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## Therapeutic applications of histone deacetylase inhibitors in sarcoma

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### Abstract

Sarcomas are a rare group of malignant tumors originating from mesenchymal stem cells. Surgery, radiation and chemotherapy are currently the only standard treatments for sarcoma. However, their response rates to chemotherapy are quite low. Toxic side effects and multi-drug chemoresistance make treatment even more challenging. Therefore, better drugs to treat sarcomas are needed. Histone deacetylase inhibitors (HDAC inhibitors, HDACi, HDIs) are epigenetic modifying agents that can inhibit sarcoma growth *in vitro* and *in vivo* through a variety of pathways, including inducing tumor cell apoptosis, causing cell cycle arrest, impairing tumor invasion and preventing metastasis. Importantly, preclinical studies have revealed that HDIs can not only sensitize sarcomas to chemotherapy and radiotherapy, but also increase treatment responses when combined with other chemotherapeutic drugs. Several phase I and II clinical trials have been conducted to assess the efficacy of HDIs either as monotherapy or in combination with standard chemotherapeutic agents or targeted therapeutic drugs for sarcomas. Combination regimen for sarcomas appear to be more promising than monotherapy when using HDIs. This review summarizes our current understanding and therapeutic applications of HDIs in sarcomas.

### Keywords

Sarcoma; Epigenetic; Histone acetylation; Histone deacetylases inhibitor (HDI)

## 1. Introduction

Sarcomas are malignant tumors that arise from transformed cells of mesenchymal origin, in contrast to carcinomas which originate from epithelial cells. Sarcomas account for over 20%

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of all pediatric solid malignancies and less than 1% of all adult solid malignancies<sup>1</sup>. More than 50 distinct histologic sarcoma subtypes exist and many of these subtypes can occur at any age and are not restricted to a specific location of the body<sup>1</sup>. The rarity of this disease combined with the diverse number of subtypes present challenges in formulating a consensus on the treatment of sarcomas. Standard treatment modalities may include surgery and radiation for local control and chemotherapy for adjuvant and palliative therapy. With the progress of modern multimodality therapies, the 5-year survival rate in some types of sarcoma, such as in osteosarcoma, has increased to over 70%. However, progress has slowed over the past 30 years, and efforts to improve outcomes with intensifying regimens or adding novel agents have brought disappointing results. Additionally, for patients with metastatic sarcoma at diagnosis and for those with relapsed disease, outcomes are remarkably poor with 4- to 5-year overall survival rate at less than 20%. Furthermore, current chemotherapeutic drugs are generally not effective in some types of sarcomas, such as chondrosarcoma or chordoma. Therefore, the development of new therapeutic strategies is critical for achieving positive results in sarcoma treatment.

The critical mechanisms that drive sarcoma cell proliferation and growth remain largely unclear. However, an increasing amount of data have shown that histone deacetylases (HDACs) influence diverse cellular processes and contribute to sarcoma growth and progression by multiple mechanisms. HDAC inhibitors (HDAC inhibitors, HDACi, HDIs) are epigenetic drugs that can regulate gene expression through action on histones as well as non-histone proteins without changing the gene sequence. HDIs have emerged over the past 10 years as potential treatment options based on their ability to inhibit tumor growth in vitro and in vivo. The pan-HDI, vorinostat (ZOLINZA™, Merck & Co., Inc.), was the first HDI approved by U.S. Food and Drug Administration (FDA) for patients with cutaneous tumor and peripheral T-cell lymphoma in 2006<sup>2</sup>. Also approved were belinostat (BELEODAQ™, Spectrum Pharmaceuticals, Inc.) for relapsed or refractory peripheral T-cell Lymphoma and panobinostat (FARYDAK®, Novartis Pharmaceuticals Co.) for multiple myeloma<sup>3, 4</sup>. Although single-agent treatment with HDI has demonstrated efficacy in hematologic malignancies, benefits in patients with sarcomas have yet to be realized. The acceptable toxicity profile has led to combinations with other currently approved agents, including chemotherapeutics, radiation, and tyrosine kinase inhibitors, in which HDIs may appear to be a promising modulator in sarcoma treatment. Here, we review the current knowledge of the expression, function of HDAC, preclinical findings and clinical trials using HDIs in sarcoma research and chemotherapy. We also discuss the mechanisms of resistance to HDIs in the treatment of sarcoma.

## 2. HDAC and HDI in sarcoma

### 2.1 Definition of histone acetylation and histone deacetylation

Histone acetylation and deacetylation are reversible processes that are regulated by histone acetyltransferases (HATs) and HDACs. Acetylation removes the positive charge on the histones, thereby decreasing the interaction of the N terminus of histones with the negatively charged phosphate groups of DNA. As a consequence, the chromatin is transformed into a more relaxed state. And in this state, the DNA is more accessible, leading to more

transcription factors being able to reach the DNA. Thus, acetylation of histones is known to increase the expression of genes, leading to gene expression in the multiple pathways involved in proliferation, migration, angiogenesis and differentiation. Deacetylation performed by HDACs has the opposite effect. By deacetylating the histone tails, the DNA becomes more tightly wrapped around the histone cores, making it harder for transcription factors to bind to the DNA. This leads to decreased levels of gene expression and is known as gene silencing (Fig. 1). Dysregulation and overexpression of HDACs have been observed in many cancer types including sarcomas. The upregulation of HDACs that inhibit or silence the growth suppressor genes is an important mechanism to promote cancer cell proliferation. As such, HDIs can be used to halt cancer cell growth.

## 2.2 Classification of HDACs

To date, eighteen distinct HDACs have been identified and they are classified into four groups based on their structural divergence, namely class I, II, III and IV HDACs (Table 1) <sup>5</sup>. They are subdivided into Zn<sup>2+</sup>-dependent (classes I, II, and IV) which are referred as the ‘classical family’, and nicotinamide adenine dinucleotide (NAD<sup>+</sup>)-dependent (class III) enzymes <sup>6</sup>. Class I HDAC consists of HDACs 1, 2, 3, and 8, that are primarily localized in the nucleus and ubiquitously expressed in all tissues. Class I HDACs have the deacetylase domain located at their N-terminus and carry a variable C-terminus depending on the specific HDAC of the class. Class II HDACs are localized both in the nucleus as well as the cytoplasm. They are divided into class IIa including HDAC 4, 5, 7, 9, and class IIb which is consisted of HDAC 6 and 10. Class II HDACs have the deacetylase domain at the C-terminus with the exception of HDAC 6, which contains two acetylase domains at both the N- and C-termini. Class III HDACs are homologues of yeast silent information regulator 2 proteins and consist of Sirtuins 1–7 <sup>7</sup>. Sirtuins are distinct from Class I and II HDACs because of their enzymatic dependence on the coenzyme nicotinamide adenine dinucleotide (NAD<sup>+</sup>-dependent) for deacetylase activity, rather than Zn<sup>2+</sup>-dependent. HDAC 11 is the only member of class IV HDAC, which has the properties of both class I and class II HDACs <sup>8</sup>.

## 2.3 Expression and clinical significance of HDACs in sarcomas

HDACs have been closely linked with malignant phenotypes in tumorigenesis. In most sarcoma cases, a high level of HDACs is associated with advanced disease and poor clinical outcomes. For instance, HDACs 1, 4, 6, 7, and 8 have been shown to exhibit a high frequency of strong immunoreactivity and were associated with a lower disease-free survival trend, which may represent potential therapeutic targets for endometrial stromal sarcoma <sup>9</sup>. HDAC 2 may also be a predictive therapeutic biomarker of endometrial stromal sarcoma, as its expression is much higher than that of HDAC 1 <sup>10</sup>. In chondrosarcoma, HDAC 4 represses vascular endothelial growth factor (VEGF) expression by modulating RUNX2 activity, resulting in tumor angiogenesis inhibition <sup>11</sup>. HDAC 4 is also one of the targets of miR-1. Upregulation of HDAC 4 contributed to chordoma pathogenesis, and downregulation of miR-1 was likely to be one factor that is involved in this process <sup>12</sup>. Besides, Scheipl et al. confirmed the expression of HDAC4 in chordomas and additionally they described the immuno-histochemical expression of HDACs 2–6 in chordoma collective with the strongest expression of HDAC 6 <sup>13</sup>. Future research is needed to address the role of HDAC6 and the

cellular functions mediated by this epigenetic enzyme in chordoma pathogenesis. In osteosarcoma, overexpression of HDAC5 could promote proliferation of cancer cells, due to the ability of upregulating mRNA expression of twist 1, which has been reported as an oncogene<sup>14</sup>.  $\beta$ -catenin is an oncogene and a substrate of HDAC 6. In osteosarcoma, rho-associated coiled-coil kinase regulated the stability of  $\beta$ -catenin by preventing TPPP1-mediated inhibition of HDAC 6 activity<sup>15</sup>.

Sirtuin 1 is a class III HDAC and it may also serve as a therapeutic predictor biomarker of sarcomas<sup>16, 17</sup>. Strong Sirtuin 1 expression was observed in leiomyoma, leiomyosarcoma, rhabdomyosarcoma and osteosarcoma<sup>18</sup>. Overexpression of Sirtuin 1, DBC1,  $\beta$ -catenin, and cyclin D1 were significantly correlated with higher clinical stage, higher histological grade, increased mitotic counts, and higher potential of distant metastasis. More importantly, the expression of Sirtuin 1 was an independent prognostic indicator for metastatic potency and disease-free survival of sarcoma patients<sup>19</sup>. Like HDAC 4, Sirtuin 1 is also one of the targets of some miRNAs. For example, miR-126, miR-133b, miR-204 inhibited the proliferation, migration, invasion and epithelial-mesenchymal transition of osteosarcoma cells via targeting Sirtuin 1<sup>20–22</sup>.

## 2.4 Classification of HDIs

HDIs include a broad class of compounds that can be divided into pan- HDIs and specific HDIs according to their specificity of action (Table 2). Pan-HDIs are compounds that can inhibit the activity of more than one class of HDACs. The most common pan-HDIs are vorinostat, panobinostat, belinostat and trichostatin A. In contrast, specific HDIs work only on one class or a single specific HDAC. For instance, tenovin-6 is a specific class III HDI that inhibits the activity of Sirtuin 1 and Sirtuin 2. Romidepsin (Istodax®, Gloucester Pharmaceuticals Inc.), also known as FK-228, FR901228, and depsipeptide, is a specific class I HDI that has been approved by the U.S. FDA for the clinical treatment of cutaneous T cell lymphoma<sup>23</sup>. There are also some novel HDIs, like PCI-48012 and PCI-34051, which specifically inhibit the activity of HDAC 8. Both pan-HDIs and specific HDIs are in development, and there is uncertainty which will be more successful in sarcoma treatment.

## 3 Molecular mechanisms of HDIs in sarcoma

### 3.1 HDIs upregulate tumor suppressor genes and downregulate oncogenes

Unbalance of the tumor suppressor *p53* and the oncogene *MDM2* feedback loop contributes to tumorigenesis. In osteosarcoma, HDI sodium butyrate inhibited the proliferation of tumor cells by enhancing *p53* expression, and conversely, decreasing *MDM2* expressing<sup>24</sup>. HDIs, panobinostat and vorinostat, upregulated the expression of tumor suppressor gene *PTEN* and *p21* in well-differentiated liposarcoma. The same combined treatment resulted in dephosphorylation and depletion of *MDM2* and *TP53*, irrespective of *p53* mutational status in *MDM2*-amplified liposarcoma<sup>25</sup>. HDIs have also enhanced the transcriptional function of *p53* by directly stabilizing the acetylation of *p53*. For example, HDI trichostatin A promoted *p53*-*p300* interaction and recruitment of *p53* Lys-382 to promoter regions of its target genes *p21* and *PUMA*, consequently upregulating their expression in Ewing sarcoma cells<sup>26</sup>.

Polo-like kinase 2 (*Plk2*) has tumor suppressor functions which were regulated by the tumor microenvironment. Trichostatin A upregulated *Plk2* gene expression by directly enhancing GATA-1 acetylation in human osteosarcoma <sup>27</sup>. In epithelioid sarcoma, pan-HDIs, vorinostat and entinostat, induced widespread gene expression changes, and among these, EZH2 was significantly downregulated leading to abrogated cell growth in vitro <sup>28</sup>. Retinoid X receptors (RXRs) and retinoic acid receptors (RARs) are nuclear receptors that mediate the biological effects of retinoids by their involvement in retinoic acid-mediated gene activation. HDI valproic acid restored the expression of RXR $\alpha$  target genes *RAR $\beta$* , *CRABP1* and *p21*, and conversely, repressed the expression of fusion oncogenes, *EWS-ERG* and *EWS-Fli1* in Ewing sarcoma cells <sup>29</sup>. Plakoglobin is a member of the catenin protein family and a homologue to  $\beta$ -catenin. Promoter regions (P1–P3) of plakoglobin gene were associated with hypoacetylated H4 histone in embryonal rhabdomyosarcoma <sup>30</sup>. HDI trichostatin A activated the Tcf/Lef target promoter partly by upregulation of plakoglobin expression in human fibrosarcoma <sup>31</sup>.

Many sarcomas bear fusion oncogenes like *SS18-SSX* in synovial sarcoma, *EWS-Fli1* in Ewing sarcoma and *Pax3:Foxo1* in embryonal rhabdomyosarcoma. These sarcomas were more sensitive to HDI treatment than other sarcomas lacking known translocations. The underlying mechanism may be related to HDI's inhibiting fusion oncogene activity by suppressing gene transcriptional activity, or directly acetylating the fusion oncogene proteins. For example, HDIs, entinostat and romidepsin, decreased the expression of fusion oncoprotein EWS-ATF1 in clear cell sarcoma <sup>32</sup>. SS18-SSX while serving as a bridge between activating transcription factor 2 (ATF2) and transducin-like enhancer of split 1 (TLE1), resulted in repression of ATF2 target genes. Besides, the fusion oncoprotein SS18-SSX via TCF/LEF, TLE1 and HDAC interaction leads to an upregulation of AXIN2, which is involved in the WNT pathway but without direction interaction with the pathway <sup>33</sup>. Romidepsin significantly suppressed the growth of synovial sarcoma cells compared with that of osteosarcoma, as it impacted SS18-SSX target gene expression by preventing TLE1 complex recruitment <sup>34,35</sup>. Early growth response-1 (*EGR1*) has been suggested as a tumor suppressor as its overexpression dramatically inhibits tumor cell growth. HDI, romidepsin, reversed SS18-SSX-mediated polycomb silencing of *EGR1* in synovial sarcoma <sup>36</sup>. In rhabdomyosarcoma, HDI, entinostat, directly suppressed the activity of *Pax3:Foxo1* at the transcriptional level. As a result, *Pax3:Foxo1*-positive embryonal rhabdomyosarcoma was transformed fusion-negative like state in rhabdomyosarcoma <sup>37</sup>.

### 3.2 HDIs induce apoptosis in sarcoma cells

Apoptosis, programmed cell death, is probably one of the most widely-studied subjects among cancer cell biologists <sup>38</sup>. Using HDIs to treat sarcomas can not only directly induce apoptosis through the extrinsic death receptor pathway and intrinsic mitochondrial pathway, but also enhances the susceptibility of sarcoma cells to apoptosis (Fig. 2). Fas and TRAIL are cell surface death receptors belonging to the tumor necrosis factor super family, which trigger apoptosis upon ligand binding <sup>39</sup>. HDIs vorinostat and sodium butyrate induced tumor cell death and enhanced the apoptosis-inducing activity of TRAIL in Ewing's sarcoma <sup>40</sup>. Additionally, HDIs also increased Fas expression and regulated the localization of Fas on tumor cell membranes. Entinostat, a specific class I HDI, promoted Fas expression

by increasing histone acetylation of the Fas gene promoter and mRNA expression<sup>41</sup>. HDI valproic acid enhanced human osteosarcoma cell susceptibility to Fas-induced cell death by decreasing the secretion of soluble Fas and increasing the sensitivity to Fas mediated cell death<sup>42</sup>. However, another study has demonstrated that entinostat sensitized osteosarcoma cells to Fas L treatment is not mediated through inducing Fas expression on the cell surface, instead lipid rafts are required<sup>43</sup>. Cellular FLIP(c-FLIP) is a master anti-apoptotic regulator which can decrease the localization of Fas in lipid rafts. Some sarcoma cells exhibit a phenotype resistant to death receptor-mediated apoptosis by expressing c-FLIP. However, HDIs sensitize such resistant sarcoma cells by directly downregulating c-FLIP expression. For example, HDI romidepsin downregulate c-FLIP by inhibiting generation of FLIP mRNA, rather than stimulating degradation at either the protein or mRNA level<sup>44</sup>. In vivo, oral HDI entinostat administered to mice with osteosarcoma lung metastases resulted in decreased c-FLIP in tumor nodules and tumor regression<sup>45</sup>.

The intrinsic mitochondrial apoptotic pathway is closely regulated by a group of proteins belonging to the BCL-2 family, which can be either pro-apoptotic including BAX, BAK and BOK among others or anti-apoptotic such as BCL-XL and BCL-W<sup>46</sup>. Combination treatment of HDI valproic acid and chloroquine for human osteosarcoma cells led to an increase of BAX expression and a decrease of BCL-2 and BCL-XL expression<sup>47</sup>. HDIs have also induced apoptosis through common caspase pathway in sarcoma. In addition to upregulating Fas expression, romidepsin also induced apoptosis by activating caspase-8 and caspase-3 in osteosarcoma<sup>48</sup>. In osteosarcoma, trichostatin A induced apoptosis of tumor cells partly via the activation of procaspase-3 and cleavage of PARP<sup>49</sup>. In uterine sarcoma, vorinostat induced immediate apoptotic cell death as supported by upregulation of caspase-9 as well as activation of caspases-3, caspases-7 and PARP cleavage<sup>50</sup>.

HDIs have also induced apoptosis in sarcoma cells through the gene signaling pathway. In synovial sarcoma, PTEN is crucial for EGR1-induced apoptosis. EGR1 reactivation by HDIs promoted cell death in sarcoma by PTEN tumor suppressor<sup>51</sup>. HDI plus phosphoinositide 3-kinase (PI3K) inhibitors were identified as specific activators of E2F1/FOXO transcription. Combining the HDI vorinostat with a PI3K inhibitor led to enhanced FOXO-dependent apoptosis in osteosarcoma cells<sup>52</sup>. In addition, HDI trichostatin A significantly inhibited the human osteosarcoma cells growth and promoted apoptosis in a dose-dependent manner through p53 signaling pathway activation<sup>53</sup>.

### 3.3 HDIs induce cell cycle arrest in sarcomas

HDAC 1 and 2 directly bind to the promoters of the cell cycle associated *p21* gene and negatively regulate its expression<sup>54</sup>. HDAC 2-siRNA knockdown led to p21 increment and arrested endometrial stromal sarcoma cell proliferation<sup>55</sup>. Epigenetically, accumulation of acetylated histones and induction of p21 expression were observed in human rhabdomyosarcoma cells and uterine sarcomas cells exposed to HDACI vorinostat<sup>56, 57</sup>. A recent study has shown HDI trichostatin A induced G1 cell cycle arrest in osteosarcoma cells via the p53-independent activation of p21 promoter through the specific Sp1 sites<sup>58</sup>. Fusion oncoprotein EWS-Fli1 downregulated the expression of p21 by inhibiting the p300-

mediated transactivation of the p21 gene<sup>59</sup>. However, HDI romidepsin strongly induced p21 expression by inhibiting the expression of *EWS-Fli1* at protein and mRNA levels<sup>60</sup>.

Two new HDIs, PCI-34051 and PCI-48012, specifically inhibited the activity of HDAC 8 leading to marked S-phase cell cycle arrest in human malignant peripheral nerve sheath tumors cells<sup>61</sup>. In osteosarcoma, vorinostat arrested the cell cycle in G1 and G2/M phase, while HDI sodium butyrate arrested the cell cycle in G2/M phase<sup>62</sup>. In chondroma, trichostatin A arrested the cell cycle in G2/M phase but valproic acid arrested the cell cycle in G1 phase<sup>63</sup>. Gadd45, a p53-regulator and DNA damage inducible protein, has recently been demonstrated to play a role in the G2-M checkpoint in response to DNA damage<sup>64</sup>. Trichostatin A increased gadd45 mRNA and protein levels directly through targeting its promoter without the need of functional p53, leading to G2/M cell cycle arrest in human osteosarcoma cells<sup>65</sup>.

### 3.4 HDIs decrease invasion, metastasis and angiogenesis in sarcomas

HDIs attenuated the expression of hypoxia inducible factor 1 alpha (HIF-1 $\alpha$ ) that led to a decrease of chordoma cell invasion<sup>66</sup>. Invadopodia are specialized membrane protrusions that are associated with degradation of the extracellular matrix in cancer invasiveness and metastasis. In fibrosarcoma, HDAC 6 served as a key participant of hypoxia-induced cell invasion. One study has shown hypoxia induced invadopodia formation in a biphasic manner, which involves the activation of HDAC 6 deacetylase activity by EGFR<sup>67</sup>. HDI vorinostat inhibits the metastatic potential of highly metastatic osteosarcoma cells by reducing invadopodia formation and decreasing the expressions of metastasis-associated factors, including *mTOR* and *ALDH* gene<sup>68</sup>. Furthermore, vorinostat suppresses embryonal rhabdomyosarcoma growth by reducing the self-renewal and migratory capacity of tumor cell in vitro. In this process, EFNB1 is regulated by vorinostat to modulate migratory behavior of embryonal rhabdomyosarcoma cells<sup>69</sup>. Matrix metalloproteinase 1 (MMP-1) is involved in the miR-10b and brain-derived neurotrophic factor (BDNF)-mediated chondrosarcoma cell migration and invasion. Treatment with DNA methyltransferase (DNMT) inhibitor 5-aza-dC and HDACI 4-phenylbutyric acid cause markedly reduced of MMP-1 expression, which further suppressed the migratory and invasive capacities of chondrosarcoma cells<sup>70</sup>. Overexpression of gelatinase A, also known as MMP-2, is associated with pulmonary metastasis and related to the prognosis of osteosarcoma<sup>71</sup>. HDI trichostatin A has acted to control metastatic potential by reducing the ability of metastatic cells to recruit stromal cells to secrete MMP-2 in fibrosarcoma<sup>72</sup>.

### 3.5 HDIs inhibit sarcoma growth through regulating autophagy

The role of autophagy in sarcoma is complex and is likely dependent on sarcoma type-associated genetic context. HDI vorinostat inhibits sarcoma growth that is associated with autophagy upregulation, however, the class III HDI tenovin-6 impairs the autophagy process which induces cell death in sarcoma<sup>73</sup>. MTOR is one of the factors involved in the process of upregulating autophagy under vorinostat treatment. In osteosarcoma, vorinostat upregulates autophagy by decreasing *mTOR* gene expression and increasing LC3 expression<sup>68</sup>. In addition, vorinostat significantly inhibits the proliferation of endometrial stromal sarcoma cells via activation of autophagy by decreasing mTOR and phospho-mTOR

expression<sup>74</sup>. Furthermore, in rat chondrosarcoma cells, vorinostat induces autophagy-associated cell death which was confirmed by the detection of autophagosome-specific protein and specific ultrastructural morphology in the cytoplasm<sup>75</sup>.

### 3.6 HDIs induce reactive oxygen species (ROS) generation in sarcomas

ROS is a double-edged sword in cancer and most of the chemotherapeutic and radiotherapeutic agents kill cancer cells by augmenting ROS stress<sup>76</sup>. An increase of ROS generation was also found in sarcoma cells after treatment with HDIs. In osteosarcoma, the cytotoxic effect of the combination treatment of chloroquine and HDI valproic acid trigger the generation of ROS, leading to an increase of the apoptosis related gene expression and a decrease of anti-apoptosis related gene expression<sup>47</sup>. Melatonin inhibits osteosarcoma cell growth, targeting Sirtuin 1 signaling partly by increasing the ROS generation. Sirtinol, a known Sirtuin 1 inhibitor, further enhances the antitumor activity of melatonin<sup>77</sup>. Pan-HDIs panobinostat and vorinostat induce the generation of ROS, which are regarded as highly effective inhibitors of rhabdomyosarcoma in vitro and in vivo<sup>78</sup>. In a later study, combination treatment of HDI quisinostat and proteasome inhibitors lead to elevated endoplasmic reticulum stress, activation of pro-apoptotic effector proteins and increased levels of ROS in synovial sarcoma<sup>79</sup>.

### 3.7 HDIs induce sarcoma cell differentiation

Treating malignant tumor by induction of cell differentiation has been an attractive concept, but clinical development of differentiation-inducing agents to treat malignant tumor, especially for sarcomas, has been limited<sup>80</sup>. Long-term treatment with low dose HDI depsipeptide induces cell differentiation in chondrosarcoma<sup>81</sup>. HDAC 2 inhibition by valproic acid in endometrial stromal sarcoma cells lead to differentiation and arrested cell proliferation<sup>55</sup>. Low-dose panobinostat acts predominantly as a potent “differentiating” agent, as differentiation of alternative mesenchymal lineages is induced when treating osteosarcoma cells<sup>82</sup>. Notch1- and EphrinB1-mediated pathways are directly regulated by HDACs to inhibit myogenic differentiation and enhance migratory capacity of embryonal rhabdomyosarcoma cells. Epigenetic treatment with trichostatin A or vorinostat can reduce the expressing of Notch1 and EphrinB1 in embryonal rhabdomyosarcoma cells<sup>69</sup>. Moreover, a new class I and II HDI, MC1742, promote the sarcoma stem cells to osteogenic differentiation at nontoxic doses<sup>83</sup>.

## 4 Preclinical studies of HDIs in sarcoma

### 4.1 Sensitizing sarcoma to radiotherapy

HDIs could be radiation sensitizers that augment the response of sarcomas to radiation. Addition of vorinostat to radiotherapy has been shown to induce apoptosis and cause cell cycle arrest through elevated p53, p21, and Fas expression in osteosarcoma cell lines<sup>84</sup>. Moreover, vorinostat enhances radiation in osteosarcoma and rhabdomyosarcoma cells by inhibiting the expression of radiation-induced DNA repair proteins such as Rad51 and Ku80<sup>85</sup>. Heavy ion radiotherapy has been shown certain therapeutic advantages including potentially reducing undesirable side-effects, lower risk of secondary malignancies and improvement of clinical outcome and quality of life when compared with conventional



photon radiotherapy. In osteosarcoma xenografts, the combination of vorinostat and heavy ion radiotherapy induce a significant delay of tumor growth through the increased rate of apoptosis, increased expression of p53 and p21, and inhibition of proliferation and angiogenesis compared to osteosarcomas treated with heavy ion radiotherapy only <sup>86</sup>.

#### 4.2 Sensitizing sarcoma to chemotherapy and reverse multidrug resistance

HDI's such as vorinostat, quisinostat and abexinostat have been shown to increase drug sensitivity in the cells of specific sarcomas. Vorinostat and doxorubicin synergistically induce apoptosis of fibrosarcoma cells and inhibited fibrosarcoma xenograft growth more than when treated with either agent alone<sup>87</sup>. HDI's vorinostat and quisinostat sensitize rhabdomyosarcoma cells to doxorubicin-induced apoptosis by changing the ratio of pro- and anti-apoptotic BCL-2 family proteins with the downregulation of MCL-1 and BCL-XL, dephosphorylation of BCL-2 and upregulation of BimEL, thus shifting the balance towards apoptosis <sup>88</sup>. In addition, the combination treatment of vorinostat and doxorubicin cooperate to induce caspase activation and caspase-dependent apoptosis <sup>89</sup>. Temozolomide is an oral chemotherapeutic drug that methylate DNA primarily at O6-guanine. Vorinostat enhances cytotoxicity of temozolomide by downregulating cyclin D1 to induce G0/G1 cell cycle arrest and promoting apoptosis by cleavage of caspase-3 and PARP in Ewing sarcoma cells<sup>90</sup>. Doxorubicin and etoposide are both topoisomerase II enzyme inhibitors. Vorinostat antagonistically affects the antiproliferative effect of doxorubicin in majority Ewing sarcoma cells, but synergistically enhances the antiproliferative activity of etoposide<sup>91</sup>. One explanation of the different combined effects was that the molecular mechanisms of the two chemotherapeutics may be different. Doxorubicin performs DNA double-strand damage repair through pathways other than etoposide. Etoposide and doxorubicin also show different repair kinetics<sup>92</sup>. The manifestation may also be explained by the different biological background of sarcomas. Some sarcomas are with chromatin translocations resulting in abnormal fusion proteins like Ewing sarcomas, synovial sarcoma, and embryonal rhabdomyosarcoma. The fact was that these sarcomas with fusion oncogenes are more sensitive to HDI's treatment than sarcomas lacking known translocations. Abexinostat, an HDI, exhibits significant anti-sarcoma proliferative activity in vitro, by inducing S phase depletion, G2/M cell cycle arrest, and increasing apoptosis. Superior effects are seen when combined with chemotherapy. An abexinostat-induced reduction in Rad51, a major mediator of DNA double-strand break homologous recombination repair, has been shown as a mechanism underlying abexinostat chemo-sensitization <sup>93</sup>. The most effective tumor growth inhibition in vitro was observed with doxorubicin and the HDI vorinostat. In the in vivo xenograft mouse model, the combination treatment of doxorubicin and vorinostat did not reduce the tumor growth<sup>94</sup>. In this study, xenograft animal models were established by subcutaneously injecting the stable oligoclonal cell cultures from freshly tumor species of two patients into immuno-deficient mice. So, the treatment results in the xenograft model were more reliable than in tumor derived cell cultures, as tumor derived cell cultures may do not reflect the actual treatment condition that is observed in clinical patients. Future in vivo models are required to test HDI's and potential chemotherapeutics for the treatment of sarcomas prior to clinical use.

The roles of HDIs in multi-drug resistant sarcomas have been investigated recently. Abexinostat has a synergistic effect on doxorubicin-induced apoptosis in bone sarcoma cells<sup>95</sup>. Instead of influencing the protein expression level of P-gp, abexinostat reverses drug resistance in multidrug-resistant sarcoma cells by inhibiting of Rad51 expression and inducing the Gadd45 $\alpha$  expression<sup>96</sup>. In doxorubicin-resistant osteosarcoma cells, treatment involving both DNMT inhibitors and HDIs inhibit tumor growth by inducing cell growth arrest and reprogramming multidrug resistance osteosarcoma cells to osteoblast differentiation<sup>97</sup>.

### 4.3 Synergistic effect of HDIs on kinase inhibitor pazopanib

Patients with alveolar soft part sarcoma, undifferentiated pleomorphic sarcoma and synovial sarcoma have shown promising clinical outcomes after treatment with pazopanib<sup>98</sup>. Combination treatment of pazopanib and HDI vorinostat have a synergistic effect on killing sarcoma cells. In vitro, co-treatment increases autophagy, inactivates mTOR and activates AKT in sarcoma cells. Knockdown of autophagy associated Beclin1 or Atg 5 reduces drug combination toxicity. In vivo, treatment of xenografted mice carrying sarcomas with pazopanib and valproic acid results in a greater than additive reduction in tumor volume compared with single treatment with either drug<sup>99</sup>.

## 5 HDIs interact with other agents on sarcoma

### 5.1 Synergistic effect of HDIs and DNMT inhibitors

DNMT inhibitor is another group of epigenetic drugs that has proven useful for the treatment of cancers. Deregulation of epigenetic silencing by histone acetylation and DNA hyper-methylation might play a fundamental role in the etiology of uterine sarcomas<sup>50</sup>. Comparable effects are achieved in osteosarcoma cells using vorinostat and the DNMT inhibitor zebularine, with significantly more pronounced cytotoxicity in cells whose molecular phenotypes are indicative of aggressive biological behavior<sup>100</sup>. The HDIs entinostat, trichostatin A, phenylbutyrate, LAQ824 and depsipeptide enhance the anti-neoplastic action of 5-aza-dC on Ewing sarcoma cells (Table 3). Combination of 5-aza-dC and entinostat shows marked synergy, and is correlated with significant reactivation of the expression of two tumor suppressor genes, E-cadherin and tumor suppressor lung cancer-1<sup>101</sup>. In addition, targeting of DNMT and HDAC activities is highly effective in preventing formation of *Ptch*-associated tumors. For example, in a study that adopted heterozygous *Ptch* knockout mice, 5-aza-dC and HDI valproic acid combination therapy reactivates wild-type *Ptch* expression by reducing methylation of the *Ptch* promoter and induction of histone hyperacetylation, effectively preventing rhabdomyosarcoma formation<sup>102</sup>.

### 5.2 HDIs augment NK cell immunotherapy of sarcomas

Recent studies have indicated that HDIs can augment NK cell immunotherapy of sarcomas (Table 3). HDI entinostat enhances NK cell killing of sarcoma cells through upregulation of both NKG2D on human NK cells and its ligands MICA/B expression on sarcoma cells<sup>103</sup>. Both hydralazine and HDI valproic acid enhance the susceptibility of osteosarcoma cells to NK cell-mediated cell death, and combination treatment further enhanced the effects.

Mechanistically, valproic acid and hydralazine increase the expression of cell-surface MICA and B in osteosarcoma cells, and their combination induces a greater increase in their expression. In addition, valproic acid inhibits the production of both soluble MICA and MICB in osteosarcoma cell lines<sup>104</sup>. Chemotherapy-resistant Ewing sarcoma exhibits reduced susceptibility to resting NK cells. Pretreatment with HDI entinostat induces the expression of NKG2D ligands in an ATM/ATR-dependent manner and sensitizes chemotherapy-resistant Ewing sarcoma cells for NKG2D-dependent cytotoxicity<sup>105</sup>.

### 5.3 HDIs interact with PI3K and mTOR inhibitors

Vorinostat combined with inhibitors of PI3K and mTOR may represent an efficient therapy option for patients with endometrial stromal sarcoma or synovial sarcoma (Table 3). Single vorinostat treatment induces autophagy in endometrial stromal sarcoma cells via inhibition of mTOR activation and reduces the growth of endometrial stromal sarcoma cell lines by inhibiting protein kinase B AKT and mTOR/p70S6K cascade activation. Combination of vorinostat and PI3K and mTOR inhibitors reduces p-p70S6K/p-4E-BP1 and cell growth to the lowest measured levels in endometrial stromal sarcoma cells lines<sup>106</sup>. Ridaforolimus is a small-molecule inhibitor of mTOR. In synovial sarcoma cell lines, the combination of ridaforolimus and vorinostat demonstrates in vitro synergism, as vorinostat abrogates ridaforolimus-induced AKT activation which may be a possible mechanism of resistance to mTOR inhibitors<sup>107</sup>.

## 6 Clinical trials of HDIs in sarcoma

Based on the significant anti-sarcoma activities of HDIs as single agents in preclinical studies, beneficial performance of HDIs in clinical treatment for patients with sarcomas is anticipated (Table 4). In one case report, a female with Ewing sarcoma developed inoperable progressive lung metastases and was treated with the panobinostat. During 18 months of treatment, no new lesions appeared<sup>108</sup>. In another case of a female with leiomyosarcoma who had progressed through multiple chemotherapeutic agents achieved a partial response to vorinostat treatment<sup>109</sup>. A phase II study was conducted to assess the clinical efficacy of monotherapy of oral panobinostat for patients with advanced soft tissue sarcoma. In this cohort study of 47 patients, 17 patients had stable disease and six patients were progression-free at 6 months<sup>110</sup>. SB939 is an oral inhibitor of classes 1 and 2 HDAC. In a phase II trial, eligible patients with recurrent or metastatic translocation-associated sarcoma were treated with SB939. Fourteen patients were assessable for response with confirmed specific chromosomal translocations. Among these, 8 patients achieved stable disease with a median duration of 5.4 months. The 3-month progression-free survival rate was 49%<sup>111</sup>. Clinical outcomes of vorinostat in patients with locally advanced or metastatic soft tissue sarcomas, that failed first-line treatment with anthracycline-based chemotherapy, was investigated in a phase II trial. Stable disease was seen in a subgroup of 23% of 40 patients. Median progression-free survival was 3.2 months<sup>112</sup>. Even though there was a modest clinical outcome of HDI monotherapy for sarcomas, further exploration of combination regimens is warranted.

A phase I study evaluated the clinical parameters of oral abexinostat when administered to metastatic sarcoma patients in combination with doxorubicin. The maximum tolerated dose of abexinostat and doxorubicin was significantly higher when patients received granulocyte-colony stimulating factor (G-CSF). More importantly, the maximum tolerated dose of abexinostat and doxorubicin exceeded doses for maximal histone acetylation, so that the effectiveness of this combination would not be limited by inadequate dosing. Clinical benefit of this combination resulted in 7 out of 17 evaluable participants maintained stable disease for at least 5 cycles of chemotherapy<sup>113</sup>. Another study evaluated the response rate of belinostat in combination with doxorubicin for patients with soft tissue sarcoma. The combination therapy was well tolerated in these patients. Response rate was moderate but median time to progression was 6.0 months which is superior to some reports using single-agent doxorubicin<sup>114</sup>. Clinical outcomes of combining panobinostat and epirubicin for refractory sarcoma patients suggested that the combination treatment is well tolerated and may reverse anthracycline resistance. In this clinical trial, 12 out of 20 patients derived clinical benefit (1 partial response and 11 stable disease, median overall survival 8.3 months), including 8 patients who previously progressed on topoisomerase II therapy<sup>115</sup>.

HDI in combination with multityrosine kinase inhibitor pazopanib, mTOR inhibitor sirolimus, cyclin dependent kinase inhibitor flavopiridol and therapeutic proteasome inhibitor bortezomib were also evaluated in clinical trials. In a latest reported phase I trial, the addition of HDI abexinostat to pazopanib is well tolerated and resulted in durable responses in patients who experienced prior progression during treatment with VEGF inhibitors. Peripheral blood histone acetylation and *HDAC2* gene expression were associated with durable response to treatment, which supports epigenetically mediated reversal of treatment resistance<sup>116</sup>. In a phase I dose-escalation study of the mTOR inhibitor sirolimus and the HDI vorinostat in patients with advanced malignancy, stable disease was observed in patient with fibromyxoid sarcoma<sup>117</sup>. A phase I study was conducted to examine the effect of pulse-dose vorinostat plus flavopiridol treatment in 34 patients, 7 of which were sarcoma patients. Clinical outcomes support the strategy of combining intermittent high dose vorinostat with other anti-cancer agents where higher vorinostat levels may enhance efficacy<sup>118</sup>. In a phase I trial of vorinostat and bortezomib, the combination was generally well-tolerated at doses that achieved clinical benefit in relapsed or refractory sarcoma patients. Bortezomib did not affect the pharmacokinetics of vorinostat<sup>119</sup>. A later study from the same center was conducted to evaluate an intermittent dosing schedule of vorinostat with bortezomib. Stable disease was found in two pre-treated sarcoma patients<sup>120</sup>.

## 7 Mechanisms of HDIs resistance in sarcoma

Molecular mechanisms inducing resistance to HDIs in sarcomas have not been fully documented. However, understanding the mechanisms of resistance are crucial to overcome this resistance with combination treatments or to develop new HDIs. Combination treatment of HDIs with agents targeting those signaling pathways synergistically induces the death of sarcoma cells. The effect of HDIs, like standard chemotherapeutic agents, can be affected by drug efflux, target overexpression and desensitization, epigenetic silencing, anti-apoptotic or pro-survival mechanisms. Drug efflux is caused partly by overexpression of ATP binding

cassette transporter superfamily, which includes P-gp. In fact, romidepsin resistance of osteosarcoma cells was related to P-gp overexpression and MAPK pathway activated by romidepsin. Treatment of romidepsin-resistant clones with the P-gp inhibitor verapamil restored romidepsin sensitivity. Besides, the combined treatment of romidepsin and a MEK inhibitor effectively overcame romidepsin resistance<sup>121</sup>. It is also suggested that other epigenetic modulators can lead to resistance to HDIs in sarcomas. The combination of vorinostat and TRAIL synergistically enhanced the intrinsic apoptosis pathway. However, apoptotic resistance was caused by reduced expression of caspase-8 and TRAIL-R1 in sarcoma cells, due to epigenetic silencing by DNA hyper-methylation of gene promoter sequences. Treatment with 5-aza-dC restored gene expression and increased the sensitivity of both cell lines against TRAIL-induced apoptosis<sup>50</sup>. Expression levels of pro-apoptotic and anti-apoptotic BCL-2 protein families are also important for sensitivity to HDIs. Indeed, overexpression of anti-apoptotic BCL-2 family proteins BCL-2 and BCL-XL attenuated the activity of HDIs and inhibited the synergistic induction of apoptosis induced by combination treatment of HDIs and chemotherapy agents in sarcoma<sup>88, 89</sup>.

## Conclusion and prospective

Overall, HDIs display several desirable properties for a novel therapeutic, notably a broad range of biologic effects across cellular pathways including apoptosis, cell cycle, autophagy, cell differentiation and ROS generation in sarcomas. These molecular evidences reveal that HDIs may be a promising treatment agent for patients with sarcomas and lay foundation on the clinical application of HDIs in sarcomas. What's more, evidences from preclinical studies showed that HDIs can not only sensitize sarcomas to radiotherapy and chemotherapy, but also increase the treatment efficacy when combine chemotherapeutic drugs or other targeted drugs in sarcomas. However, the problem is that HDIs may be too blunt of an instrument to modulate very subtle nuances in cellular signaling. In some subtypes of sarcomas, combination treatment with HDIs and chemotherapy was different between animal models and cell culture models. New and better animal models are required to test HDIs for the treatment of sarcomas prior to clinical use. Another challenge is that sarcomas have diverse subtypes and genetic alterations involve oncogenic somatic mutations in some subtypes and chromosomal translocations in other subtypes. In some context, sarcomas with chromosomal translocations can be more sensitive to HDI treatment than sarcomas lacking known translocations. As HDIs can regulate gene expression either via HDACs or by directly inhibiting these fusion oncogenic fusion proteins, further studies are needed to focus on the gene level of regulation of HDIs in sarcomas. Four HDIs have been approved by the FDA for treatment of hematopoietic neoplasm based on evidence showing benefit from HDIs in vivo and in vitro models, and in clinical trials. However, HDIs as monotherapy do not appear to be effective for sarcomas patients. Compared to HDIs on hematopoietic neoplasm, clinical trials in sarcomas revealed that combination therapy with other therapeutics including chemotherapy drugs and small-molecular inhibitors such as pazopanib, sirolimus, flavopiridol and bortezomib seem more promising than HDIs as monotherapy. An improved understanding of the disease specific targets and the development of appropriate biomarkers will improve the efficacies of HDIs in future human sarcoma trials to reverse drug resistance.

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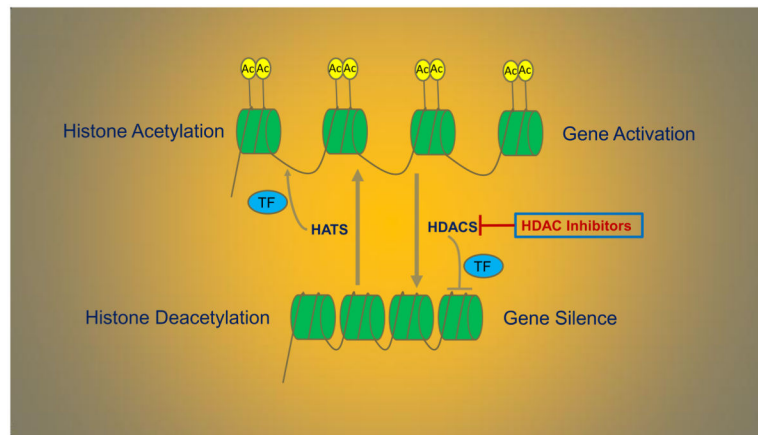
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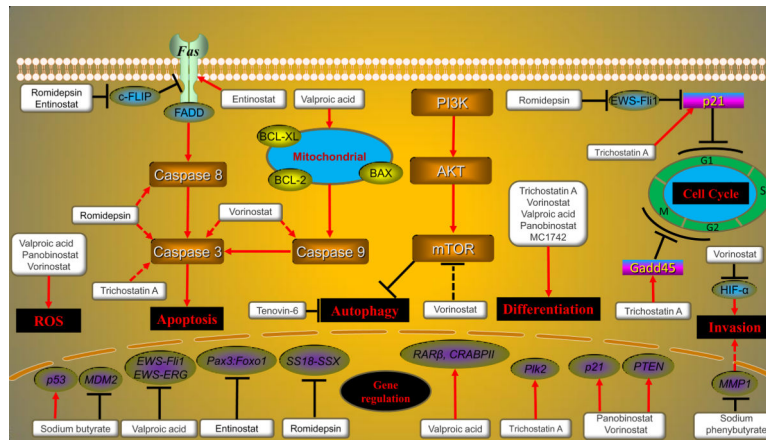
### Highlights

- Sarcomas are a diverse group of difficult-to-treat malignant tumors.
- Histone deacetylase inhibitors are epigenetic drugs that can inhibit sarcoma growth in vitro and in vivo.
- Histone deacetylase inhibitors can increase treatment efficiency when combined with chemotherapeutic drugs.
- Histone deacetylase inhibitors represent a promising new class of compounds for the treatment of sarcomas.



**Fig. 1.** The processes of histone acetylation and histone deacetylation that regulated by HATs and HDACs. As a result, acetylation increase levels of gene expression and deacetylation performed by HDACs has the opposite effect.

 : Directly inhibit modification      TF: transcription factor



**Fig. 2.** Molecular actions of HDIs in sarcoma. HDIs upregulate some tumor suppressor genes and downregulate oncogenes gene expression. HDIs also induce apoptosis by extrinsic death receptor pathway, intrinsic mitochondrial pathway and caspase common pathway. Besides, HDIs cause cell cycle arrest, regulate autophagy, increase ROS generation, reduce invasion ability and induced cell differentiation in sarcomas.

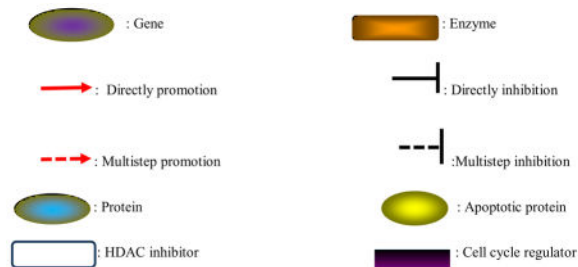




Table 1

The classification and characteristic of HDACs

Class	Name	a.a number	Localization	Function	Zn <sup>2+</sup> /NAD <sup>+</sup>
I classic	HDAC 1	482	Nucleus	Deacetylase	
	HDAC 2	488	Nucleus	Deacetylase	
	HDAC 3	428	Nucleus/cytoplasm	Deacetylase	Zn <sup>2+</sup> -dependent
	HDAC 8	377	Nucleus	Deacetylase	
	HDAC 4	1084	Nucleus/cytoplasm	Deacetylase	
	HDAC 5	1122	Nucleus/cytoplasm	Deacetylase	
	HDAC 7	991	Nucleus/cytoplasm	Deacetylase	Zn <sup>2+</sup> -dependent
	HDAC 9	1069	Nucleus/cytoplasm	Deacetylase	
	HDAC 6	1215	Mainly cytoplasm	Deacetylase	
	HDAC10	669	Mainly cytoplasm	Deacetylase	Zn <sup>2+</sup> -dependent
IIa classic	Sirtuin 1	747	Nucleus/cytoplasm	Deacetylase	
	Sirtuin 2	389	Nucleus	Deacetylase	
	Sirtuin 3	399	Nucleus/Mitochondria	Deacetylase	
	Sirtuin 4	314	Mitochondria	ADP-ribosyltransferase	
III Sirtuins (human)	Sirtuin 5	310	Mitochondria	Demalonylase Desuccinylase Deacetylase	NAD <sup>+</sup> -dependent
	Sirtuin 6	355	Nucleus	Demyristoylase Depalmitoylase Weak deacetylase ADP-ribosyltransferase	
	Sirtuin 7	400	Nucleus	Weak deacetylase	
IV classic	HDAC11	420	Nucleus/cytoplasm	Deacetylase	Zn <sup>2+</sup> -dependent

**Table 2**

Characteristics of HDAC inhibitors in sarcoma

Drug Name	Molecular Formula	Target HDACs
Vorinostat(SAHA)	C <sub>14</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub>	Pan-HDI of class I and IIa
Panobinostat(LBH589)	C <sub>21</sub> H <sub>23</sub> N <sub>3</sub> O <sub>2</sub>	Pan-HDI of class I, II, and IV
Belinostat(PXD101)	C <sub>15</sub> H <sub>14</sub> N <sub>2</sub> O <sub>4</sub> S	Pan-HDI of class I, II, and IV
Romidepsin(FK-228, FR901228, Depsipeptide)	C <sub>24</sub> H <sub>36</sub> N <sub>4</sub> O <sub>6</sub> S <sub>2</sub>	Specific-HDI of class I, specially HDAC 1,2
Entinostat (MS-275, SNDX-275)	C <sub>21</sub> H <sub>20</sub> N <sub>4</sub> O <sub>3</sub>	Specific-HDI of HDAC 1, 3
Valproic acid (VPA, Valproate)	C <sub>8</sub> H <sub>15</sub> NaO <sub>2</sub>	Pan-HDI of Class I and IIa
Abexinostat(PCI-24781)	C <sub>21</sub> H <sub>23</sub> N <sub>3</sub> O <sub>5</sub>	Pan-HDI of class I and IIb
Pracinostat(SB939)	C <sub>20</sub> H <sub>30</sub> N <sub>4</sub> O <sub>2</sub>	Pan-HDI of class I, II and IV
Sodium phenylbutyrate	C <sub>10</sub> H <sub>11</sub> O <sub>2</sub> Na	Pan-HDI of Class I and IIa
Quisinostat(JNJ-26481585)	C <sub>21</sub> H <sub>28</sub> Cl <sub>2</sub> N <sub>6</sub> O <sub>2</sub>	Pan-HDI of class I, II and IV
LAQ824	C <sub>22</sub> H <sub>25</sub> N <sub>3</sub> O <sub>3</sub>	Pan-HDI of class I, II, IV
Sodium Butyrate	C <sub>4</sub> H <sub>7</sub> NaO <sub>2</sub>	Pan-HDI of class I, IIa, and IV
Trichostatin A (TSA)	C <sub>17</sub> H <sub>22</sub> N <sub>2</sub> O <sub>3</sub>	Pan-HDI of class I and II
Tenovin-6	C <sub>25</sub> H <sub>34</sub> N <sub>4</sub> O <sub>2</sub> S	Specific-HDI of Sirtuin 1,2,3
Pyroxamaide (NSC696085)	C <sub>13</sub> H <sub>19</sub> N <sub>3</sub> O <sub>3</sub>	Specific-HDI of HDAC 1
MC1742	C <sub>21</sub> H <sub>21</sub> N <sub>3</sub> O <sub>3</sub> S	Pan-HDI of Class I and II
MC2625	/	Pan-HDI of class I, II, IV
PCI-34051	C <sub>17</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub>	Specific-HDI of HDAC 8
PCI-48012	/	Specific-HDI of HDAC 8

\* FDA approved for hematologic neoplasms not sarcomas Po:by mouth i.v.: intravenous infusion

**Table 3**

HDAC inhibitors have synergistic effect with other moleculars

HDAC Inhibitor	Combination Therapy	Molecular Name	Effect on tumor inhibition
Trichostatin A	DNMT inhibitor	5-aza-cdR	Inhibit cell proliferation Reduce cell invasion Reverse multidrug resistance
Valproic acid	DNMT inhibitor	5-aza-cdR	Prevent Ptch-associated tumors formation
Entinostat	DNMT inhibitor	5-aza-cdR	Synergistic antitumor effect
Vorinostat	DNMT inhibitor	Zebularine	Synergistic antitumor effect
Vorinostat	mTOR/PI3K inhibitor	Ridaforolimus/LY	Synergistic antitumor effect
Valproic acid	-	Hydralazine	Enhanced the susceptibility of osteosarcoma cells to Fas-/NK cell-mediated cell death
Vorinostat	NK cell	-	Enhance NK cell killing cancer cell
Vorinostat	TRAIL	-	Synergistic antitumor effect
Valproic acid	-	Chloroquine	Synergistic antitumor effect
Quisinostat	Proteasome inhibitor	-	Synergistic antitumor effect

**Table 4**

Clinical trials of HDAC inhibitors in sarcoma

HDAC	Combination therapy	Phase	Number of sarcoma patients	Dose
Panobinostat	-	II	47	20–40mg thrice weekly
Pracinostat	-	II	22	60 mg/day every other day for 3 of 4 weeks
Vorinostat	-	II	40	400 mg po qd for 28 day followed by a treatment-free period of 7 day
Abexinostat	Doxorubicin	I	22	Abexinostat: 45 mg/m <sup>2</sup> twice daily administered on days 1 through 5 Doxorubicin: 75 mg/m <sup>2</sup> on day 4 of a 3- week cycle
Belinostat	Doxorubicin	I/II	41	Belinostat: 1000 mg/m <sup>2</sup> /day Doxorubicin: 75mg/m <sup>2</sup>
Panobinostat	Epirubicin	I	21	Panobinostat: 50 mg Epirubicin: 75 mg/m <sup>2</sup>
Abexinostat	Pazopanib	I	6	Abexinostat: 30–45 mg/m <sup>2</sup> twice Daily Pazopanib: 400–800 mg Daily