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Histone deacetylase inhibitors: SAHA (Vorinostat). A treatment option for advanced cutaneous T-cell lymphoma

Introduction

Deactylases (DACs) belong to one of three highly conserved classes of 18+ known enzymes whose function is to remove acetyl groups from various proteins. Histone acetylases are enzymes that acetylate proteins and have been found to be mutated in cancer cells. There is a balance between deacetylation and acetylation which controls gene transcription. Although best known for their acetylation of DNA histone proteins, these enzymes predate histones and are responsible for acetylating a large number of other proteins, including tumors suppressors such as p53. Epigenetic variations of DNA are important as signals for gene transcription or silencing. Epigeneic modulation of gene transcription through de-methylation and acetylation. is a novel approach for the treatment of cancer. HDAC inhibitors are small molecule inhibitors of HDACs (Marks 2000). They represent a new class of antitumor agents that can induce a number of important anti-proliferative effects: differentiation, cell cycle growth arrest, or apoptosis in various cancer cell lines (Richon 2006).

Mechanism of action

Based on the chemical structure, four categories of HDAC-inhibitors are currently recognized and prior to SAHA, only valproic acid, an anti-epileptic, was available (Table 1).

Vorinostat (suberoylanilide hydroxamic acid; Zolinza®; Merck; Whitehouse Station, USA) also known as SAHA, is a competitive oral inhibitor (HDAC-i) of Class I (1,2,3) and Class II (6,8) deacetylases. Acetyl groups on the lysine tails of DNA associated histone proteins interact with the phosphate backbone of DNA and in doing so allow access of transcription factors to DNA. Deacetylation of histone proteins by HDACs results in compacted and therefore, unaccessible chromatin. In the presence of HDAC-inhibitors, chromatin remains in an opened configuration, allowing transcription factors to reach DNA promoters and facilitate transcription of tumor suppressor genes that check the growth of cancer cells. HDAC-inhibitors including SAHA have a wide variety of functions important for treating cancers including induction of apoptosis or differentiation, cell

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-275

**Activity in CTCL*

cycle arrest, and inhibition of angiogenesis (Richon 2006). Major known activities of SAHA are shown in Figure 1 but the full mechanism of action is still under investigation. SAHA was able to induce apoptosis and cell cycle arrest in CTCL cell lines and in malignant cells from Sézary Syndrome patients' blood (Zhang 2005). Furthermore, there was nuclear expression of the transcription factor, p-Stat-3 at baseline and it became cytoplasmic in skin lesions from patients with clinical responses to SAHA (Duvic 2007). There was reduction in the superficial vasculature of treated lesions as measured by CD31 staining in treated skin lesions., which may result from the negative effect of SAHA on VEGF.

Clinical trials of Vorinostat

SAHA (suberoyl hydroxamic acid) is a novel, oral hydroxamic acid inhibitor of Class I and II histone deacetylases (HDACs) with broad biologic effects on cancer cell lines (Richon 2001; Richon 2006) (Duvic 2007; Duvic 2007). In Phase I studies, clinical benefits were noted in patients with hematologic malignancies and mesothelioma and the optimal dose of 400 mg/d was observed (Kelly, et al. 2005; O'Connor 2006; O'Connor 2006).

SAHA 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 (Olsen 2007). 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(Duvic 2007; Olsen 2007). In the dose ranging Phase II trial of 33 patients the overall response rate was 24%; four of 12 patients receiving 400 mg/day had responses (30.8%),

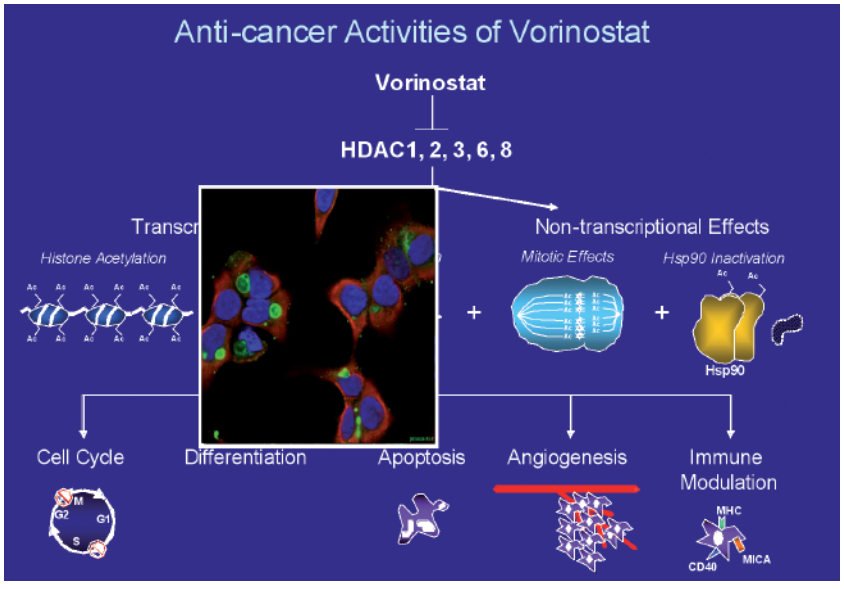

Figure 1. Proposed mechanism of action of SAHA.

and there was a median of five prior therapies (Duvic 2007).

The larger, pivotal multicenter Phase IIB trial enrolled 74 patients who had received a median of 3 prior systemic therapies and who received 400 mg/day(Olsen 2007). 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. 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 of patients in the Phase IIB multicenter 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 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%), anorhexia (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

Table 2. Phase II B registration trial (74 patients)(Olsen 2007) and Phase IIA (33 patients) (Duvic 2007).

Population 400 mg/day	*N*	*n (%)*	*Med time to response (days)*	*Med duration 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 (Duvic 2007) Variable	*Cohort 1 (13) 400 mg q.d.*	*Cohort 2 (11) 300 mg b.i.d. x 3-5 d/wk*	*Cohort 3 (9) 300 mg b.i.d. 400 mg. q d.*
Partial Response Marked	4 pts = 31% 2	1 pts = 9% 1	3 pts =33% 3
Median PR Duration (range)	15 wks (8-24 wks)	16 wks (8-24 wks)	13 wks (3-21 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

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 vein thrombosis with 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. If thrombocytopenia develops the drug should be stopped and can be restarted at 300 mg.

Cardiac effects are possible since Class II, HDAC-6 is expressed in heart muscle, therefore, EKG studies prior to starting drug are recommended. Non-specific ST-T wave changes are seen and rarely there can be prolongation of the QT intervals on EKG which could be exacerbated by low potassium levels. Vorinostat should not be given to pregnant or nursing women. Patients on valproic acid (anti-seizure medication) should not take vorinostat since it is also an HDAC-inhibitor. The ability to give vorinostat (SAHA) orally offers a great clinical advantage compared with other IV agents in preventing line sepsis. Sezary patients who are commonly colonized by Staphylococcus aureus(Talpur 2008) have the highest response rates to vorinostat and are most in need of its anti-pruritic effects.

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 SAHA (Richon 2006) and other HDAC-inhibitors. Skin biopsies collected from the clinical trial were used to study biomarkers predictive of SAHA sensitivity and resistance (Fantin 2008). 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 resistance to SAHA. A pan-Janus-activated kinase (Jak) inhibitor was synergistic with SAHA's ability 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 appears to be synergistic to vorinostat *in vitro* (reference) and is being examined in a multi-center trial. Other agents which have been combined with vorinostat are topoisomerase I (Bruzzese 2007) and the methylation inhibitors. SAHA is known as a radiosensitizer which suggests it may be combined with phototherapy or radiation in patients with mycosis fungodies. The exploration of HDAC-inhibitor biology and clinical applications is just beginning and the future seems very bright for these agents.

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