




# Epigenetic Modifications in Myeloma: Focused Review of Current Data and Potential Therapeutic Applications

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## Abstract

Multiple myeloma is a common hematologic malignancy with an incidence of 1 per 100,000 population and is characterized by a nearly 100% risk of relapse, necessitating treatment with newer therapeutic agents at each instance of progression. However, use of newer agents is often precluded by cost and accessibility in a resource-constrained setting. Description of newer pathways of disease pathogenesis potentially provides opportunities for identification of therapeutic targets and a better understanding of disease biology. Identification of epigenetic changes in myeloma is an emerging premise, with several pathways contributing to pathogenesis and progression of disease. Greater understanding of epigenetic alterations provides opportunities to detect several targetable enzymes or pathways that can be of clinical use.

## Keywords

- ▶ myeloma
- ▶ lenalidomide
- ▶ pomalidomide
- ▶ transplant
- ▶ relapse

## Introduction

Epigenetics is defined as heritable changes in genetic function that occur without alteration of the underlying genetic code. Since its original description, it is now clear that epigenetic mechanisms are vital for cellular growth, development, and establishment of a distinct cellular identity.<sup>1</sup> The term “epigenome” describes the epigenetic regulatory mechanisms across the entire genome; and unlike the genome, it varies across different cell types. Abnormalities in epigenetic control can lead to a wide variety of cellular dysfunction, and have been specifically found to contribute to carcinogenesis.<sup>2</sup> Epigenetic alterations are early events in the pathogenesis of certain cancers and contribute to disease progression, analogous to clonal cytogenetic evolution.<sup>3</sup> Epigenetic modifications are of interest in several

malignancies, and have been utilized most notably for the treatment of acute myeloid leukemia in the form of hypomethylating agents. Significant molecular data are also emerging on the role of epigenetics for several other malignancies, and is of specific importance in multiple myeloma (MM).

MM is a common hematologic malignancy with a universal risk of relapse and has undergone advances in survival over the past two decades.<sup>4</sup> However, it still carries a nearly 100% risk of relapse, necessitating newer agents to be initiated at each instance of progression.<sup>5</sup> As a result, no functional cure is defined, and therapy must continue indefinitely. Although several newer novel agents have been introduced in the past 5 years, costs continue to be prohibitive, especially in resource constrained settings.<sup>6</sup> Thus, there is an ongoing need to identify newer cellular pathways and targetable genetic

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lesions to ensure the availability of treatment options at each relapse.

Epigenetic mechanisms have recently emerged as critical mediators of disease pathogenesis and progression in myeloma. Several mechanisms are linked to drug responsiveness and known to modify survival. As seen in the case of acute myeloid leukemia (AML), several components of epigenetic mechanisms are eminently targetable and will likely offer an opportunity for a new approach to drug development. An added impetus for studying epigenetic pathways would be definition of differences in disease biology based on geographic variation. For instance, significant differences in age of incidence of myeloma have been noted in Indian patients compared with Western data, with higher likelihood of end organ damage at diagnosis.<sup>7</sup>

We provide a succinct snapshot of the current role of epigenetic mechanisms in disease pathogenesis in MM, and highlight potential therapeutic applications. We also provide a snapshot of potential drug targets and current ongoing trials targeting epigenetic pathways in MM.

## Summary of Basic Mechanisms of Epigenetic Control

Epigenetic control is facilitated by several biochemical processes, of which the best understood mechanisms are (a) DNA methylation, (b) histone modification, and (c) microRNA (miRNA) expression. These three processes are most widely described and are targets of several investigational approaches to treatment.

### DNA Methylation

DNA methylation consists of addition of a methyl group to the 5' carbon of cytosine on DNA. The cytosine bases that are

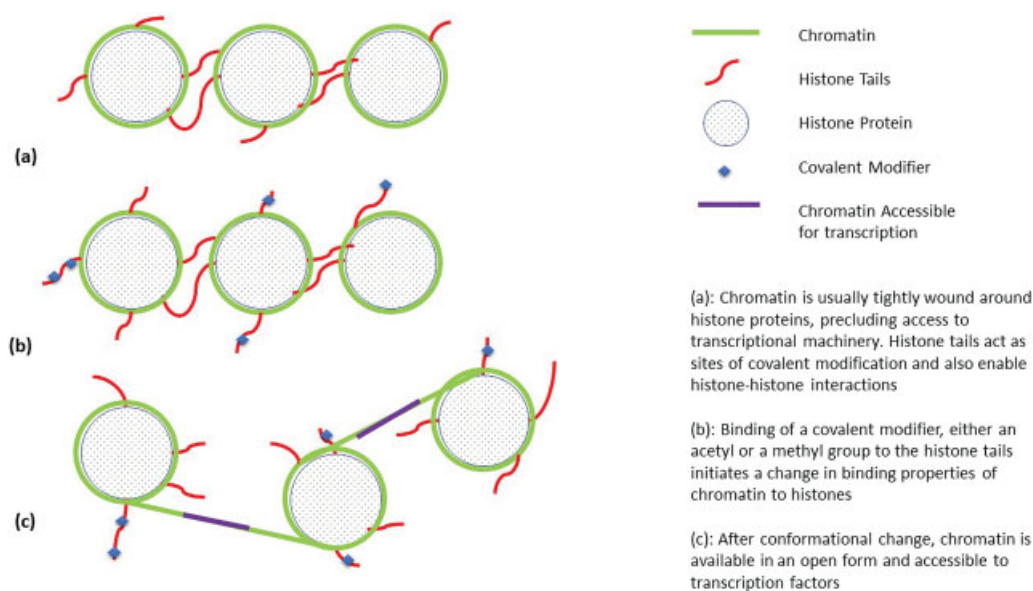
involved in methylation are usually located in regulatory (enhancer or repressor) sequences, and can recruit other proteins to functionally silence downstream genes.<sup>8</sup> DNA methylation of promoter genes, therefore, leads to silencing of the downstream genes regulated with the same. DNA methylation is performed by enzymes called DNA methyltransferases (DNMT) and typically occurs at sites of CpG (cytosine-phosphate-guanine) dinucleotides, which are concentrated in sites known as CpG islands, mostly at 5' ends of DNA.<sup>8</sup> There are ~28 million CpGs in the human genome, and ~60% to 80% are methylated.

### Histone Modification

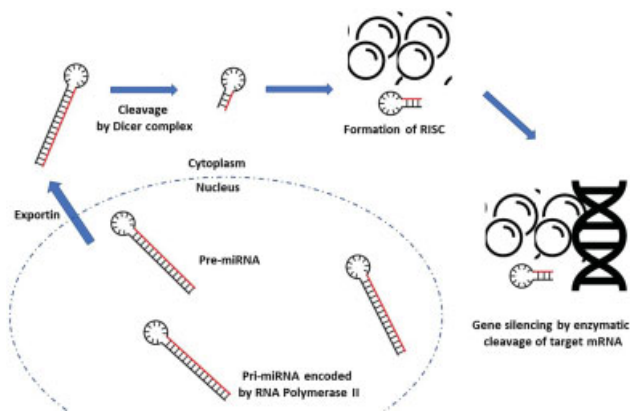
Intracellular DNA is tightly wound around proteins called histones, which are octamers consisting of eight subunits. This process is best summarized by its original definition given in the 1960s, "dynamic and reversible mechanism for activation as well as repression of RNA synthesis."<sup>9</sup> It is known that modification of histone proteins by biochemical changes enables regulation of DNA expression by increasing or decreasing chromatin accessibility. The first mechanism to be elucidated was histone acetylation and deacetylation, following which several other mechanisms have been described over the last decade.<sup>10</sup> As genetic regulation needs to be reversible and dynamic, most of the processes are reversible and involve reciprocal "effector" and "eraser" enzymes. These include acetylation, methylation, and phosphorylation, and the use of so-called "erasers," or enzymes with reciprocal activity to the ones above. ► **Fig. 1** summarizes the basic process of histone modification.

### Noncoding RNA Expression

Noncoding (ncRNAs) are RNAs that are not coded into proteins. They are classified by their size into small and large



**Fig. 1** Histone modifications in DNA. Histone proteins (dotted blue) are tightly wound to DNA (green), preventing access of transcriptional machinery. Reversible binding of covalent modifiers can occur either on the histone protein core or on one of the histone tails (red). Binding or a modifier, for example, an acetyl group (blue), leads to a conformational change, opening the chromatin structure and enabling access to transcriptional machinery.



**Fig. 2** Summary of micro-RNA (miRNA)-mediated control of genetic expression. Starting from lower left, RNA polymerase II encodes primary miRNA (pri-miRNA) that is converted to pre-miRNA and exported out of the nucleus by Exportin. After cleavage by the Dicer (helicase with RNase motif) complex, the miRNA forms an RNA-induced silencing complex (RISC) that binds to complementary miRNA, silencing its expression. This repressor function of miRNA against has demonstrated antimyeloma activity in vitro.

ncRNAs. The small ncRNAs include miRNA, piwi interacting RNA, transfer RNA, and small nucleolar RNA.<sup>11</sup> They are involved in regulation of DNA expression by several mechanisms, out of which epigenetic alteration is one of them. **►Fig. 2** summarizes mechanisms of miRNA control with potential therapeutic interest.

## Genetic Changes in Myeloma

Plasma cell (PC) clones in MM acquire several genetic abnormalities while progressing from the stage of monoclonal gammopathy of undetermined significance (MGUS) to symptomatic disease. Broadly, these are categorized as primary and secondary cytogenetic abnormalities. Primary cytogenetic abnormalities arise at the stage of MGUS and have a significant impact on disease phenotype, organ damage, and rate of progression. These are mutually exclusive, with each clone having only one primary cytogenetic anomaly.<sup>12</sup> Common primary genetic changes include hyperdiploidy and immunoglobulin heavy chain promoter (IgH) translocations. Hyperploidy of at least one odd numbered chromosome is noted in a majority of patients with myeloma, commonly presenting as trisomy.<sup>13</sup> IgH translocations are usually a result of errors in DNA modification as a part of physiological processes of V(D)J recombination and class switching. These translocations commonly place an oncogene under control of the highly active IgH locus.<sup>14</sup> Both these changes can be observed in PCs at all stages of disease, indicating the fundamental nature of these changes.

Recent advances in next-generation sequencing (NGS) have also identified several patterns of single gene mutations in patients with newly diagnosed myeloma. Mutations in BRAF, TP53, KRAS, NRAS, and DIS3 are some of the commonest recurrently mutated genes. Single gene mutations have already been used to classify patients into groups with distinct phenotypes and open up avenues for individualized medicine.<sup>15</sup>

On the other hand, secondary cytogenetic abnormalities arise at the time of disease progression and clonal evolution, with each cell possibly having multiple secondary abnormalities. In routine practice, either of these can be detected in ~50% of patients by conventional karyotyping and 40 to 50% by fluorescence in situ hybridization.<sup>16</sup> Commonly described secondary cytogenetic abnormalities include del(17p), gain (1q), and myc translocations.

## Summary of Epigenetic Changes in Myeloma

All three epigenetic processes, namely DNA methylation, histone modification, and miRNA processing, are active in MM. The following text is a summary of each of these processes.

### DNA Methylation

#### DNA Methylation in Myeloma Is Heterogeneous

The potential role for DNA methylation in MM was elucidated in 2004, when it was shown that methylation of promoter regions of tumor suppressor genes (leading to inactivation) was increased in patients with MM.<sup>17</sup> Subsequently, it has been observed that the pattern of genome methylation in myeloma is not uniform, and consists of specific *hypo*- and *hypermethylated* regions. Several in vitro studies have highlighted differential effects of methylation at different stages in the pathogenesis of myeloma. As with several solid organ malignancies, the overall methylation pattern in initial phase consists of hypermethylation, with advanced myeloma demonstrating global DNA *hypomethylation*, reflecting genomic instability.<sup>18,19</sup> DNA hypomethylation is postulated to activate several transcriptional units or oncogenes that are usually repressed and provide a survival advantage.<sup>20</sup> Hypomethylation is frequently observed on repetitive DNA elements and progressively increases while moving from healthy controls to myeloma to PC leukemia.<sup>21</sup> It is essential to note that global hypomethylation is not uniform, and several loci are hypermethylated in advanced or relapsed disease.<sup>22</sup> Interestingly, DNA methylation in MM and MGUS has been seen to occur at sites distant from CpG rich promoters, specifically in areas known for binding to B cell promoters.<sup>18</sup> This presents a new area of potential research, as these promoters may have a role in driving myeloma cell proliferation and may be targetable by newer therapies.

Unlike methylation, no single *mechanism* describes the process of hypomethylation. The simplest method described is lack of maintenance of methylation, which occurs with downregulation of DNMT. These changes mirror alterations in enzymes mediating DNA methylation, that is, DNMT1, which is shown to increase in expression when moving from MGUS to symptomatic disease.<sup>21</sup> Expression of DNMT3a has been shown to be downregulated in patients with MGUS and MM, reducing baseline maintenance methylation.<sup>23</sup> Putative biochemical mechanisms of DNA hypomethylation are still not clear and are summarized in this excellent review.<sup>24</sup> These mechanisms are clinically relevant as myeloma cell lines are susceptible to DNMT inhibition with currently available hypomethylating agents, providing a potential therapeutic target.

## MicroRNA

### Diverse Changes in miRNA Expression Characterize Myeloma

miRNAs were first identified in 1993, defined as small molecules with antisense 3' UTR specificity.<sup>25</sup> It was later identified that miRNAs are small, ncRNAs that regulate gene expression at the posttranslational level. Depending on their location, miRNAs are of two major subtypes, that is, intragenic (intronic) and intergenic, with most showing clustering with a common promoter. The major mechanism of gene regulation is at the posttranslational level, by binding to the corresponding miRNAs, typically resulting in repression. The entire process can be summed up as miRNAs are transcribed by RNA polymerase II, which creates a pri-miRNA, which is converted to pre-miRNA and finally exported as miRNA into the cytoplasm. This final miRNA forms a complex called RNA-induced silencing complex, which leads to binding and inhibition of target miRNAs.<sup>26</sup> Deregulation of miRNA function, both through genomic and epigenomic mechanisms, is increasingly seen to occur in several malignancies, including myeloma. In MM, deregulation of miRNA function can be noted at all disease stages, from MGUS to MM. Multiple studies have demonstrated deranged miRNA function in PCs from MGUS and MM cells compared with normal PCs.<sup>27</sup> Remarkably, the changes seen in a particular miRNA are seen to proceed in the same trend with disease progression, raising the possibility that specific changes have a part to play in pathogenesis and disease progression.<sup>28</sup>

### Histone Modification in Myeloma Is a Complex Multistep Process

Histone proteins are subject to several covalent posttranslational modifications, of which over 15 have been described in detail.<sup>10</sup> Histone modification is reversible, and the processes involved have effector and eraser functions with a set of reciprocally active enzymes. Acetylation, phosphorylation,

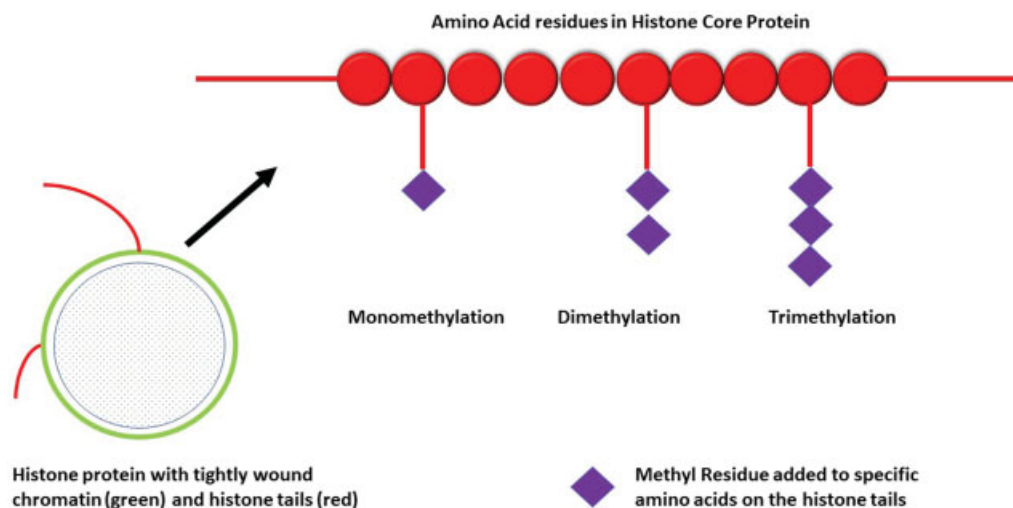
and methylation are the most well described, with the first two being clear markers of active transcription<sup>29</sup> Methylation, on the other hand, is more complex, as the final effects of methylation depend on the amino acids modified and the degree of methylation (mono, di, or tri).<sup>30</sup> Histone methylation is seen to occur at basic amino acids, namely arginine, histidine, and lysine, and may have a variable effect on transcription depending on context (i.e., type and site of amino acid that is methylated).<sup>31</sup> A common nomenclature is used to define histone modifications, comprising the name of histone protein (e.g., H2), abbreviation and position of amino acid (K or M), chemical group added (e.g., methyl = Me or Acetyl = Ac), and copies of the modifier added (1, 2, or 3). Therefore, addition of a dimethyl group to a lysine residue in position 36 on histone protein H3 is abbreviated as H3K36Me<sub>2</sub>. This process is summarized in ▶Fig. 3.

#### Histone Dimethylation

Histone dimethylation is catalyzed by enzymes containing the SET domain with methyltransferase activity. In this well-characterized group, mutations in MMSET, NSD1, and NSD2 are found in several patients at diagnosis and relapse. MMSET is one of the most well defined, and catalyzes the addition of H3K36me<sub>2</sub>, associated with active transcription.<sup>32</sup> It is seen to be associated with the t(4;14) translocation, leading to transcriptional activation and is associated with a poorer outcome in patients with MM.<sup>33</sup> Over the past decade, there has been an increasing recognition of the biological contribution of MMSET to pathogenesis of myeloma. MMSET is shown to activate the MAF gene through the MAP kinase pathway, indicating indirect activation of an oncogene. MMSET is also implicated in activation of several downstream mitogenic pathways, including c-myc and IRF4.<sup>34</sup>

#### Histone Trimethylation

Histone trimethylation is brought about by enzymatic units of enhancer of Zeste homologue 2 (EZH1/2) complexes, which deposit H3K27me<sub>3</sub> on target genes. EZH2 is part of



**Fig. 3** Patterns of histone methylation. Methylation occurs on specific amino acids in the histone core protein and is mediated by histone methyl transferases. Depending on the number of methyl groups added, it can either occur as mono-, di-, or trimethylation.



the polycomb repressor complex (PRC), which silences several downstream genes. The role of EZH2 varies in different malignancies, and in MM it is shown to be upregulated and correlates with a poor outcome.<sup>35</sup> Similar to MMSET, EZH2 overexpression induces disease progression through a variety of mechanisms. EZH2 is shown to induce proliferation of MM cells independent of interleukin 6 (IL-6). It is also shown to control the expression of tumor suppressor miRNAs that target c-myc, BLIMP-1, and IRF4.<sup>36</sup>

### Histone Monomethylation

Histone monomethylation at lysine sites deposits H3K9me and is essential for gene repression at promoter regions. H3K9 transferring enzymes are elevated in MM patients. KDM3A leads to IRF4 supported cell growth in MM.<sup>37</sup>

### Histone Acetylation

Histone acetylation is seen to occur at lysine residues, which opens up chromatin and makes it transcriptionally active. This process is reversibly regulated by lysine transferases (KATs) and deacetylases (histone deacetylase inhibitors [HDACs]).<sup>38</sup> Mutated KAT genes are found in several patients with MM, especially at relapse, possibly loss of function mutations. HDACs are overexpressed in myeloma and are associated with poor prognosis.<sup>39</sup>

### Role of Epigenetic Mechanisms in Disease Progression and Drug Resistance

Epigenetic processes have been found to play a significant role in two important processes mediating disease progression, that is, PC plasticity and drug resistance.

Analysis of malignant PCs in myeloma reveals a heterogeneous population comprising PCs (CD138+/CD19-), pre-plasma cells (pre-PCs: CD19-/CD138-), and plasmablasts (CD19+). A vast majority of the cells are CD19-/CD138+ and predominant mediators of disease phenotype, including immunoglobulin secretion and organ damage. A clone of pre-PCs with a CD19-/CD138- represents a small minority, representing a quiescent population. This clone confers resistance to therapy and is in dynamic equilibrium with CD138+ PCs.<sup>40</sup>

Several characteristics of this process indicate a significant role played by epigenetic processes. Pre-PC populations demonstrate high concentrations of several epigenetic regulators, including PRC, MLL transcriptional activating complex, demethylases, HDACs and KDM5C/D, indicating epigenetic control. Additionally, the absence of irreversible genetic mutations or change in CD138 miRNA levels makes genetic level (transcriptional/ translational) control unlikely.<sup>41</sup> These cells are intrinsically resistant to proteasome inhibitors, and may indicate a reservoir that can proliferate at relapse.

Additionally, epigenetic mechanisms are observed to mediate many aspects of drug resistance in patients with myeloma. Epigenetic inactivation of RASD1 is essential in mediating glucocorticoid resistance. Several miRNAs have been found to mediate drug resistance through diverse mechanisms, including modulation of target genes, upregu-

lation of oncogenes, and modification of drug efflux mechanisms. A review by Rastgoo et al provides a succinct overview of drug resistance mediated by miRNA.<sup>42</sup> The control of cell adhesion-mediated drug resistance by altering microenvironment interactions and modification of anti-apoptotic signals also have significant epigenetic control. The description of all allied mechanisms is out of scope of this review and is summarized elegantly by Furukawa et al.<sup>43</sup>

### Therapeutic Applications of Epigenetic Modifications in Myeloma

All the mechanisms described above provide potential therapeutic targets. The following discussion summarizes salient features of some of the commonly described mechanisms. Many mechanisms have been translated to clinical applications, and a summary of recent or ongoing trials utilizing the same is provided in **Table 1**.

#### Promotor Region Hypermethylation Is a Potential Therapeutic Target

Promoter region hypermethylation of several tumor suppressor genes has been documented in myeloma (summarized in **Table 2**). The use of *hypomethylating* agents, azacytidine (Aza) and decitabine, thus has a potential role in reversing this phenomenon.<sup>44</sup> The in vitro efficacy of Aza against MM cell lines was demonstrated in 2008, where it was shown that Aza led to demethylation of p16, theoretically restoring its tumor suppressor function. It also inhibited IL-6 production and expression of IL-6 receptor, leading to apoptosis of MM cell lines.<sup>45</sup> Further, Aza has been shown to have synergistic activity with several other chemotherapeutic agents in MM. Bortezomib and doxorubicin have been shown to sensitize PCs in MM to the effects of Aza by synergistic induction of double strand DNA breaks.<sup>46</sup> Thus, Aza, possibly in combination with other commonly used chemotherapeutic agents, may have a potential therapeutic role in MM.

#### Preclinical Efficacy of Targeting miRNA Mechanisms

Several miRNAs have been shown to have therapeutic efficacy against PCs from MM cells in preclinical studies. MiR-29b is shown to have anti-MM activity through multiple mechanisms, including inhibition of IL-6 and JAK STAT signaling.<sup>47</sup> It has been shown to inhibit MM cell growth in vitro and potentiates the antitumor efficacy of bortezomib.<sup>48</sup> Many miRNAs have been found to have tumor suppressor functions. For instance, miR-192, -194, and -215 upregulate P53 expression, and their downregulation is involved in disease progression.<sup>49</sup> Similarly, miR-34 is also shown to mediate similar activity by controlling cell proliferation and differentiation. Synthetic miR-34 constructs were found to have significant anti-MM activity, and found to potentiate the same in combination with other anti-MM agents.<sup>50</sup> Anti-MM cell activity is also noted with miR-15a and 16a through multiple mechanisms, including inhibition of BCL2, IL-17, and angiogenesis (via vascular endothelial growth factor). Downregulation of antitumor activity is observed to correlate with advanced disease stages.<sup>51</sup>

**Table 1** A snapshot of currently ongoing clinical trials utilizing epigenetic pathways for treatment of myeloma, predominantly in the relapsed/refractory setting

Mechanism targeted	Drug/Preclinical molecule	Trial phase and design	Salient findings
DNMT	Oral azacytidine <sup>86</sup>	Oral azacytidine in combination with Len/Dex for RRMM	ORR was 37.5%; clinical benefit rate was 50%. Median OS was 10.3 mo; median PFS was 2.6 mo
	Injectable azacytidine <sup>87</sup>	Phase 1b, twice a week S/C azacytidine with Len/Dex for RRMM	The median PFS was 3.1 mo (95%CI: 2.1–5.1 mo), median TTP 2.7 mo (95% CI: 2.1–7.5 mo), and median OS 18.6 mo (95% CI: 12.9–33.0 mo)
HDAC	Panobinostat <sup>53</sup>	Phase 3, panobinostat + Vd vs. Vd alone for RRMM	PFS advantage, 11.99 mo (95% CI: 10.33–12.94) vs 8.08 mo (7.56–9.23); HR 0.63, 95% CI 0.52–0.76; $p < 0.0001$ . OS difference not yet clear
	Tefinostat <sup>88</sup>	Phase 1, dose escalation trial of oral drug	Safety and maximal tolerated dose defined. Further clinical studies planned
	Romidepsin <sup>89</sup>	Phase 2 trial in thirteen patients	Initial safety and clinical improvement noted, Phase 3 trials planned
		Phase 1/2 trial in combination with Len for refractory disease	Active, completed recruitment December 2020. Updates available at NCT01755975
	Vorinostat <sup>90</sup>	Bortezomib alone or with Vorinostat in patients with RRMM	Median PFS was 7.63 mo (95% CI: 6.87–8.40) in the Vorinostat group and 6.83 mo (5.67–7.73) in the placebo group (HR 0.77, 95% CI: 0.64–0.94; $p = 0.0100$ . OS difference not yet clear
	Ricolinostat <sup>91</sup>	Phase 1/2 trial of ricolinostat + bortezomib/DeX	ORR 37%, responses in bortezomib refractory 14%
BET	CPI203 <sup>92</sup>	In vitro	Increased myeloma cell kill in vitro when added to Len/Dex combination
	Molibresib <sup>93</sup>	Phase 1	Trial including patients with multiple hematological and solid organ cancers. Safety and tolerability established
	CPI-0160	Phase 1	Recruitment completed; results awaited. Details at NCT02157636
	RO6870810 <sup>94</sup>	Phase 1	Safety established Partial responses in 16%, including daratumumab resistant patients.
EZH	EPZ 6438 <sup>95</sup>	In vitro	EPZ6438 in combination with Len represses myc and activates tumor suppressor genes
	Tazemetostat	Phase 1	Currently recruiting for solid tumors and lymphomas

Abbreviations: CI, confidence interval; Dex, dexamethasone; DNMT, DNA methyl transferases; EZH, enhancer of Zeste homologue; HDAC, histone deacetylase inhibitor; HR, hazard ratio; Len, lenalidomide; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; RRMM, relapsed/refractory multiple myeloma; Vd, bortezomib-dexamethasone.

**Table 2** Summary of pathologic genes hypermethylated in multiple myeloma

Name	Normal function	Frequency	Comments
P16	Inhibits CDK 4 and 6	19–53%	Associated with poor outcomes Involved in progression from MGUS to myeloma <sup>82</sup>
SHP and SOCS <sup>83</sup>	Inhibition of JAK/STAT function	20–79%	Overactivity of IL-6 stimulated JAK/STAT
E-Cadherin	Cell adhesion inhibits cellular mobility	27–56%	Disease progression marker for high-risk disease, immature morphology
DAPK	Proapoptotic kinase	12.5–67%	Associated with high-risk disease, poorer response to therapy
DNA damage repair genes	Multiple genes <sup>84</sup>		More information needed for exact function

Abbreviation: IL-6, interleukin 6; MGUS, monoclonal gammopathy of undetermined significance.

Source: Adapted from Sharma et al.<sup>84</sup>

### Histone Modification Has Potential Therapeutic Applications

As there are multiple pathways leading to histone modification in MM, several potential therapeutic approaches exist to exploit these pathways for antimyeloma efficacy. Out of the various drugs studied, HDACs are some of the most well characterized with a proven clinical efficacy.<sup>52</sup> Panobinostat is a nonselective HDAC inhibitor that has been approved for the treatment of relapsed/refractory myeloma based on phase 3 data.<sup>53</sup> Two important features noted with the use of HDAC inhibitors. First, a high rate of cardiac adverse events and cytopenia is noted, indicating several off target epigenetic effects that are still to be elucidated. The use of more selective HDAC inhibitors is expected to reduce these serious adverse events. Second, the efficacy of these drugs, even the newer selective HDAC inhibitors, is only modest as single agents. The best results are seen in combination with other anti-MM agents.<sup>54</sup> Several clinical scores based on gene expression profile to predict response to HDAC inhibitors have been described.<sup>55</sup> Histone acetylation is always activating, but histone methylation may be activating or deactivating. Several small molecule modifiers of histone methylation are still in preclinical or phase 1 trials (NCT02082977).<sup>56</sup>

### Mediators of Histone Methylation Are Forthcoming Therapeutic Targets

Histone proteins are found in the nucleus of all eukaryotic cells and exist as octamers, around which double-stranded DNA is wrapped. Each cell has two copies of each of the histone proteins H2A, H2B, H3, and H4 that form nucleosomes around which DNA is compactly packed. Release of DNA from histone proteins leads to chromatin modification and activation of transcription, which is otherwise suppressed.<sup>57</sup> Histone modifications act by influencing the recruitment of nonhistone proteins and the levels of chromatin compaction, altering the accessibility of transcription factors to DNA. Posttranslational modification of histone proteins is an essential biologic process, required for normal development and cellular functioning.<sup>58</sup> The most important and clinically relevant mechanisms of histone modification consist of acetylation and methylation. We will focus on histone methylation for this discussion, which is amenable to therapeutic intervention in myeloma.

### Histone Methylation

Histone methylation occurs on basic amino acids, that is, arginine, lysine, and histidine, and varies according to the amino acid in question. Lysine can be mono-, di-, or trimethylated, arginine mono- or demethylated, and histidine monomethylated. Histone methylation is context dependent, and effects on genetic expression may vary based on the location of the target residue and degree of methylation.<sup>58</sup> An extensive review by Greer and Shi summarizes histone modification in detail, and a relevant summary of these changes is provided below.<sup>58</sup>

Enzymes mediating histone changes are thought to be attracted to DNA through specific sequences, the most prominent being the polycomb repressor group. The methylation

status of histones is read by proteins with methyl binding domains. Many mechanisms are thought to be in play, but most elegantly, the positive charge created by methyl residues is thought to increase the binding of hydrophobic proteins.<sup>59</sup> Histone methylation is mediated by a group of enzymes called histone methyltransferases, of which arginine and lysine methyltransferases are the most relevant.

Arginine methyltransferases comprise nine members in all, as a part of the protein arginine methyltransferase family (PRMT), which mediate methylation of arginine.<sup>60</sup> These enzymes catalyze two types of dimethylation and one monomethylation. Monomethylation is regarded as an intermediate metabolite in the formation of di-ch<sub>3</sub>-arginine. PRMTs are constitutively active and involved in cell growth and development, and multiple mechanisms exist for regulation of activity of PRMTs. PTM, including methylation and phosphorylation, inhibit PRMT function. In addition, PTM already on the substrate prevents further addition of methyl groups. For example, phosphorylation blocks methylation and acetylation stimulates methylation.<sup>61</sup> Expression of PRMTs has been altered in several malignancies.

Lysine methyltransferases are part of a more extensive family and are characterized by the presence of a catalytic SET domain, which is conserved across all members of the family. The SET domain, named after drosophila proteins from which it was isolated, executes the final catalytic activity of these enzymes.<sup>62</sup> SET containing proteins can be further subclassified based on sequence homology around the catalytic domain. Six families, namely, SUV39, EZH, SET2, PRDM, SMYD5, and KMT4 (an exception with no SET domain), have been identified. Interestingly, SET containing proteins also have the property of being able to read PTMs, indicating a linkage of reader and writer functions in mediating overall control of histone modification.<sup>63</sup>

Salient details about the clinically important members from both of the above two groups are listed below, which also form the focus of our study. ► **Table 3** summarizes important enzymes involved in histone methylation.

**MMSET:** MMSET catalyzes the addition of H3K36me<sub>2</sub>, which is associated with active chromatin. The significance of MMSET activation is seen in myeloma with t(4;14) translocation, which shows universal activation of this gene.<sup>64</sup> MMSET expression has been found to promote myeloma tumor growth in vitro, with MMSET knockdown leading to growth arrest.<sup>65</sup> MMSET also indirectly activates the activation of several oncogenic proteins that act as transcription activators and play a role in carcinogenesis, including IRF4, MAF, and c-MYC. Recently, it has also been shown to induce degradation of p53, thus increasing cellular proliferation.<sup>66</sup> MMSET also enhances the ability of malignant PCs to repair DNA damage, leading to resistance to alkylating agent-based chemotherapy.<sup>67</sup>

**EZH2:** EZH2 catalyzes the addition of a trimethyl mark H3K27me<sub>3</sub>, which is associated with repression of gene expression. EZH2 is part of a PRC, which contains EZH2, ASXL1, EED, and other accessory proteins.<sup>68</sup> EZH2 overexpression has been found to be associated with dysregulation

**Table 3** A summary of enzymes involved in histone methylation and their therapeutic correlates

Methylation modifier	Prooncogenic effect	Others	Drug resistance	Adverse effect on survival	Preclinical small molecule inhibitor
MMSET	↓ P53 function	↑ IRF-4, MAP, c-MYC	By resistance to DS-DNA breaks	Yes	No
EZH2	↓ TS miRNA, ↓ TS Genes (CDKN1C, RBPMS, LTB)	↑ Oncogenes, c-Myc, JUNB, BLIMP1	By cell adhesion mediated drug resistance	Yes	Yes, E7438, UNC1999 and GSK 126 and shRNA
PRMT5	↑ NF-kB pathway	↓ IKKβ	No	Yes	Yes, EPZ015666
KDM1A	↓ p53	↑ c-MYC	No	Yes	Tranylcypromine, GSK-LSD1, and ORY-1001
KDM3A	↑ KLF2, IRF4, MALAT1	↓ Apoptosis	No	Yes	No
KDM6A	↓ Tumor suppressor function (self)	Coordinates with EZH2	No	Yes	Sensitizes to EZH2 inhibition
KDM6B	↑ ELK, FOS, MAP-k	–	No	Yes	–

Source: Adapted from Sharma et al<sup>84</sup> and Anderson et al<sup>85</sup>

of cell cycle control and overall inferior outcomes. The effects of EZH2 overexpression are context dependent, and lead to activation of transcription in DLBCL/Follicular lymphoma and silencing in myeloma.<sup>69</sup> Multiple downstream effects of EZH2 include suppression of tumor suppressor miRNAs and upregulation of antiapoptotic and pro-oncogenes.<sup>70</sup> EZH2 also leads to poorer outcomes by repression of p21 and p15 via H3K27me3, leading to uncontrolled cyclin D overexpression and cellular proliferation.<sup>35</sup>

**PRMT5:** PRMT5 is an arginine methyltransferase and catalyzes dimethylation of arginine. PRMT5 is overexpressed in myeloma and is associated with inferior outcomes. It activates multiple oncogenic pathways, most notably the nuclear factor-kappa B pathway.<sup>71</sup> Pharmacologic inhibition of PRMT5 is a potential therapeutic target.

**Demethylases:** Histone demethylases consist of a large group of enzymes, of which important members are listed below.

**Table 4** A summary of studies describing the prognostic impact of epigenetic modifications in myeloma

Study	Parameters	Specific genes	Salient findings
Kaiser et al <sup>96</sup>	Global methylation status of tumor suppressor genes in myeloma	GPX3	Median OS high vs. low methylation status, 16 vs. 46 mo, $p = 0.0001$
		RBP1	23.9 vs. 47.7 mo, $p < 0.0001$
		SPARC	19.4 vs. 47.7 mo, $p < 0.0001$
		TGFB1	25.7 vs. 50.9 mo, $p < 0.0001$
Barwick et al <sup>97</sup>	Global DNA methylome		Low vs. high methylation status, median OS: 2 y vs. not reached, $p = 8.7e-8$
Mithraprabhu et al <sup>39</sup>	Histone deacetylase expression	Class I HDACs, HDAC 1,2,3, and 8 and Class II HDACs, HDAC5 and 10	High vs. low expression shorter PFS ( $p = 0.07$ ) and OS ( $p = 0.003$ )
		KDM6A mutation	Mutated vs. wild-type PFS: 16.8 vs 26.6 mo; proportion alive at 2 y mutated vs. wild type: 51 vs. 80%
Pawlyn et al <sup>98</sup>		DNA methylation modifier	Mutated vs. wild-type PFS: NR vs. 26.6 mo; percent alive at 2 y mutated vs. wild-type: 58 vs. 80%

Abbreviations: HDAC, histone deacetylase inhibitor; OS, overall survival; PFS, progression-free survival.



**KDM1A:** KDM1A (also known as LSD1), demethylates H3K4. It coordinates with several other cellular proteins, including MMSET and HDAC. KDM1A has several nonhistone targets, notably p53, whose function it inhibits, and has been associated with a poorer prognosis for multiple malignancies.<sup>72</sup> In myeloma, inhibition of KDM1A has been found to inhibit interaction with epithelium and osteoclastogenesis. It has also been associated with activation of multiple pathways, including the c-myc pathway that contributes to pathogenesis and inferior overall survival.<sup>73</sup>

**KDM3 family:** It consists of KDM3A, KDM3B, and JMJD1C. It has been found to be associated with MM cell survival as part of the KDM3A-KLF2-IRF4 axis, and levels are seen to be increased in MM patients compared with controls.<sup>37</sup> It has been shown that hypoxia inducible KDM3A knocks out KDM3A induced apoptosis and leads to an antiapoptotic phenotype in malignant MM cells.<sup>74</sup>

**KDM6A:** Also known as UTX. It removes H3K27me2 and me3, methyl marks correlated with genomic silencing. KDM6A mutations have been implicated in several malignancies, including ALL/CMML and bladder cancers.<sup>75</sup> KDM6A mutations have been noted in over 10% of patients with MM and have been associated with a poorer prognosis. UTX loss is associated with increased proliferation and clonogenicity of MM cells.<sup>76</sup> Closely related KDM6B is also noted to play a role in MM cell survival and leads to activation of MAP-K pathway genes.<sup>77</sup>

### Clinical Analysis of Epigenetic Modifications

Changes in epigenetic modifications, including methylation and acetylation status, can be quantitatively measured and have been shown to have several prognostic implications. A basic understanding of clinical evaluation of epigenetic modifications is essential to appraise studies describing epigenetic pathways. DNA methylation has been traditionally tested with bisulfite sequencing, in which bisulfite is used to convert unmethylated cytosine to uracil, differentiating it from methylated cytosine. Recently, CpG island microarray has been utilized to provide a high throughput method for methylation status.<sup>78</sup> Histone modification is traditionally measured by ChIP sequencing, which can detect any protein as long as a specific antibody is available.<sup>79</sup> This has been upscaled with combination with chip microarray.<sup>80</sup> There is no single standard method of choice for miRNA analysis, and a comparative analysis can be found in this excellent review.<sup>81</sup> An overview of studies indicating prognostic impact of epigenetic changes at diagnosis is summarized in ► **Table 4**.

### Conclusions

Epigenetic pathways are being observed to play an increasingly important role in pathogenesis and disease progression in myeloma and have a significant prognostic impact. There are two critically important clinical correlates of the above advances in basic science. First, these pathways provide a new approach to therapeutic intervention in MM, and can be targeted by multiple approaches. Considering the incurable

nature of disease and paradigm for continuous therapy, identifying a newer therapeutic approach is an important step forward. Second, epigenetic mechanisms can explain geographic and ethnic differences in disease phenotype and outcomes not explained by conventional cytogenetics, improving our understanding of disease biology. The current review provides a short overview of the above, with clinical correlates, and will be a useful primer for better understanding of this intriguing approach.

### Conflict of Interest

None declared.

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