofibrosis; Bort, bortezomib; CR, complete response; HI, hematologic response.

dose 5-azacytidine with escalating MGCD0103 in patients with AML/MDS (67), 5-azacytidine with vorinostat (68), 5-azacytidine and entinostat (69), and 5-azacytidine and belinostat (70). These studies have already shown that such combinations are tolerable at doses near single agent MTD. Preliminary results are promising with respect to response and CR rates even in poor risk cytogenetic groups. Long-term outcomes are however still awaited, as are the results of an ongoing randomized study comparing 5-azacytidine alone with 5-azacytidine and entinostat (Tables 1 and 2; ref. 71). For greater detail on the preclinical and clinical aspects of demethylating agents please refer to the articles by Issa and colleagues (72) and McCabe and colleagues elsewhere in this issue (73).

Expert Opinion

Without doubt, HDACi have established antitumor activity in selected malignancies and from this early clinical data some key questions emerge. T-cell lymphoma seems to respond to most of the newer HDACi. Is this an HDACi "class-effect" and if so, what is the mechanism of action? By doing detailed correlative studies we will not only advance our understanding of what biological mechanisms drive these diseases, but also in turn these studies will further focus our treatment targets and lead to the development of more specific HDACi. In the short term however, we are asking: which will be the most effective (and hence most prescribed) HDACi for CTCL and or PTCL? Ultimately as more HDACi become approved for this indication, the answer is going to be based on the balance between the antitumor efficacy, convenience, side effect profile, and the capacity to partner HDACi to other drugs active in T-cell lymphomas. Clearly these are important questions as we move these drugs earlier in the treatment paradigm. Similar questions seem to be arising for HL and the myeloid malignancies.

Another question is how important is drug specificity? Indeed, as more isotype-specific HDACi develop, are we going to see more specific disease-targeted activity and reduced toxicities such as thrombocytopenia, diarrhea, and fatigue? MGCD0103 is the prototype example of a highly isotype-selective HDACi with clear evidence of antitumor activity in HL, with the implication that class I HDACs may be the most important target in cancer, however the efficacy of this drug is yet to be compared with the pan-HDACi such as vorinostat and panobinostat. Such comparisons of clinical efficacy and biological targets will be fascinating.

The arena of epigenetic modification and cancer therapeutics is moving rapidly (74). New HDACi are being developed with all the current drugs in varying stages of drug development with each pharmaceutical company targeting different tumors, varying schedules, and a variety of drug combinations. Indeed, combination studies will be the next major area of investigation with partner drugs including chemotherapy agents,

Table 3. Potential biomarkers for correlation with clinical response in clinical studies of HDACi

Biomarker	Disease	HDACi
Tumor and/or PBMC hyperacetylation (variably by immunohistochemistry, RNA analysis, flow cytometry)	AML	Vorinostat (22), MGCD0103 (45, 48), panobinostat (51), entinostat (61), belinostat (59, 60)
	CTCL	Vorinostat (19), romidepsin (35), panobinostat (55)
	PTCL	Romidepsin (40)
Inhibition of whole cell total HDAC activity in PBMC	AML	MGCD0103 (44)
Tumor STAT1 localization and STAT3 phosphorylation	CTCL	Vorinostat (21)
Tumor reactive oxygen species generation by gene array	AML	Vorinostat (23)
Overexpression of p21 and p53 responsive genes	AML	Vorinostat (24), entinostat (61)
MDR-1 gene expression and blood fetal hemoglobin	CTCL and PTCL	Romidepsin (35)
Plasma IL-6	B-cell NHL	Belinostat (60)
Plasma TARC level	HL	MGCD0103 (47), vorinostat (50)

Abbreviations: MDR-1, multidrug resistance 1.

demethylating agents, monoclonal antibodies, proteasome inhibitors, and other small molecules (75).

In addition to T-cell lymphomas, HL and myeloid malignancies are being investigated relatively extensively with promising data. It is somewhat surprising that B-cell neoplasms have not been studied so extensively. Indeed, the results of larger studies with mantle cell lymphoma will be particularly interesting-this tumor is driven by cell cycle abnormalities that are clear targets of HDACi-however results to date have been modest. Again appropriate correlative studies incorporated into the trials' designs will be critical.

Lastly, of course, why are responses seen far more often in hematologic malignancies rather than solid tumors? Preclinical data have shown nanomolar activity of HDACi against solid tumor cell types, albeit generally higher than those seen in most hematologic cancer cell lines. Despite this, clinical responses have been disappointing. Potential reasons for the somewhat surprising lack of response in these tumors *in vivo* include differences at both the cellular and microenvironment level. For example, it is known that different HDACi have been shown to have differential activities against cells that over- or under-express pro- and antiapoptotic molecules, such as p53 and Bcl-2 and many others. Specific HDAC expression of cancer cells may also affect clinical responses to HDACi, with reports suggesting high class I HDAC expression associated with advanced, proliferative solid tumors and adverse clinical outcome (76). An example of how the microenvironment may be profoundly affected by these drugs is suggested by the often profound early metabolic responses seen with MGCD0103 and panobinostat in HL, which may be due to alterations in the "reactive nonmalignant" infiltrates rather than direct cytotoxic effects on Reed-Sternberg cells (47).

Future Perspectives

There is evidence of potential synergy of HDAC inhibition in combination with many chemotherapeutic and biologically active anticancer compounds in preclinical studies. This observation suggests that combination strategies should be a major focus in future studies. Diseases such as PTCL and CTCL currently have a high relapse rate after standard chemotherapy and one can envisage HDACi being incorporated in front-line studies in such diseases.

The early phase clinical trial data indicate that HDACi have clinical activity in myeloid disorders. It remains to been seen whether specific cytogenetic or molecularly defined subgroups can predict for response or resistance, and this should be a focus of future studies. A number of studies examining combination strategies with chemotherapy and demethylating agents are underway but only large phase III studies will determine efficacy. In addition it will be crucial that studies should be designed to allow testing of these drugs in older frailer patients to improve survival as well as examining whether HDACi improve remission- and cure-rates when combined with aggressive chemotherapy in younger patients.

Although no major long-term toxicities have been recognized with the HDACi, one needs to recognize that there are very few patients who have been treated continuously for prolonged periods of time. Long-term effects will need monitoring with a focus on lymphocyte, hematopoietic, hormonal function, and viral reactivation.

Finally, we need to be mindful that there is still much to be learned about which of the targets of these agents that lead to responses. Although biomarkers such as tumor and PBMC histone acetylation have some value in correlating dose to the level and duration of hyperacetylation, it does not generally predict for response. Indeed, it remains unclear whether the intensity or duration of histone acetylation is key to tumor response or whether nonhistone targets are more important. Examples of potentially useful biomarkers used to date are listed in Table 3. It is critical that extensive biomarker studies examining tumor and nontumor targets, such as immune effectors, continue to be incorporated into all early phase clinical trials with these agents.

Disclosure of Potential Conflicts of Interest

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Clinical Studies of Histone Deacetylase Inhibitors

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