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## Clinical experience with intravenous and oral formulations of the novel histone deacetylase inhibitor suberoylanilide hydroxamic acid in heavily pretreated patients with hematological malignancies

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**H**DACs are a novel class of anti-cancer drugs whose primary mechanism of action revolves around altering chromatin structure, and subsequently, gene expression. At present, there are several classes of compounds that have been identified that inhibit HDACs and cause transformed cell growth arrest, differentiation and/or apoptosis. These compounds have been shown to inhibit the activity of partially purified HDACs and to inhibit the growth of several types of cancers in tumor bearing models. Alterations in histone acetyl transferases (HATs) have been reported in several types of cancer, as genes encoding HATS have been translocated, amplified, over-expressed and/or mutated in different malignancies. Conversely, specific alterations in HDACs have not been reported in cancer, though HDACs are known to be associated with oncogenes and tumor suppressor genes. It is not entirely clear how HDACs facilitate cancer cell death or cytostasis, though it is conceivable that the mechanism may differ from cancer to cancer, depending upon the molecular lesions that dominate the phenotype. In lymphoma, one important mechanism of action may include inactivation of BCL6, primarily by promoting the accumulation of an inactive, acetylated form of the transcription factor.

The potential therapeutic benefit created by facilitating gene transcription has led to the development of HDAC inhibitors (HDACIs). HDACIs have the capacity to induce cytodifferentiation, cell cycle arrest and apoptosis in transformed cells. To date, four major classes of HDACIs have been identified, including: short chain fatty acids (e.g. 4-phenylbuterate and valproic acid), hydroxamic acids (e.g. trichostatin [TSA] and SAHA), cyclic tetrapeptides (e.g. depsipeptide [FR901228]), and benzamides (e.g. MS-275). Preclinical studies of HDACIs have demonstrated anti-tumor activity both *in vivo* and *in vitro* suggesting that the HDACIs may be potentially important nov-

el anti-cancer therapeutics. As discussed above, the broader therapeutic benefit of these compounds in hematologic malignancies may go well beyond 'simple' chromatin remodeling, and may also include effects on the acetylation status of BCL6, and other essential transcription factors.

Suberoylanilide hydroxamic acid (SAHA; Figure 1) is a hydroxamic acid based histone deacetylase (HDAC) inhibitor that is able to induce differentiation of murine erythroleukemia (MEL), human bladder transitional cell carcinoma (T24), and human breast adenocarcinoma (MCF-7). SAHA induces apoptosis in human myeloma cells (ARP-1) and human neuroblastoma cells (KCN-69n) and suppresses the growth of CWR22 human prostate xenografts. SAHA inhibits HDAC resulting in the accumulation of acetylated histones H2a, H2b, H3 and H4. SAHA's inhibition occurs through a direct interaction with the catalytic site of the enzyme.

Clinically significant activity of the histone deacetylase inhibitor SAHA in heavily pre-treated patients with advanced B-cell lymphoproliferative malignancies have been reported in 2 initial phase I studies assessing 2 formulations (IV and oral). The results of these studies are notable for a number of reasons. First, regardless of the formulation, SAHA was well tolerated with manageable toxicities, though the toxicity profile was different between the formulations. These differences may be related to differences in the pharmacokinetic profiles (i.e. C<sub>max</sub>/AUC). Second, in both studies, patients with HD experienced clinically meaningful benefit that included durable remissions of disease, which in 1 patient continued for well over 9 months. Thirdly, in the oral study, only patients with heavily pre-treated transformed lymphomas (n=2) experienced dramatic clinical improvements which included a complete remission and a partial remission. One patient with refractory cutaneous T-cell lymphoma also experienced a partial remission of dis-



**Figure 1. SAHA (Suberoylanilide hydroxamic acid).**

ease.

As mentioned above, HDACs are known to play a critical role in the pathogenesis of certain types of lymphoma, namely diffuse large B-cell lymphoma (DLBCL), where they may alter the regulation of the transcriptional repressor BCL6. BCL6 has been identified as the gene located at the recurrent chromosomal breakpoint on 3q27, which may occur in as many as 35% of these cases. Consistent with the involvement of BCL6 in malignant transformation, over-expression of BCL6 in RAT-1 fibroblasts results in anchorage independent growth and the ability to form colonies in soft agar. Expression of BCL6 inhibits differentiation of germinal center B cells and inhibits apoptosis.

HDACs are known to be recruited by BCL6. The BCL6 proto-oncogene was originally identified because of its involvement in 3q27 chromosomal translocations in DLBCL. BCL6 encodes a POZ/zinc finger transcriptional repressor expressed in mature germinal center B-cells. BCL6 protein suppresses genes involved in lymphocyte activation, differentiation, cell cycle arrest and apoptosis. Interestingly, the regulation of BCL6 activity is controlled at a number of different levels, which include the ubiquitin-proteasome pathway (BCL6 is a substrate for proteolytic degradation) and through histone acetylation/deacetylation mediated in part through the HAT p300. Expression of BCL6 inhibits differentiation of germinal center B cells and inhibits apoptosis. At the transcriptional level, acetylation of BCL6 down-regulates its activity by inhibiting its transcriptional repression function.

What makes BCL6 a potentially attractive target in lymphoma, is the fact that BCL6-mediated transcriptional repression is known to be dependent on HDAC, as trichostatin A (TSA), a potent and specific HDAC inhibitor for example, results in a dose-dependent inhibition of BCL6-mediated repression. Furthermore, treatment with TSA leads to the accumulation of acetylated BCL6 in the RAMOS B-cell lymphoma cell line. A second mechanism for deacetylation of BCL6 is through the SIR-2 pathway which is inhibited by niacinamide (NIA). Treatment of cells with NIA results in accumulation of acetylated BCL6, and combined treat-

ment with NIA and TSA is additive.

These findings have led to the hypothesis that treatment of lymphomas over-expressing BCL6 with HDAC inhibitors could inactivate BCL6, promoting apoptosis and differentiation, leading to tumor regression. Hence, in lymphoma, the inhibition of HDAC may have a broader panoply of effects on tumor cell biology compared to other malignancies. These effects are likely to revolve around both the chromatin remodeling effects and differential gene expression, as well as the effects mediated by repression of transcriptional activation mediated by BCL-6. Taken together, there is a strong mechanistic rationale for the use of HDACi in lymphoma.

Some of the earliest clinical experiences with HDACi employed phenylbutyrate (PB), which is metabolized to phenylacetate. Clinical trials of PB employed prolonged intravenous infusion schedules to maintain steady-state plasma concentrations of PB but were often associated with somnolence and confusion as the major DLTs. Clinical improvements in patients with leukemia and myelodysplastic syndrome were documented along with improved pain control and disease stabilization in patients with advanced solid tumor malignancies. Depsipeptide, or FR228, is a HDAC inhibitor isolated from *Chromobacterium violaceum* and has activity even at nanomolar concentrations. The major DLTs have included fatigue, malaise, vomiting, and thrombocytopenia. Other major toxicities have also included severe gastrointestinal toxicity, skin toxicity and cardiac toxicity. Early observations from the phase I experience recently led to a case report from the National Cancer Institute, where they presented 4 patients with CTCL who had been treated on a Phase I study of depsipeptide (FR901228) who attained major remissions of their disease. A phase 2 study of depsipeptide was initiated in patients with only CTCL using a slightly different schedule, administering depsipeptide as a 4-hour infusion on days 1, 8 and 15 of a 28 day cycle. Eight of 14 evaluable patients achieved a major remission, including 3 who achieved complete remission. In a similar study, Duvic began a Phase II of SAHA in patients with CTCL at a dose of 400 mg per day. Five of 13 patients on study achieved partial remissions as measured by the Physician Global Assessment, 5 had stable disease and 3 experienced progression of disease on study. Interestingly, major partial remissions were noted in patients with large cell transformation of their cutaneous tumors. The mean duration of these responses was about 15 weeks (8 to 24 weeks). More than 50% of patients reported clinically significant decreases in pruritus.

In conclusion, there is mounting scientific and clinical evidence that targeting HDAC is a potentially

important therapeutic target in the treatment of lymphoma. SAHA clearly has activity at well tolerated doses in very heavily pre-treated patients with lymphoma. Coupled with the mechanistic rationale and effect on Bcl-6, it is clear future studies in patients with lymphoproliferative malignancies should address this underlying biology across other sub-types of lym-

phoma, perhaps as a function of different doses and schedules. At this time, it is also unclear precisely how to integrate these agents in the conventional treatment paradigms of lymphoma. Future pre-clinical and clinical studies are warranted in order to clarify how best to exploit this promising new class of drugs in the treatment of lymphoma.