M. Duvic

Department of Dermatology, University of Texas, MD Anderson Cancer Center, Houston, TX, USA

Epigenetic approaches. Histone deacetylase inhibitors: vorinostat. A new treatment option for advanced cutaneous T-cell lymphoma

Introduction

Deacetylases (DACs) belong to one of three highly conserved classes of 18+ known enzymes whose functions are to remove acetyl groups from various proteins, including DNA associated histones (HDACs). Histone acetylases are enzymes that acetylate proteins and have been found to be mutated in cancer cells. The balance between acetylation (mediated by HATs) and deacetylation (mediated by HDACs) controls gene transcription and imbalance can lead to malignant transformation. Although HDACs are known for their abilities to acetylation the DNA associated histone proteins, DACs actually predate histones and are highly conserved proteins. DACs acetylate a large number of other proteins, including tumors suppressors (p53, p21). Acetylation and methylation are called epigenetic variations of DNA and are important signaling devices for transcription factors. Manipulating gene transcription by epigenetic mechanisms, including demethylation and acetylation, is a novel approach for the treatment of cancer.

Mechanism of action

Histone deacetylase inhibitors (HDAC-i) are small molecule inhibitors of Class I or II histone deacetylases and are under intense development as anti-neoplastic agents.[1] When acetyl groups are present on the lysine tails of the DNA associated histone proteins, they inhibit lysine (+) interactions with the (-) charged phosphate backbone of DNA. Thus, acetylated lysine tails of histones make the DNA backbone more accessible to transcription factors, allowing DNA transcription to occur. Deacetylation of histone proteins by HDACs causes compaction of the chromatin and gene silencing. HDAC-inhibitors derepresss gene transcription, allowing chromatin to remain in an opened configuration. HDAC-I suppression of HDACs facilitates the transcription of multiple tumor suppressor genes whose products inhibit cancer cell poliferation. Thus, HDAC-I are novel anti-tumor agents that can mediate a large number of anti-proliferative processes including cellular differentiation, cell cycle growth arrest, inhabitation of angiogenesis and apoptosis both in multiple cancer cell lines and in xenograft models.[2] Four categories of HDAC-inhibitors, based on their chemical structures, are currently recognized. Prior to the approval of vorinostat in October 2006, only valproic acid, an anti-epileptic, was available (Table 1).

Table 1. Structure of HDAC-inhibitors.

Short chain fatty acids
Butyrate, phenylbutyrate, valproic acid AN-9
Hydroxamic acids
Trichostatin A, Vorinostat (SAHA)*
pyroxamide, LBH-589*, PXD-101*, LAQ-824
Cyclic -Romidepsin* (depsipeptide)
Benzamides - MS-572

**Activity in CTCL*

Vorinostat (SAHA - suberoylanilide hydroxamic acid; Zolinza®; Merck; Whitehouse Station, USA) is a competitive oral inhibitor (HDAC-i) of Class I (1,2,3) and Class II[6,8] deacetylases. Vorinostat has broad biologic effects on cancer cell lines.[2-5] Vorinostat enhances differentiation, promotes apoptosis and cell cycle arrest, and inhibits angiogenesis.[2] Although many known activities of Vorinostat are shown in Figure 1, its mechanism of action is still under investigation. We showed that vorinostat at concentrations of 1 - 5 uM was induced apoptosis and cell cycle arrest in CTCL cell lines and selectively in malignant cells from Sézary Syndrome patients' blood.[6] Furthermore, in skin biopsies, nuclear expression of the transcription factor, p-Stat-3 was observed at baseline but was cytoplasmic in patients whose had a clinical response.[7] The superficial vasculature in treated lesions (as measured by CD31 immunostaining) was significantly decreased in responding patients. The negative effect on angiogenesis could be mediated by downregulation of VEGF.

Clinical trials of vorinostat

In parallel Phase I studies assessing the intravenous and oral formulations, clinical benefits were noted in many patients with hematologic malignancies and mesothelioma. Patients with cutaneous T-cell lymphoma had durable responses. An optimal dose of 400 mg/d given without interruption was observed.[8-10] Dose limiting thrombocytopenia was also observed with fatigue, nausea, and vomiting identified as most common side effects.

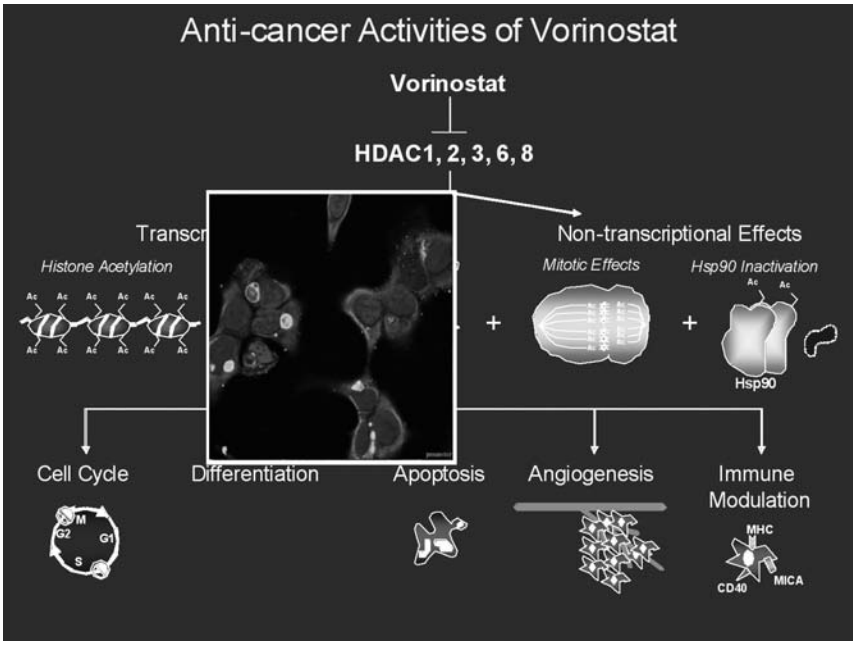

Figure 1. Proposed mechanism of action of SAHA.

Based on two Phase II clinical trials, Vorinostat was approved by the FDA in October of 2006 for the treatment of the cutaneous manifestations of cutaneous T cell lymphoma (CTCL) in patients with progressive, persistent or recurrent disease on or following two prior systemic therapies.[7,11] The primary response in skin was based on a modified SWAT score (skin weighted assessment tool). The response rates in the two clinical Phase II trials evaluated 33 and 74 patients, respectively.[7,11] In the dose ranging Phase II trial of 33 patients who had received a median of five prior systemic therapies, the overall response rate was 24%; four of 12 patients receiving 400 mg/day had responses (30.8%) and four of eleven patients with Sézary syndrome had partial responses.[7]

A second, pivotal multi-center, one arm Phase IIB trial enrolled 74 patients who had received a median of 3 prior systemic therapies, and received an oral dose of 400 mg/day.[11] The overall response rate was 30% (29.7%) with responses for specific stages of patients are shown in Table 2. The response rate in Sézary Syndrome (patients with erythroderma and blood involvement) was 33% in this trial and 36% in the first trial. Examples of tumor and Sézary Syndrome patients' responses show rapid tumor responses and improvement of erythroderma and keratoderma in Sezary Syndrome. Sézary patients are commonly colonized by *Staphylococcus aureus*[12] and thus are at risk for sepsis with intravenous and immunosuppressive therapies. SS patients have the highest response rates to vorinostat and are most in need of its anti-pruritic effects. The ability to give vorinostat (SAHA) orally offers a great clinical advantage compared with other IV agents in preventing line sepsis.

Lymph node tumor burden and in subjective measurements of pruritus by VAS also improved. Almost all patients showed some degree of clinical benefit or disease stabilization, without reaching the 50% partial response by SWAT. Further clinic monitoring

Table 2. Phase II B registration trial (74 patients)[11] and phase IIA (33 patients).[7]

Population *400 mg/day*	*N*	*n (%)*	*Med time*	*Med duration* *to response (days)*	*response (days)**
All Phase IIA	74	22 (30%)	55 (28-171)	118+ (34-322+)	
Stage IB/IIA	13	4 (30.8%)	42.5 (30-57)	80.5+ (48-322+)	
Stage ≥ IIIB	61	18 (29.5%)	56 (28-171)	126+ (34-280+)	
Sézary Syndrome	30	10 (33%)	56 (28-171)	115.5+ (34-244+)	

Phase IIA trial[r] *Variable*	*Cohort 1 (13)* *400 mg q.d.* *x 3-5 d/wk*	*Cohort 2 (11)* *300 mg b.i.d.* *400 mg. q d.*	*Cohort 3 (9)* *300 mg b.i.d.*
Partial response	4 pts = 31%	1 pts = 9%	3 pts =33%
Marked	2	1	3
Median PR Duration (range)	15 wks (8-24 wks)	16 wks (8-24 wks)	(3-21 wks) 13 wks
Mean time to PR	11 wks	4 wks	11 wks
LN regression at 4 wks	7 of 9 pts	3 of 8 pts	5 of 9 pts
> 50%↓ pruritus	9 of 11 pts	8 of 12 pts	4 of 8 pts

of patients in the Phase IIB multi-center trial has shown that six of the initial 74 patients have safely remained on Vorinostat for over two years, including one IIB patient with a complete response, one with stable disease and four patients with partial responses (Duvic *et al*., ASH Abstract 2007)

Side effects of vorinostat and HDAC-inhibitors

Histone deacetylase inhibitors are generally well-tolerated and their most common side effects are generally grade 1-2. The most frequently encountered side effects observed in clinical trials, regardless of causality were fatigue (52%), and GI related diarrhea (52%), nausea (41%), dysguesia (28%), thrombocytopenia (26%), anorexia (24%), weight loss (21%), and muscle spasms (20%).
Hyperglycemia may occur so glucose levels should be carefully monitored in patients with diabetes. Electrolytes, especially potassium which may be altered by diarrhea, should be corrected prior to giving the drug.

The most common serious adverse events regardless of causality were pulmonary emboli (4.7%), squamous cell carcinoma (3.5%), and anemia (2.3%). Deep venous thrombosis (DVT) and pulmonary emboli were encountered in a few patients (4.7%), especially those with Sezary syndrome who are prone to have clotting problems. One patient with a DVT remained on SAHA long term (Duvic *et al*., ASH 2007). Thrombocytopenia was dose related, reversible, and related to maturation arrest of megakaryoctyes. Thrombocytopenia was first encountered in the Phase I trial in patients with hematologic malignancies and in the Phase IIb trial at doses of 300 mg bid for two weeks but was rarely seen at the recommended dose of 400 mg/day. Thrombo-cytopenia is dose related and reversible in several days. Vorinostat should be stopped and restarted at 300 mg when the thrombocytopenia resolves.

Since Class II, HDAC-6 is expressed in heart muscle, cardiac effects from HDAC-inhibitors have been monitored closely in clinical trials. EKG studies should be done prior to starting drug and patients with prolonged QT intervals or significant heart disease should not be treated. Non-specific ST-T wave changes are seen when patients are on HDAC-I therapy. There can be prolongation of the QT intervals on EKG which could be exacerbated by low potassium or magnesium levels, which should be corrected prior to therapy.

Vorinostat should not be given to pregnant or nursing women as it could cause possible fetal abnormalities. Patients on valproic acid (anti-seizure medication) should not take vorinostat since it is also an HDAC-inhibitor.

Future directions

Although HDAC-inhibitors, including SAHA, may show dramatic anti-tumor effects, especially in patients with transformed tumors of mycosis fungoides and SS, the partial response rates are relatively low in the 30% range, complete responses are rare, and clinical responses are not always durable. Unfortunately, patients ultimately progress. Gene array profiling has been useful in identifying a number of target genes for determining both sensitivity and resistance to Vorinostat[2] and other HDAC-inhibitors. Skin biopsies collected from the clinical trial were used to study biomarkers predictive of SAHA sensitivity and resistance.[13] Persistent activation of the stat (signal transducer and activator of transcription) signaling pathways were implicated. Stats 1, 3, and 5 activation are associated with resistance to SAHA. Nuclear expression of p-stat-1 and p-stat-3 were associated with resist-

ance to SAHA. A pan-Janus-activated kinase (Jak) inhibitor was synergistic with the ability of Vorinostat to induce differentiation and to down-regulate anti-apoptotic genes.

In vitro, cancer cells including malignant T-cells become resistant to the apoptotic effects of HDAC-inhibitors. There are likely to be differences in the class or specific activity of HDACs for optimal inhibition or development of resistance. Thus, rational combinations of SAHA with other agents are under investigation. As above Jak-stat inhibitors might be rationally combined with HDAC-inhibitors and are currently in clinical trials as single agents. Bexarotene also appears to be synergistic to vorinostat *in vitro* (reference) and was examined in a multi-center dose escalating multi-center trial that was prematurely terminated. Vorinostat is known as a radiosensitizer and thus could be combined with phototherapy or radiation in patients with mycosis fungodies. Other agents which have been combined with vorinostat are topoisomerase I[14] and methylation inhibitors. The combination of azacitidine and vorinostat gave a response rate of 86% in 18 of 21 patients with myelodysplastic syndrome (Silverman *et al.* Blood 2008; 112: abs 3656). Small pilot studies have demonstrated that vorinostat can also be combined with lenalidomide for a 50% response rate (Siegel *et al.* Blood 2008; 112: abs 3705) and also with bortezomib for a response rate of 43% in 9 of 21 patients with relapsed or refractory multiple myeloma (Badros *et al.* Haematologica 2008;93(S1):0642). These studies suggest that it may be possible to increase the response rate to vorinostat as well by combining it with these or other agents in patients with CTCL. The exploration of HDAC-inhibitor biology and clinical applications is just beginning and the future seems very bright for these agents.

References

1. Marks PA, Richon VM, Rifkind RA. Histone deacetylase inhibitors: inducers of differentiation or apoptosis of transformed cells. J Nat Cancer Inst 2000;92:1210-6.
2. Richon VM. Cancer biology: mechanism of antitumour action of vorinostat (suberoylanilide hydroxamic acid), a novel histone deacetylase inhibitor. Br J Cancer 2006; 95(Suppl 1):S2-S6.
3. Richon VM, Zhou X, Rifkind RA, Marks PA. Histone deacetylase inhibitors: development of suberoylanilide hydroxamic acid (SAHA) for the treatment of cancers. Blood Cells Mol Dis 2001;27:260-4.
4. Duvic M, Vu J.Vorinostat: a new oral histone deacetylase inhibitor approved for cutaneous T-cell lymphoma. Expert Opin Investig Drugs 2007;16:1111-20.
5. Duvic M, Vu J.Vorinostat in cutaneous T-cell lymphoma. Drugs Today (Barc) 2007;43:585-99.
6. Zhang C, Richon V, Ni X, Talpur R, Duvic M. Selective induction of apoptosis by histone deacetylase inhibitor SAHA in cutaneous T-cell lymphoma cells: relevance to mechanism of therapeutic action. J Invest Dermatol 2005;125:1045-52.
7. Duvic M, Talpur R, Ni X, Zhang C, Hazarika P, Kelly C, et al. Phase II trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). Blood 2007;109:31-9.
8. O'Connor OA, Heaney ML, Schwartz L, Richardson S, Willim R, MacGregor-Cortelli B, et al. Clinical experience with intravenous and oral formulations of the novel histone deacetylase inhibitor suberoylanilide hydroxamic acid in patients with advanced hematologic malignancies. J Clin Oncol 2006;24:166-73.
9. O'Connor OA. Clinical experience with the novel histone deacetylase inhibitor vorinostat (suberoylanilide hydroxamic acid) in patients with relapsed lymphoma. Br J Cancer 2006;95:S7-12.
10. Kelly WK, O'Connor OA, Krug LM, Chiao JH, Heaney M, Curley T 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.
11. Olsen E, Kim YH, Kuzel T, Pacheco TR, Foss FM, Parker S, et al. A Phase IIb multicenter trial of vorinostat (suberoylanilide hydroxamic acid, SAHA) in patients with persistent, progressive, or treatment refractory mycosis fungoides or Sézary syndrome subtypes of cutaneous T-cell lymphoma. J Clin Onc 2007;25:3109-15.
12. Talpur R, Bassett R, Duvic M. Prevalence and treatment of Staphylococcus aureus colonization in patients with mycosis fungoides and Sézary syndrome. Br J Dermatol 2008;159:105-12.
13. Fantin VR, Loboda A, Paweletz CP, Hendrickson RC, Pierce JW, Roth JA, et al. Constitutive activation of signal transducers and activators of transcription predicts vorinostat resistance in cutaneous T-cell lymphoma. Cancer Res 2008;68:785-94.
14. Bruzzese F, Rocco M, Leone A, Piro G, Avallone A, Di-Gennaro E, et al. Synergistic antitumor interaction between histone deacetylase inhibitor vorinostat and topoisomerase I inhibitor topotecan in small cell lung cancer (SCLC) cells. AACR 2007;48:167.