Suberoylanilide hydroxamic acid (SAHA, MW = 264) is a linear hydroxamic acid that inhibits histone deacetylase activity (HDAC) (1, 2). It causes hyperacetylation of all core histone proteins, H2A, H2B, H3, and H4, and inhibits purified HDAC activity in vitro (3). Crystallographic analysis of an HDAC enzyme, co-crystallized with SAHA, revealed the inhibitor, accompanied by a zinc ion, directly bound to the enzyme’s catalytic active site (4).

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Of the three classes of HDACs, SAHA inhibits most human Class I and Class II enzymes, including Class I human HDACs, and Class II human HDACs (2, 5). SAHA does not inhibit Class III HDACs (homologues of yeast Sir2), which require NAD⁺ for activity.

SAHA was identified originally by its ability to induce differentiation of murine erythroleukemia cells at µM concentrations (1, 3). Subsequently, it was found to induce differentiation or arrest growth of a wide variety of human carcinoma cells. To date, SAHA activity has been reported in transformed hematopoietic cells, including U937, HL-60, Jurkat (6), CEM cells (7), AR-1 cells (2), NB4 acute promyelocytic leukemia (APL) cells (8) and other APL models (9), ALL cells (10), Bcr-Abl⁺ CML cell lines K562 and LAMA-84 (11, 12), multiple myeloma cells (13-15), Waldenström’s macroglobulinemia cells (16) and cutaneous T-cell lymphoma cells (17). Reports of activity in cell lines representing other tumor types include T24 bladder transitional cell carcinoma (18), MCF7, MDA-MB-231, and MDA-MB-435 breast cancer lines (19-21), LNCaP, PC-3, TSU-Pr1, and CWR22 prostate cancer lines (22), head and neck squamous carcinoma cell lines (23), and LoVo colon carcinoma cells (7). The activity of SAHA was investigated using the 60 cell line screen at the National Cancer Institute (NCI) and IC50s ranging from approximately 500 nM to 5 µM were obtained for the growth inhibitory activity of SAHA.

The antitumor activity of SAHA was demonstrated in several in vivo models of cancer, including a xenograft model of human CWR22 prostate cancer cells (22), a mouse model of APL containing the promyelocytic leukemia zinc-finger-retinoic acid receptor α fusion gene (PLZF-RARα) (9), and an N-methylnitrosourea-induced mammary tumor model in rodents (24). SAHA showed activity when administered daily by intraperitoneal (IP) injections in the CWR22 and PLZF-RARα models, and by oral (PO) administration in the carcinogen-induced mammary tumor model.

**Gene Expression Affected by HDAC Inhibition**

DNA methylation and the hypoacetylation of core nucleosomal histone proteins lead to the tight coiling of chromatin, thereby silencing the expression of a variety of genes, including those implicated in the regulation of cell survival, proliferation, differentiation, and apoptosis (25). HDAC inhibitors alone or in combination with DNA hypomethylating agents, such as 5-aza-cytidine (5-AZA) or 5-aza-2-deoxycytidine (DAC), restore expression of silenced genes by remodeling the tightly coiled chromatin, leading to the subsequent induction of differentiation, arrest in the progression of the cell cycle, or apoptosis.

The gene expression profiles of T24 bladder and MDA breast carcinoma cells treated with SAHA or other HDAC inhibitors were studied to define a common set of genes induced or repressed by HDAC inhibition (26). As expected from major phenotypic changes observed with HDAC inhibitors in carcinoma cells, many of these affected genes are involved in regulation of cell cycle progression, proliferation, and apoptosis. They suggest that targeting transcriptional lesions may allow therapeutic intervention at the apex of the malignant transformation process (27).
**Induced Genes**

SAHA elevates expression of the cyclin-dependent kinase inhibitors \(p21^{\text{CIP1/WAF1}}\) (18), \(p27^{\text{KIP1}}\) (post-translationally) (19, 28) and \(p16^{\text{INK4A}}\) (7). Their expression seems crucial for the cell growth arrest and/or apoptosis inducing activity of HDAC inhibitors. Another gene possibly involved in cell cycle arrest is **\(\text{tob1}\)**, which may function in the absence of functional \(p21^{\text{CIP1/WAF1}}\) (26).

SAHA alone, and/or in combination with all-trans retinoic acid (ATRA), elevated the transcription of **retinoic acid receptor beta (RAR\(\beta\))** leading to cell differentiation and apoptosis (29). DAC further stimulated the effects of SAHA and ATRA on RAR\(\beta\) transcription.

SAHA induces two nuclear proteins of unknown function, **Hep27** and **TRPM-2** (26). Hep27 may be involved in the metabolism of nuclear hormones that regulate induction of differentiation or cell cycle progression. The nuclear form of TRPM-2 may also be a major factor in the induction of apoptosis in carcinoma cells.

SAHA induces the expression of the vitamin D-regulated protein **thioredoxin-binding protein-2 (TBP-2)** in transformed cells, blocking thioredoxin function (30). Simultaneous with TBP-2 expression, thioredoxin expression is decreased.

SAHA treatment induced expression of tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) cell surface death receptors **TRAIL-R1/DR4** and **TRAIL-R2/DR5** in PC-3 and DU-145 prostate cancer cells (31), in U937, HL-60, and Jurkat leukemia cells (32), and in ALL cells (10, 33).

Other genes with elevated expression due to SAHA were **metallothionein 1L, \(\alpha\)-fucosidase, histone H2B, \(\alpha\)-tubulin**, and **glutaredoxin** (26).

**Repressed Genes**

SAHA repressed two genes directly involved in DNA synthesis, **thymidilate synthetase** (TS) and **CTP synthetase**. These enzymes are targets for anti-metabolites commonly used in chemotherapy, such as 5-fluorouracil (5-FU) (26).

SAHA blocks the expression of various **target genes** under the control of signal transducer and activator of transcription-5 (STAT5), suggesting its use in STAT5-associated cancers (34).

SAHA alone decreased the mRNA and protein levels of the **Bcr-Abl** fusion gene, leading to apoptosis of Bcr-Abl+ K562 and LAMA-84 cells (12). The addition of imatinib caused greater reduction of Bcr-Abl levels and apoptosis of these cell types (11, 12).

SAHA and other HDAC inhibitors blocked angiogenic signaling in human umbilical cord endothelial cells (HUVEC) by inhibiting vascular endothelial growth factor (VEGF) induced expression of the VEGF receptors **VEGFR1, VEGFR2**, and **neuropilin-1**. SAHA also increased the expression by HUVEC of **semaphorin III**, a VEGF competitor, at both mRNA and protein levels (35). This effect was specific to endothelial cells and was not observed in human fibroblasts or in vascular smooth muscle cells.

A single oral administration of SAHA to mice reduced circulating levels of the inflammatory cytokines **TNF-\(\alpha\), IL-1\(\beta\), IL-6**, and **IFN-\(\gamma\**) induced by lipopolysaccharide (LPS) (36). Human peripheral blood mononuclear cells (PBMC) stimulated with LPS in the presence of SAHA released 50% less TNF-\(\alpha\), IL-1\(\beta\), IL-12, and IFN-\(\gamma\). SAHA also reduced levels of IL-12, IFN-\(\gamma\), IL-6, and IL-10 mRNA and protein levels in splenocytes from the murine MRL-lpr/lpr systemic lupus erythematosus model (37).
SAHA Combined with Other Agents

5-AZA or DAC
Accumulating evidence has confirmed the hypothesis that combining HDAC inhibition and DNA hypomethylating agents is synergistically effective in inducing apoptosis, differentiation and/or cell growth arrest in various cancer cell lines (29, 38).

5-FU or Raltitrexed (ZD-1694, Tomudex®)
Protein expression of TS, a target for anti-metabolite agents such as 5-FU and Raltitrexed, was diminished by SAHA treatment in colorectal cancer cell lines (39). SAHA combined with 5-FU enhanced cell growth inhibition compared to either agent alone regardless of cell p53 status. 24-hour pretreatment with SAHA followed by 5-FU produced a greater synergistic inhibition of cell growth, which may be explained by SAHA-induced repression of TS gene expression (26).

Imatinib mesylate (Gleevec®, STI571)
Studies suggest that combining SAHA with imatinib may be effective in CML cells that resist imatinib through increased Bcr-Abl expression (11, 12). SAHA alone decreased mRNA and protein levels of Bcr-Abl, leading to apoptosis of Bcr-Abl+ K562 and LAMA-84 cells (12). The addition of imatinib caused greater reduction of Bcr-Abl levels and apoptosis of these cells (11, 12). Combined exposure in these cells also blocked SAHA-mediated induction of p21cip1/waf1 (11). The combination interacted synergistically to induce apoptosis in imatinib-resistant cells that display increased Bcr-Abl protein expression. Similarly, SAHA treatment in vitro reduced Bcr-Abl levels and induced apoptosis in CD34+ leukemia blast progenitor cells from patients experiencing progressive blast crisis of CML while receiving imatinib (12).

Bortezimib (Velcade™, PS-341)
Minimally toxic concentrations of the proteasomal inhibitor bortezimib combined with SAHA resulted in increased apoptosis in human leukemia cells (40). These events were accompanied by down-regulation of the Raf-1/MEK/ERK pathway and diminished expression of Bcr/Abl and cyclin D1, cleavage of p21cip1/waf1 and pRb, and induction of the stress-related kinases JNK and p38 MAPK.

Hsp 90 Acetylation
HDAC inhibitors deplete the levels of several oncoproteins that are normally stabilized by binding to heat shock protein (Hsp) 90 in cancer cells (41). HDAC inhibitors induce acetylation of Hsp 90 protein, inhibiting its binding to ATP and impairing the binding of its chaperone proteins, mutant p53, c-Raf-1, Her-2, and AKT. A consequence of Hsp 90 acetylation is the depletion of Her-2 and increased apoptosis induced by taxotere, trastuzumab, epothilone B, and gemcitabine in breast cancer cell lines (42). In Bcr-Abl over-expressing CML cells, HDAC inhibition blocked chaperone association of Bcr-Abl with Hsp 90 thereby promoting its proteasomal degradation (43).

Anti-Bel-2 Agents
Overexpression of Bel-2 or Bel-XL in leukemia and multiple myeloma cell lines abolished SAHA-induced apoptosis but did not affect its differentiation or cell cycle regulatory effects (7, 15, 44). This suggests that agents blocking the expression or function of Bel-2 could be effectively combined with SAHA.

Retinoic Acid
In cells from RARα-PLZF/RARα-PLZF transgenic mice and in cells harboring t(15;17) (RARα-PML fusion genes), SAHA induced significant apoptosis and growth inhibition, effects that were increased by adding ATRA (9). SAHA, combined with ATRA, overcame the transcriptional repression exerted by these RARα fusion oncoproteins. This combination of agents induced leukemia remission and prolonged survival in murine APL models, without apparent toxic side effects. Similar effects of SAHA alone, and combined with ATRA and DAC, were reported in the expression of RARβ2 in NB4 cells, ATRA-resistant NB4 variants, and in primary APL patient samples (29).

Etoposide, Ellipticine, Doxorubicin, and Cisplatin
Pre-treating four human cancer cell lines with SAHA increased the killing efficiency of etoposide, Ellipticine, doxorubicin or cisplatin, but not of the Topo I inhibitor camptothecin (45). Treating cells in the reverse order, anticancer drug first then TSA or SAHA had no more cytotoxic effect than the drug alone, suggesting that relaxing chromatin structure by histone acetylation can increase the efficiency of several anticancer drugs targeting DNA.
**P-gp Multidrug Resistance**
SAHA treatment is equally effective in multi-drug resistant tumor cells expressing P-glycoprotein (P-gp) as it is in cells lacking P-gp (7, 46). RT-PCR and immunoblot evidence suggests that SAHA overcomes resistance, in part, by diminishing the overexpression of P-gp in drug-resistant cells (47).

**Phase I Clinical Experience**

A Phase I study reported the experience of SAHA administered IV in patients with advanced solid tumors and hematological malignancies (48). SAHA was administered by 2-hour IV infusion for 3 days every 21 days in part A, and 5 days for 1-3 weeks in part B. No dose-limiting toxicities (DLT) were observed in 8 patients enrolled in part A at doses of 75, 150, 300, 600, and 900 mg/m²/day. Among 12 hematological and 17 solid tumor patients enrolled in part B (300, 600, and 900 mg/m²/day), therapy was delayed ≥1 week for grade 3/4 leukopenia and/or thrombocytopenia in 2 of 5 hematological patients at 600 mg/m²/day × 5 days for 3 weeks. Thus the maximum-tolerated dose (MTD) for hematological patients was 300 mg/m²/day × 5 days for 3 weeks. One solid tumor patient on 900 mg/m²/day × 5 days for 3 weeks developed acute respiratory distress and grade 3 hypotension. Among 5 other patients at this dose no additional DLTs were observed. Mean terminal half-life (t½) ranged from 21 to 58 minutes, and increasing dose produced linear increases in the area under the concentration versus time curve (AUC). Acetylated histones were detected in PBMCs up to 4 hours post-infusion at higher dose levels. This was confirmed by immunohistochemical analyses of post-therapy tumor biopsies. Four patients, 2 with lymphoma and 2 with bladder cancer, experienced objective tumor regression with clinical improvement in tumor related symptoms.

As of June 2003, SAHA capsules have been administered to 95 patients with advanced solid tumors or hematologic malignancies in two phase 1 trials and two phase 2 trials (49). The MTD of oral SAHA is 400 mg q.d., or 200 mg b.i.d., or 300 mg b.i.d. × 3 days per week in patients with either solid tumors or hematologic malignancies. The DLTs are non-hematological (anorexia, dehydration, diarrhea, and fatigue). The most common hematologic adverse events are anemia and thrombocytopenia. These are rapidly reversible after study drug interruption.

Pharmacokinetic (PK) analysis demonstrated a bioavailability of approximately 46% for oral SAHA. Doses in the range of 200 to 600 mg were linearly proportional with maximum concentration (Cmax) and drug exposure (AUC). Mean t1/2s of oral SAHA (range: 92 to 150 minutes), are longer than the mean t1/2s after IV administration of the oral equivalent doses (range: 27 to 38 minutes), suggesting absorption rate limited disposition. Preliminary data suggest that the administration of SAHA with food does not appear to substantially alter the rate or extent of absorption. Inhibition of HDAC activity was achieved in PBMCs at the 200 mg dose level. At dose levels of 400 mg and 600 mg, duration of HDAC inhibition lasted at least 10 hours.

Significant antitumor activity in hematologic malignancies and solid tumors has been observed in these early studies. In the ongoing phase 1 study of oral SAHA in patients with advanced solid tumor and hematologic malignancies, 7 patients with heavily pre-treated diffuse large B cell lymphoma (DLBCL) were entered. One complete response (CR) and one PR were observed. In addition, one patient has had a significant PET scan response. The CR is ongoing with a duration exceeding 12 months. The PR lasted 5 months, and the PET scan response lasted 6 months. Antitumor activity has also been observed in patients with mesothelioma with decrease in tumor mass, pleural effusion, and improvement of tumor-related pain or shortness of breath. In the ongoing phase 2 study of SAHA in patients with heavily pretreated cutaneous T-cell lymphoma (CTCL) or peripheral T-cell lymphoma (PTCL), objective responses have been observed.

**Requests for Letters of Intent for Trials**

CTEP is soliciting for a single-agent pediatric phase 1 study of SAHA and single-agent phase 2 studies. Program priorities for the initial round of phase 2 studies include non-Hodgkin’s lymphoma, renal cell carcinoma, bladder cancer, breast cancer, lung cancer, mesothelioma, glioma, and thyroid cancer.

CTEP is also soliciting for phase 1 studies of SAHA in combination with 5-fluorouracil (5-FU) and other possible agents which may include anthracycline, platinum, taxane, imatinib mesylate, retinoids, trastuzumab, gemcitabine, hypomethylating agents, radiation therapy, and agents that block the function of Bcl-2. For these phase 1 combination studies, PK analyses of SAHA and the second agent should be obtained. Priority will be given to...
proposals with correlative studies directed at gaining insight into the molecular mechanisms involved in the induction of apoptosis in tumors by SAHA. Questions regarding this solicitation may be addressed to:

James Zwiebel, M.D., Associate Chief, Biologics Evaluation Section
Investigational Drug Branch, CTEP, NCI
Phone: 301-496-8798
E-mail: jz43j@nih.gov

Submit Letters of Intent (LOIs) for all trials by January 9, 2004. CTEP review of the submissions will be conducted over 4 to 6 weeks following that date, depending upon the number of LOIs submitted.

Investigators are encouraged to carefully consider the proposed prior therapy requirements for each group of patients and discuss with CTEP before LOI submission if there are questions. Please do not submit an LOI that will require revision of the prior therapy requirements if approved for a CTEP protocol. Protocols that do not adhere to the prior therapy commitments in the LOI are subject to disapproval.

Please do not submit trials for tumor types not included on this list without consulting with CTEP. All trials should include the RECIST response criteria.

Investigators who intend to request Translational Research Fund (TRF) support for laboratory correlative studies should submit an estimated budget with the LOI.

We ask that investigators who submit an LOI for this study indicate how soon after LOI approval they can submit a protocol. Phase 1 and 2 U01 grant holders are encouraged to participate in collaborations that can speed patient accrual.

Submit LOIs electronically to the LOI Coordinator at the CTEP Protocol Information Office (PIO), by January 9, 2004:

LOI Coordinator
E-mail: PIO@ctep.nci.nih.gov
Phone: 301-496-1367

Please use the most recent versions of the LOI form and the TRF Cost Estimate Worksheet available on the CTEP Website at: http://ctep.cancer.gov/guidelines/index.html

CTEP requests that investigators submit a protocol within 4 weeks of notification of LOI approval and work towards prompt clinical trial activation by responding promptly to the consensus review.

References


