# Chapter 7
# Histone Deacetylase Inhibitors


**Opelo Sefhore and Silvia CW Ling**

**Abstract** Histone Deacetylase Inhibitors (HDACi) inhibits deacetylases of histones and nonhistones. As such it has potential widespread biological effects. However, cancer cells are preferentially affected than normal cells making it a useful targeted therapy in cancer. HDACi has therapeutic effects in hematological malignancies like acute myeloid leukemia and multiple myeloma. The molecular effect is dependent on the cancer type, the specific class of histone deacetylase, and the chemical structure of the HDACi. This chapter focuses on the basics of HDAC classification and the specific molecular effects in multiple myeloma.

**Keywords** Multiple myeloma · Histone deacetylase · Histone deacetylase inhibitor · Pan-HDAC inhibitor · Histone deacetylase inhibitor resistance

## Abbreviations

| | |
|---|---|
| ABC | ATP binding cassette |
| ATF6 | Activating transcription factor 6 |
| CCND1 | Cyclin D1 |
| CDK | Cyclin-dependent kinase |
| CR | Complete response |
| CRBN | Celebron |
| DNMT1 | DNA-methyltransferase 1 |
| HDAC | Histone deacetylase |

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| | |
|---|---|
| HDACi | Histone deacetylase |
| HSP | Heat shock protein |
| IGF-1 | Insulin-like growth factor 1 |
| IL | Interleukin |
| IMiD | Immunomodulatory imide drug |
| IRF4 | Interferon 4 |
| MAPK | Mitogen-activated protein kinase |
| MEF2 | Myocyte enhancer factor 2 |
| MM | Multiple myeloma |
| N-CoR | Nuclear receptor corepressor |
| PAI-1 | Plasminogen activator-inhibitor 1 |
| PERK | Protein kinase R-like ER kinase |
| PI | Proteasome inhibitor |
| RRMM | Relapsed/refractory multiple myeloma |
| SAHA | Suberoylanilide hydroxamic acid |
| siRNA | Small interfering RNA |
| SMRT | Silencing mediator for retinoic acid for thyroid hormone receptors |
| STAT | Signal transducer and activator of transcription pathway |
| TAFI68 | TATA-box binding protein associated factor 1 |
| TIF | Transcription initiation factor |
| TRAIL | Tumor necrosis factor-related apoptosis-inducing ligand |
| TSA | Trichostatin A |

## 7.1 Introduction

The introduction of new therapeutic agents such as proteasome inhibitors (PI) and immunomodulatory imide drugs (IMiD) in the last 1–2 decades has dramatically improved the outcome of multiple myeloma (MM) patients. Multiple myeloma is characterized by heterogeneous and complex genetic alterations such as structural chromosomal abnormalities, point mutations, and epigenetic alterations. Epigenetic alterations refer to changes in gene expression without changes in the DNA code. Examples of epigenetic alterations include DNA methylation, acetylation, phosphorylation, ubiquitination, and sumoylation which modify the posttranslational structure of histone. In cancer, dysregulation in epigenetics affects the expression of proteins involved in tumor suppression, cell cycling, DNA repair, apoptosis, protein homeostasis, and tumor immunity.

Histone Deacetylase inhibitors (HDACi) is a promising new group of anti-myeloma therapy that acetylates histone and nonhistone proteins. There have been encouraging clinical responses observed with HDACi used in combination with other targeted therapies such as proteasome inhibitors in MM. To further understand the efficacy of HDACi, it is important to explore the biology of histone deacetylases and their roles in MM.

## 7.2 Histone Deacetylases

Human DNA is organized into basic structural units called nucleosomes which are packed and wound around two copies each of four different histone proteins (H2A, H2B, H3, and H4). The accessibility of the DNA to regulatory proteins is dependent on the covalent modifications of these histones and the position of the nucleosomes [1].

Histone deacetylases (HDAC) belong to a class of enzymes that removes acetyl groups from lysine within the tail of histones. This process, known as deacetylation, allows DNA to tightly coil around histones to form chromatin. The reverse process, termed acetylation, is mediated by histone acetyltransferases and leads to the uncoiling of DNA, exposing promoter genes to transcription factors. The balance between acetylation and deacetylation determines the degree of gene accessibility to transcription factors [2]. There is growing evidence that deacetylation plays an important role in silencing tumor suppressor genes, dysregulating cellular function, and contributing to cancer development and resistance to chemotherapy [2]. Hence, research has been invested to develop drugs targeting epigenetic regulation.

There are 18 HDACs in humans, which are divided into four classes (I–IV) based on their homology to the yeast enzyme Rpd3, their intracellular localization, and organization with the DNA-binding complexes. Class I, II, and IV HDACs require zinc as a cofactor for their deacetylase activity [3–6]. Class III HDACs, also referred to as sirtuins, are homologs of the yeast enzyme Sir2 and are dependent on NAD+ for their activity rather than zinc [3, 7–16].

### *7.2.1 Class I Histone Deacetylases*

Class I HDACs are homologous to the yeast enzyme Rpd3 and consist of four subtypes (HDAC1, 2, 3, and 8). They are predominantly found in the nucleus, ubiquitously expressed in all tissues, with the main function of histone deacetylation. HDAC1 and HDAC2 are similar with 86% homology and require multiple protein cofactors for enzyme activity [1]. For example, HDAC1 and HDAC2 are part of the multiple protein complexes with Sin3, Rb-associated protein 48, and RbAp46 which function as transcription repressors [17–19]. HDAC1 and HDAC2 also bind directly to DNA-binding proteins, such as YY1, retinoblastoma protein (pRb), pRb-binding protein 1, Sp1, and breast cancer-associated susceptibility protein 1 [19–23]. HDAC1 and HDAC2 activity can be modulated by phosphorylation, increasing enzyme activity but mediating dissociation from multiprotein complexes [24]. HDAC3 is evolutionarily most closely related to HDAC8, with 34% overall sequence identity. SMRT (silencing mediator for retinoic acid and thyroid hormone receptors) and N-CoR (nuclear receptor corepressor) are necessary factors for HDAC3 activity [25, 26]. Besides histone deacetylation, HDAC1, HDAC2, and HDAC3 localize in the endoplasmic reticulum where they deacetylase nonhistone proteins,

such as GRP78, the major molecular chaperone. Inhibition of HDAC1, HDAC2, and HDAC3 leads to the acetylation of the GRP78 and activation of the protein kinase R-like ER kinase (PERK) and activating transcription factor 6 (ATF6) arm of the unfolded protein response [27].

Class I HDAC overexpression has been studied in both solid and hematological malignancies, with prognostic implications, discussed later in this chapter [2]. HDAC1 overexpression has been observed in renal cell carcinoma and ovarian cancers [3, 4]. Another main function of Class I and II HDACs is regulation of tissue regeneration [5–8].

### *7.2.2 Class II Histone Deacetylases*

Class II HDACs are homologous to the yeast enzyme Hda1, which consists of a N-terminal deacetylase domain and a long C-terminal extension. This class is subdivided into IIa (HDACs 4, 5, 7, and 9) and IIb (HDAC6 and 10). Class IIa HDACs possess a conserved N-terminal extension that binds myocyte enhancer factor 2 (MEF2) and 14–3-3 proteins. Class IIa HDACs can shuttle between the nucleus and the cytosol in response to different stimuli. HDAC4, 5, and 9 are expressed predominantly in heart, cardiac, and skeletal muscle, whereas HDAC7 is found in CD4/CD8 double-positive thymocytes [28], endothelial and smooth muscle cells [29]. Class IIa HDACs do not bind chromatin directly. Their activity is dependent on their association with other multiprotein complexes and other HDACs.

HDAC6 is a class IIb HDAC which contains tandem deacetylase domains and a C-terminal zinc finger which is homologous to the non-catalytic domain of ubiquitin-specific proteases (USPs). HDAC6 is localized in the microtubular network of the cytoplasm and acts as a deacetylase of tubulin. It binds ubiquitin via its zinc finger with high affinity. HDAC6 is important in multiple myeloma as it is necessary for the clearance of misfolded proteins via aggresomes and aggrephagy.

The other class IIb HDAC member is HDAC10, which has an N-terminal half similar to the first deacetylase domain of HDAC6, but its C-terminal half is leucine-rich. HDAC10 deacetylases polyamines, such as spermidine and spermine, which are critical in the regulation of the function of biological macromolecules [30]. In addition, HDAC10 overexpression is a poor prognostic marker in neuroblastoma, as it mediates lysosomal exocytosis of doxorubicin in neuroblastoma cells causing resistance to doxorubicin [31].

### *7.2.3 Class III Histone Deacetylases (Sirtuins)*

Class III HDACs (sirtuins) consist of seven subclasses (SIRT 1–7) [32]. This class has sequence homology with the yeast gene silent information regulator, Sir2 [33, 34]. Its enzyme activity is $NAD^+$ dependent whereas other HDACs require $Zn^{2+}$ as a

cofactor [35, 36]. Sirtuins are deacetylases and mono-ADP-ribosyl transferases [32]. Sirtuins are insensitive to inhibitors of "classical" (Class I, II, and IV) HDACs.

SIRT1 targets both histone and nonhistone proteins. SIRT1 deacetylases the lysine residues at positions 9 and 26 of histone H1, position 14 of H3, and position 16 of H4. Nonhistone targets of SIRT1 include p53 and $TAF_I68$ [TBP (TATA-box binding protein)-associated factor I] [68]. Deacetylation of p53 leads to its suppression and hence inactivation of apoptosis in response to DNA damage and oxidative stress [37]. $TAF_I68$ is a subunit of TIF (transcription initiation factor)-IB/SL, which regulates transcription of RNA polymerase I [38]. Deacetylation of $TAF_I68$ leads to the repression of RNA polymerase I. SIRT1 has been shown to act as an oncogene or a tumor suppressor gene in vitro and in vivo, depending on the specific cancer type. SRT1720 is a novel first-in-class SIRT1 activator which triggers apoptosis in MM cell lines via the activation of the DNA repair pathway, ATM-CHK2 [39].

SIRT6 is highly expressed in human multiple myeloma and is virtually absent in normal human mononuclear cells. High SIRT6 levels are associated with an adverse prognosis in MM. SIRT6 downregulates the expression of mitogen-activated protein kinase (MAPK) pathway genes. It inactivates ERK2/p90RSK signaling, allowing DNA repair via Chk1 hence conferring resistance to DNA damage treatment [40].

### *7.2.4 Class IV Histone Deacetylases*

HDAC11 is the sole class IV HDAC. It is isolated in tissues such as the heart, muscle, and kidney but there is limited knowledge about its function. HDAC11 is important in the development of plasma cells. HDAC11 knockout mice exhibited an 88% decrease in bone marrow plasma cells compared to wild-type mice. Selective inhibition of HDAC11 pharmacologically and by small interfering RNA (siRNA) reduced the viability of MM cell lines [41]. *Mithraprabhu* et al. demonstrated variable expression of HDAC11 in primary MM cells and human MM cell lines. Overexpression of HDAC11 along with HDAC1, HDAC2, HDAC4, HDAC6, was associated with poor prognosis in human MM patients [42].

Dysregulation of HDAC expression has been implicated in the pathogenesis of many cancers. Specific to hematological malignancies, HDAC dysregulation has been reported in peripheral T cell lymphomas, cutaneous T cell lymphomas, diffuse large B cell lymphomas, pediatric acute lymphoblastic leukemia, myeloproliferative neoplasms, and MM.

## 7.3 Histone Deacetylases in Multiple Myeloma

HDACs repress gene transcription by deacetylation of histones and regulate multiple cellular pathways by deacetylation of nonhistone proteins. These pathways include cell cycling, apoptosis, DNA repair, oxidative stress response, unfolded

protein response, autophagy, and angiogenesis. In MM, inhibition of HDAC has significant synergistic therapeutic effects with proteasome inhibitors. The combination of the pan-HDAC inhibitor, panobinostat with the proteasome inhibitor bortezomib is an approved therapy in relapsed/refractory multiple myeloma (RRMM). This has led to further research and understanding of the role of HDAC in the pathogenesis and the mechanism of drug resistance in MM and other cancers. Despite the therapeutic effects of HDAC inhibition, the specific roles of HDAC in MM are still unclear.

### *7.3.1 Histone Deacetylases and Protein Clearance*

Multiple myeloma is highly dependent on the ubiquitin proteasome pathway for the disposal of misfolded and unfolded proteins. Proteasome inhibitors are the backbone of anti-myeloma therapy. The aggresome/aggrephagy pathway is an alternate pathway for protein degradation when the ubiquitin proteasome pathway is overwhelmed or inhibited by drugs and is dependent on HDAC6. HDAC6 localizes in and deacetylates microtubules, binding ubiquitin which tags onto misfolded protein aggregates [43–45]. It mediates the transport of protein aggregates along the microtubules to the microtubule organization center, where aggresomes and autophagosomes are formed. Autophagosomes fuse with lysosomes where proteins and polymers are degraded by hydrolases [46]. In addition, HDAC6 dissociates from heat shock protein (HSP)-90 in the presence of protein aggregates leading to the activation of HSF1 [47, 48]. HSF1 activation leads to further activation of heat shock proteins/chaperones. Therefore, HDAC6 plays a crucial role in the clearance of misfolded proteins when the proteasomes are inhibited. This underscores the synergism between HDAC and proteasome inhibition in MM and the development and approval of panobinostat, in the treatment of multiple myeloma [49–51].

### *7.3.2 Histone Deacetylase Overexpression and Increased Activity in Multiple Myeloma*

Overexpression of Class I HDACs (HDAC1, 2, 3, and 8) and Class II HDACs (HDAC5 and 10) has been observed in human MM cell lines compared with normal plasma cells [42]. In primary human MM samples, overexpression of HDAC1, 2, 4, 6, and 11 were shown to be associated with poor prognosis [42]. Elevated HDAC1 protein expression by immunohistochemistry was associated with inferior overall survival in MM [42]. It is clear that HDACs are dysregulated in MM however the role of each HDAC in the pathogenesis of MM remains unclear.

Increased HDAC activity plays a role in the growth of MM cells. In the MM cell line MOLP8, acetylation of H3K9 is markedly reduced in the c-myc proto-oncogene coding regions and the MCL1 coding regions and promoter. When treated with

vorinostat, an inhibitor of Class I, II, and IV HDACs, these regions became acetylated [52]. In addition, it has been shown that melphalan and gamma radiation-induced apoptosis was associated with hyperacetylation of MYC and cyclin D1 (CCND1) oncoprotein [53]. This suggests that the growth of MM is associated with deacetylation and cytotoxicity is associated with hyperacetylation.

Further research of the specific function of HDACs in MM is important to develop and improve the therapeutic role of HDACi in MM.

## 7.4 Histone Deacetylase Inhibitors

### *7.4.1 Types of Histone Deacetylase Inhibitors*

There are five broad categories of HDACi based on their chemical structure: aliphatic fatty acids, hydroxamic acid, benzamides, cyclic peptides, and mercaptoketone (Table 7.1).

### *7.4.2 Mechanisms of Action*

Histone deacetylase inhibitors induce growth arrest, differentiation, and apoptosis in cancer cells in vitro and in vivo. In most cancers including MM, HDACi are used in combination with other anticancer drugs. It increases the acetylation of histone

**Table 7.1** HDAC inhibitors

| Name | Target HDAC Class | Examples |
|---|---|---|
| Aliphatic fatty acids | I and IIa | Butyrate<br>Valporic acid |
| Hydroxamic acid | All classes<br>All classes<br>All classes<br>Class I and II<br>Class IIb HDAC6<br>Class IIb HDAC6 | SAHA (Vorinostat)<br>Belinostat (PXD101)<br>Panobinostat (LBH-589)<br>Givinostat (ITF2357)<br>Resminostat (4SC-201<br>Abexinostat (PCI-24781)<br>Tubacin<br>Ricolinostat (ACY-1215) |
| Benzamides | Class I<br>Class I<br>Class I<br>Class I and IV | Entinostat (MS-275)<br>Mocetinostat (MGCD0103)<br>Tacedinaline (CI-994)<br>MGCD-0103 |
| Cyclic peptides | Class I<br>Class I<br>Class I | Depsipeptide (FK228)<br>Romidepsin<br>Apicidin |
| Mercaptoketone | Class I and II | KD5170 |

and nonhistone proteins and affects the transcriptional modulation of 7%–10% of the genes in MM and lymphoma cell lines [15]. HDAC inhibitor effects on nonhistone proteins are even more extensive with at least 50 candidate nonhistone proteins including transcription factors, transcription regulators, signal transduction mediators, DNA repair enzymes, nuclear import regulators, chaperone proteins, structural proteins, inflammation mediators, and viral proteins. The efficacy of HDACi is dependent on cell type, context, dose, and chemical structure of the inhibitor. Cancer cells are more susceptible to the effects of HDACi than normal cells, supporting its development as an anticancer drug.

#### 7.4.2.1 Altered Gene Expression

Histone deacetylase inhibitors acetylate the histones of specific genes. They are able to induce the expression of cyclin-dependent kinase (CDK) inhibitor p21 (WAF1/CIP1) by increasing the acetylation of histones H3 and H4 associated with the p21 promoter region [54, 55]. This enables cell cycle arrest, repair, terminal differentiation, and prevention of DNA replication in response to DNA damage.

#### 7.4.2.2 Induction of Apoptosis

Tumor death mainly occurs via apoptosis through mitochondrial (intrinsic) and death receptor (extrinsic) pathways. These pathways converge to activate caspases and trigger cell death. HDACi is able to induce both intrinsic and extrinsic apoptosis. Apicidin, a cyclic tetrapeptide HDACi induces extrinsic apoptosis of HL60 cell lines by induction of Fas/Fas ligand and induces intrinsic apoptosis, evidenced by the translocation of Bax from the cytosol to the mitochondria and the release of cytochrome c [56]. Suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA) induce the expression of DR3 and DR4, mediating tumor necrosis family-related apoptosis-inducing ligand (TRAIL) induced extrinsic apoptosis.

HDAC inhibitors also induce intrinsic apoptosis by the upregulation of the pro-apoptotic factors (Bax, Bak, Bid, and Bim) of the Bcl-2 related proteins, relative to the pro-survival factors (Bcl-2, Bcl-xL, Bcl-w, Mcl-1, and A1). Cancer cells are more susceptible to HDACi induced apoptosis than their normal counterparts, partly because cancer is dependent on the upregulation of the pro-survival factors. In MM there is overexpression of pro-survival factors Bcl-2 or Mcl-1 and downregulation of the pro-apoptotic protein Bax [57, 58]. SAHA and TSA upregulate the pro-apoptotic factors Bim, Bak, Noxa, PUMA β/δ, and Bax and downregulate the pro-survival factors Bcl-2 and Bcl-$X_L$ [59].

The mechanism by which HDACi interferes with the balance of pro-apoptotic and pro-survival factors is heterogeneous. HDAC3 inhibition increases the acetylation and ubiquitination of DNA-methyltransferase 1 (DNMT1), leading to reduced DNMT1 expression and downregulation of the members of the XIAP family (apoptosis inhibitors) and Bcl-2 [60]. Panobinostat, a pan-HDACi, induces apoptosis

through caspase 3 mediated interferon regulatory factor 4 (IRF4) and MYC degradation [61]. Pharmacologic and genetic inhibition of HDAC4 (Class IIa) was shown to induce ATF4 and CHOP expression and upregulate intrinsic pro-apoptotic factors Bim, Puma, and Bax [62].

#### 7.4.2.3 Cell Cycle Arrest

Most, if not all, HDACi induce G0/S/G1 arrest. This is mediated by p53-dependent and independent upregulation of cyclin-dependent kinase inhibitor proteins. SAHA and TSA induce G1 growth arrest by upregulation of p21WAF1, p27Kip1, and p53 in myeloma cell lines [55, 59, 63]. Resminostat, a potent inhibitor of HDAC1, 3, and 6, induces G0/G1 cell cycle arrest in MM cell lines by decreased levels of Cyclin D1, Cdc25a, Cdk4, pRb, and upregulation of p21 [64].

#### 7.4.2.4 Inhibition of Angiogenesis

Histone deacetylases play a significant role in angiogenesis during embryogenesis, tissue repair, and cancer growth. HDAC7, a class IIa HDAC is an essential regulator of embryonic blood vessel development [9]. HDAC7 controls endothelial angiogenic functions, such as tube formation, migration, and proliferation [10–12]. Conversely, HDAC5 represses angiogenic gene expression in endothelial cells and angiogenesis [13]. HDAC5 and HDAC7 are controlled by protein kinase D-dependent phosphorylation which mediates their nuclear export [14, 15].

HDAC6 is important in endothelial cell sprouting, tube formation, and perfusion of blood vessels. It is transcriptionally activated by hypoxia and deacetylates cortactin independently of deacetylation of alpha-tubulin in the cytoplasm. Cortactin is essential for endothelial cell migration and blood vessel formation. However, HDAC6 deficiency is not embryonically lethal as HDAC10 compensates its angiogenic function [65, 66].

Class I and II HDACi inhibit angiogenesis in vitro and in animal models [16]. The pan-HDAC inhibitor AR-42 inhibits angiogenesis by hyperacetylation of histones H3 and H4, upregulation of miR-9-5p, and downregulation of CD44 [67]. CD44 is a receptor for hyaluronic acid which induces the expression of plasminogen activator-inhibitor-1 (PAI-1) [68]. PAI-1 overexpression is associated with an unfavorable prognosis in many cancers. It stimulates the endothelial cell migration from vitronectin to fibronectin, promoting vascularization and tumor invasion from the vitronectin rich perivascular space into the fibronectin rich, poorly vascularized tumor stroma as well as promoting fibrosis [69].

R306465, a hydroxamate-based, potent inhibitor of HDAC1 and HDAC8 (class I HDACs) inhibits angiogenesis in vivo and induces G1 cell cycle arrest and apoptosis in solid and hematological malignancies including MM [70]. Panobinostat, a hydroxamic, pan-HDAC inhibitor has anti-angiogenic activity in prostate cancer xenografts. It acetylates histone H3 and alpha-tubulin in human umbilical vein

endothelial cells [71]. Panobinostat also inhibits angiogenesis in Hodgkin Lymphoma cell lines by inhibition of HIF-1α expression [72]. TSA is a HDACi that induces the ubiquitination of histone acetylases, leading to the reduction of NOX4 expression and inhibition of angiogenesis [73, 74].

#### 7.4.2.5 Regulation of Cytokines

HDAC inhibitors downregulate the expression of genes involved in cytokine signaling in MM. These include insulin-like growth factor 1 (IGF-1), IGF-1 receptor, and interleukin (IL)-6 receptor, important components in the interaction between MM cells and the bone marrow microenvironment.

IL-6 is a major growth factor for myeloma cells which is produced by the tumor microenvironment [75]. IL-6 binds to soluble IL-6 Receptor to form the IL-6/IL-6R complex, which binds to CD130 (gp130) on MM cells to activate downstream STAT3 signaling and ultimately MM cell survival and proliferation. This process is called IL-6 trans-signaling [76]. The natural inhibitor of IL-6 trans-signaling is the soluble gp130 (sgp130) which is dependent on HDAC3 mediated secretion by the bone marrow stromal cells [77].

Vorinostat (SAHA) had been shown to suppress the expression of IGF-1 and its receptor IGF-1R, IL-6R receptor and its key signal transducer gp130, TNF-receptor-1 (TNF-R1), BCMA, and paracrine IL-6 secretion by BMSC [78].

#### 7.4.2.6 Suppressed DNA Damage Repair

HDAC inhibitors affect the function of certain DNA repair proteins resulting in double-stranded breaks in DNA. KD5170, a novel mercaptoketone-based HDACi induced oxidative stress and oxidative DNA damage in myeloma cells as evidenced by the upregulation of heme oxygenase-1 and H2A.X phosphorylation [79].

SAHA selectively suppressed DNA repair proteins in cancer cells but not in normal cells, and therefore preferentially causing cell death in cancer cells [80].

#### 7.4.2.7 Ubiquitin Proteasome System

Multiple myeloma is highly dependent on the ubiquitin proteasome system. Misfolded proteins are often refolded with the help of chaperones. If this process fails, misfolded proteins are ubiquitinated and are predominantly degraded by the 26S proteasome. Failure of this degradation process results in the accumulation of these proteins which are toxic to the cells. HR23B (also known as UV excision repair protein RAD23 homolog B, XP-C repair complementing complex 58 kDa protein and p58) is a protein which is situated on the proteasome and shuttles ubiquitinated proteins into the proteasome for degradation [81]. HDAC inhibitors can result in the hyperacetylation of HR23B, aberrant proteasomal activity, and cell death. HR23B is essential to the action of HDACi (TSA, SAHA, and BELINOSTAT).

In cutaneous T cell lymphoma, HR23B is a biomarker that predicts sensitivity to HDACi [82].

Panobinostat decreases DNMT1 by hyperacetylation of HSP90, disruption of the HSP90 and DNMT1 complex, and mediating proteasomal degradation of DNMT1. This contributes to the anticancer activity of panobinostat in breast cancer as DNMT1 is an essential breast cancer stem cell survival factor [83, 84].

#### 7.4.2.8 Aggresome Pathway

The aggresome pathway is an alternate protein degradation pathway to the proteasome pathway. Unfolded or misfolded proteins can form protein aggregates, which are not degradable by the proteasomes and are toxic to cells. To avoid cell death, protein aggregates are ubiquitinated and are taken up by aggresomes. Autophagosomes then engulf the aggresomes and fuse with lysosomes where their contents are degraded by lysosomal hydrolases [19].

HDAC6 is essential for the formation of aggresomes. HDAC6 localizes in the microtubule and regulates the acetylation of microtubules. HDAC6 binds to polyubiquitinated misfolded proteins and dynein motors, which transport the polyubiquitinated misfolded proteins to aggresomes at the microtubule-organizing center [46]. Histone deacetylase inhibitors induce proteotoxic stress and cell death by blocking HDAC6.

Catley et al. has shown that the proteasome inhibitor bortezomib and the pan-HDACi panobinostat synergistically induced apoptosis of myeloma cells by hyperacetylation of tubulin and accumulation of small aggresomes [49]. This finding laid the foundation to the clinical development and subsequent approval of panobinostat in combination with bortezomib in the treatment of RRMM.

## 7.5 Approved Histone Deacetylase Inhibitors

The US Food and Drug Administration (FDA) has approved four HDACi in hematological malignancies: vorinostat (Zolinza) and romidepsin (Istodax) for the treatment of cutaneous T cell lymphoma; belinostat (Beleodaq) and panobinostat (Farydak) for the treatment of peripheral T cell lymphoma and panobinostat for MM [21].

### *7.5.1 Panobinostat*

Histone deacetylase inhibitors and PIs have been shown to act synergistically to induce cell death in MM [25]. Proteasome inhibition results in the accumulation of misfolded proteins that are prone to aggregation. The presence of HDACi prevents

the removal of these protein aggregates by inhibiting the aggresome pathway, resulting in cytotoxic stress and downstream activation of cell apoptosis.

In February 2015, FDA approved panobinostat (Farydak; Novartis Pharmaceuticals), an orally administered, pan-HDACi, in combination with bortezomib for the treatment of patients with MM who have received at least two prior lines of therapy including bortezomib and IMiDs.

Panobinostat has a stronger inhibitory effect against HDAC classes I, II, and IV compared to Vorinostat [25]. At the molecular level panobinostat affects cell cycle progression and apoptosis. Ninety percent of the drug is bound to plasma protein and the peak concentration is reached within 2 h. Metabolism of panobinostat occurs via reduction, hydrolysis, oxidation, and glucuronidation. Approximately 40% of panobinostat is eliminated via CYP3A and approximately another 40% is eliminated by CYP2D6. Panobinostat is excreted from the body via the urine (29–51%) and via the feces (44–77%) [26].

Panobinostat was shown to improve survival in the PANORAMA1 trial, a multicenter, double-blinded phase III clinical trial of bortezomib, panobinostat, and dexamethasone compared with placebo, bortezomib, and dexamethasone in RRMM patients who had received one to three previous treatment regimens. Approximately 768 eligible patients were randomized. The study observed increased rates of complete response (CR) or near CR with panobinostat compared to the placebo group (27.6% vs 16.7%) [85]. It also showed a prolonged median duration of response (13.14 vs 10.87 months), median PFS (11·99 vs 8·08 months), and median overall survival (OS) (33.6 vs 30.4 months) favoring the panobinostat group [85]. A subgroup analysis showed that panobinostat was associated with an improved PFS of 12.5 vs 4.7 months (HR 0.47; 95% CI 0.31–0.72) in patients who had received two or more prior regimens including bortezomib and an IMiD [86].

Panobinostat is the first HDACi used in MM. It is not widely used as other targeted therapy probably due to its relatively small benefit compared with its added toxicities including mainly gastrointestinal effects and cytopenia.

### *7.5.2 Vorinostat*

Vorinostat was the first HDACi approved for the treatment of cancer. It was approved in October 2006 for the treatment of progressive, persistent, or recurrent cutaneous T cell lymphoma [28]. It is a SAHA and an oral nonselective inhibitor of class I and II HDACs. When combined with bortezomib, vorinostat showed mild increases in efficacy in RRMM in multiple phase I, II, and III clinical trials compared with bortezomib alone [28]. However, vorinostat is currently not approved for use in MM.

### 7.5.3 *Ricolinostat*

Ricolinostat is a selective inhibitor of HDAC6 and has been tested as monotherapy and in combination with bortezomib and dexamethasone in a phase I/II study [87]. The combination therapy was well tolerated at ricolinostat doses of up to 160 mg/day. The overall response rate (ORR) was 29% with a clinical benefit rate of 39%. The most common treatment emergent adverse events were thrombocytopenia (71%), diarrhea (67%), anemia (42%), fatigue (42%), nausea (38%), hypokalemia (33%), vomiting (29%), peripheral neuropathy (29%), hyperglycemia (25%), and renal insufficiency (21%) [87].

The combination of ricolinostat, lenalidomide, and dexamethasone was tested in an early phase clinical trial with 38 patients. Two dose-limiting toxicities were observed with ricolinostat 160 mg twice daily [88]. More clinical trials are required to further establish the safety and efficacy of this combination.

A meta-analysis has shown a weaker anti-MM effect with ricolinostat compared to vorinostat and panobinostat [89]. The highest ORR of panobinostat in RRMM was 64% versus 51% and 38% in those treated with vorinostat and ricolinostat, respectively. The main adverse events were pancytopenia, fatigue, diarrhea, and nausea which was more pronounced in patients treated with ricolinostat [89].

## 7.6 Immunomodulatory Imide Drugs and Histone Deacetylase Inhibitors

Immunomodulatory imide drugs are a class of novel targeted therapy in MM consisting of thalidomide, lenalidomide, and pomalidomide. The effects of IMiDs on MM are mediated by the protein cereblon (CRBN) through ubiquitin-dependent and ubiquitin-independent pathways [90]. Studies have shown that the knockdown of CRBN results in resistance to treatment with IMiDs. The combination of IMiDs and HDACi has been shown to have synergistic activity in MM cell lines [91]. This combination induces caspase 8 and caspase 9 cleavage, activating the intrinsic and extrinsic apoptotic pathway and downregulating the anti-apoptotic XIAP protein [91].

Vorinostat (Class I and II HDACi) and Entinostat (Class I HDACi) have synergistic effects with lenalidomide despite the downregulation of CRBN. Their cytotoxic effect is due to the downregulation of c-Myc and is independent of CRBN [91]. On the contrary, ricolinostat, a selective HDAC6 inhibitor, does not affect CRBN activity. It downregulates IKZF1 which in turn decreases IRF4 and c-Myc, inhibiting MM growth [91].

## 7.7 Potential Mechanisms of Resistance to Histone Deacetylase Inhibitors

The basis of HDACi resistance is largely unknown and complex. This is due to the ability of HDACi to cause alterations at various cellular levels. Many molecular mechanisms of resistance have been demonstrated in vitro in cutaneous T cell lymphoma and solid cancers with limited evidence in humans.

### *7.7.1 Drug Transporters*

ATP binding cassette transporter expression has been implicated in HDACi resistance. ATP binding cassette transporters (ABC transporters) are essential for many processes in the cell and are often overexpressed in cancer cells. Overexpression of these transporters results in increased drug expulsion from cells. The ABC transporter proteins that have been implicated in resistance to HDACi include ABCB1 and ABCC1. Romidepsin is a substrate for ABCB1 and ABCC1 [92, 93].

### *7.7.2 Cell Signaling*

Genome wide gene expression studies of MM cells with different sensitivities to HDACi suggest that HDACi resistance is associated with a 35-gene signature. This signature primarily involves two pathways: the regulation of the actin cytoskeleton and protein processing in the endoplasmic reticulum. Synergism between HDACi and drugs that target the regulation of the actin cytoskeleton has been observed. These drugs include MEK/ERK, PI3K, and FAK inhibitors [94].

In cutaneous T cell lymphoma, the activation of the signal transducer and activator of transcription (STAT) pathway is associated with resistance to vorinostat. STAT1, STAT3, and STAT5 are highly expressed in lymphoma cell lines that are resistant to vorinostat [95].

### *7.7.3 Antioxidant Pathway*

HDACi induced apoptosis is associated with the generation of reactive oxygen species [96]. The activation of an antioxidant signature was shown to be associated with resistance to vorinostat in acute myeloid leukemia and myelodysplasia [97]. Thioredoxin, a major reducing protein, is protective against HDACi induced cell

death. In malignant cells thioredoxin levels are relatively reduced compared with normal cells, and hence they are more sensitive to HDACi induced cell death compared with normal cells. However, when malignant cells are transfected with thioredoxin siRNA, they become more sensitive to HDACi. Therefore, antioxidant mechanisms may mediate resistance to HDACi [98].

### *7.7.4 Cell Cycle Proteins*

HDAC inhibition is associated with the induction of $p21^{CIP1}$. Leukemic cell lines transfected with antisense $p21^{CIP1}$ have increased sensitivity to HDACi, suggesting that induction of $p21^{CIP1}$ could be a potential mechanism of resistance to HDACi [99].

### *7.7.5 Nuclear Factor-Kappa B*

Resistance to panobinostat in cutaneous T cell lymphoma is associated with constitutive activation of NF-kB which activates pro-survival factors, including inhibitors of apoptotic proteins and Bcl-2 family proteins. Inhibition of Bcl-2 by ABT-737 overcomes resistance to panobinostat [100].

### *7.7.6 Anti-Apoptotic Proteins*

Overexpression of anti-apoptotic proteins is another potential mechanism of resistance to HDACi. Valproate and ITF2357 are HDACi with cytotoxic activity in hepatoma cell lines. They inhibit Bcl-xL expression and induce apoptosis. Overexpression of Bcl-xL can induce resistance to Valproate and ITF 2357 on hepatoma cell lines [101].

### *7.7.7 Altered Histone Deacetylases*

Altered expression or structures of HDAC proteins may confer HDACi resistance. Cells of the HL-60 leukemic cell line that were selected for HDACi resistance were observed to express higher levels of HDAC1, HDCA2, and HDAC4 [102]. In melanoma cell lines, overexpression of HDAC1 confers resistance to sodium butyrate [103]. In breast cancer patients, HDAC2 expression level has been correlated with vorinostat response [104].

### *7.7.8 Autophagy*

Autophagy is a cellular process that removes damaged cellular components and misfolded proteins. Aggrephagy is a type of autophagy that targets and removes protein aggregates. HDAC6 is necessary for this process, recognizing protein aggregates through its ubiquitin-binding domain. HDAC6 binds to dynein which is a motor protein mediating the retrograde transport of the protein aggregates on the microtubules to the perinuclear region at the microtubule-organizing center. The protein aggregate is then enclosed by a vimentin cage, becoming an aggresome, which further develops into an autophagosome. The autophagosome then fuses with a lysosome which contains acid hydrolases for the degradation of the enclosed protein aggregates.

The induction of autophagy has been shown to be a mechanism of resistance to other anticancer therapy. Inhibitors of autophagy such as chloroquine and hydroxychloroquine have been shown to overcome resistance to chemotherapy in vitro and in vivo. The combination of chloroquine or hydroxychloroquine and other anticancer drugs are being investigated in ongoing clinical trials for the treatment of a range of cancer types. In a clinical trial comparing gemcitabine hydrochloride and nab-paclitaxel with and without hydroxychloroquine in patients with advanced pancreatic cancer, OS was similar in both groups. However, an improved ORR was observed in the hydroxychloroquine cohort [105]. In an early-phase clinical trial of vorinostat and hydroxychloroquine in metastatic colon cancer, there was preliminary evidence of autophagy inhibition, seen in the accumulation of lysosomal protease cathepsin D and p62 in biopsies [106].

The activation of autophagy could be a potential mechanism of resistance to HDAC6 inhibition. Autophagy activation in MM can be achieved by short hairpin RNA knockdown of HDAC1 or by treatment with SAHA, which upregulates the transcription of LC3, activating the ULK1 Complex and suppressing mTOR [107].

It can be seen that there are multiple potential mechanisms of resistance to HDACi, many of which are not yet fully understood. Further deciphering the mechanisms of HDACi resistance is important as it may enable the discovery of more synergistic combination therapies, the development of novel HDACi, and the discovery of biomarkers that could predict resistance.

## 7.8 Conclusion

Multiple myeloma remains incurable with most patients either relapsing or becoming refractory to treatments. The incorporation of various novel therapies has resulted in significant survival benefits not only in newly diagnosed MM patients but also in those with RRMM disease. Despite these advances, resistance to therapy leads to eventual relapse and fatal outcomes in the vast majority of patients. There remains an unmet need for novel drugs and efficacious therapies for continued

improvement in outcomes. The incorporation of HDACi with current MM therapies may improve long-term outcomes. The use of these drugs is however limited by unfavorable side effects and drug resistance. Further studies to address this, particularly focusing on combining selective and better tolerated HDACi with PIs and IMiDs offer the possibility of improving outcomes in MM.

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