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REVIEW ARTICLE

Predictors of clinical responses to hypomethylating agents in acute myeloid leukemia or myelodysplastic syndromes

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Abstract

Azacitidine and decitabine, two hypomethylating agents, are known to be effective in the treatment of high-risk myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) patients who cannot endure intensive cytotoxic chemotherapy or are not eligible for transplantation. However, the treatment response rate is low. The molecular mechanisms underlying the resistance to demethylation therapy are unclear. Though a wide range of predictors of treatment response have been investigated, no consensus has been reached. It is imperative to identify certain parameters that can help distinguish between patients who will obtain a favorable outcome from demethylation therapy and those who will not. Here, we describe currently researched potential predictors based on clinical characteristics, DNA methylation, gene mutation, gene expression, microRNAs, and protein expression. Although these parameters are not currently used in clinical practice, this review provides new sights into available clinical and experimental research. Moreover, this paper provides useful information on AML/MDS management.

Keywords Acute myeloid leukemia · Myelodysplastic syndromes · Hypomethylating agents · Predictors

Background

Azacitidine (5-azacytidine, 5-aza-CR, AZA) and decitabine (5-aza-2′-deoxycytidine, 5-aza-CdR, DAC), two hypomethylating agents (HMAs), are known to be effective for high-risk myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) patients with bone marrow (BM) blasts at 20–30% [1–3]. AZA was also shown to be effective in AML patients with BM blasts over 30% [4]. At low doses, HMAs exert anti-neoplastic activity by demethylation, thus reactivating some methylation-silenced genes. At high doses, cytotoxicity accounts for most of their anti-neoplastic activity [5]. Accumulated data suggest that some patients undergoing demethylation therapy have better outcomes compared to conventional treatments [6]. Hence, these drugs provide a new therapy for AML/MDS patients who are not suitable for intensive cytotoxic chemotherapy or who are not candidates for transplantation. However, only a proportion of patients who do not resist demethylation therapy could get favorable outcomes and several cycles are needed before the efficacy of the therapy becomes obvious. Therefore, it is essential to discover parameters that can determine whether a patient will respond to HMAs, to avoid both delaying other treatments and unwanted adverse effects. Recently, possible predictive factors for the response to HMAs have been extensively investigated, including clinical parameters (i.e., age, gender, cytogenetics, blast percentage, prior treatment, etc.), DNA methylation, gene mutation, gene expression, micro-RNA expression, and expression of relevant proteins. However, the conclusions are controversial and a consensus has not been reached. Here, we review currently researched factors, which may be potential predictors of patient response to HMAs (Table 1).

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Table 1 Predictors of HMA response in patients with AML or MDS

Classification	Parameters	Prediction	Possible mechanisms
Clinical parameters	Gender	Male: bad response	Higher CDA activity in males [7]
	Age	Older age: poor survival	Poor conditions [8]
	ECOG > 1	Bad response and poor survival	Poor conditions [9]
	WBC, Hb, PLT	High WBC, low Hb, low PLT: bad response and poor survival	Disease severity indicator [9–11]
	Platelet change	Platelet doubling after the first cycle: good response	Sensitivity to HMAs [12]
	BM blasts	High BM blasts: bad response	Disease severity indicator [13]
	Immunophenotype	Aberrant immunophenotype of myeloid progenitors: bad response	Ineffective hematopoiesis [14]
	CD25	CD25 on $CD34^+$ cells: poor survival	Leukemia stem molecular signature [15]
	Cytogenetics	Poor cytogenetics: bad response and poor survival	Loss of genetic stability [8, 9, 11, 12, 16]
	chr7 abnormalities	Better response	Need confirmation [17, 18]
	HbF*	Better survival	A surrogate for demethylation [19]
	LDH*	Poor survival	Adverse prognostic factor [20]
	VitD*	Better survival	Anti-bacteria; regulator of innate immune responses [21]
	Prior treatment	Untreated patients: better response	Cross-resistance [10]
	Dose and duration time	AZA (75 mg/m^2/day) and DAC (100 mg/m^2/course): better response. Prolonged treatment suggested	Dose and duration influence the efficacy of HMAs [22]
Gene mutation	DNMT3A#	Better response	De novo methylation [23]
	TET2#	Better response	5-mC to 5-hmC [24]
	IDH#	Better response	α-KG to 2-HG [25]
	TP53#	Better response	TF; tumor suppression gene [26]
DNA methylation	Global DNA methylation	Decrease: good response	Gene silencing by methylation [27]
	BCL2L10	Bad response and poor survival	Pro- or anti-apoptosis [28]
	CDKN2B	Bad response	Regulator of cell cycles [29]
Gene expression	PI-PLCβ1*	Better response	PI signaling pathway [30]
	MLL5*	Better response	Regulator of HOX gene expression [31, 32]
	MYC*	Bad response	TF in cell cycle regulation [33]
	PD-1*	Bad response	Immune-inhibitor [34, 35]
	NKD2*	Better survival	Negative regulator of Wnt pathway [36]
	BNIP3L*	Better survival	Pro-apoptosis [37]
Micro-RNAs	miRNA-126**	Better response	Anti-DNMT1 miRNA [38]
	miR-29b*	Better response	Anti-DNMT3 miRNA [39]
	miR-21*	Bad response and poor survival	Oncogenic miRNA [40]
	miR-181*	Bad response and poor survival	Associated with FAB AML type [41]
	miR-331*	Bad response and poor survival	Cancer-associated miRNA [42]
	miR-29c*	Bad response	Anti-DNMT3 miRNA [43]
Protein expression	hENT1*, hCNT1*	Better response	Transporter of HMAs [44–46]
	UCK*, DCK*	Better response	Activate HMAs [46, 47]
	CDA*	Bad response	Hydrolyze HMAs [7, 48]
	FAS*	Better response	Apoptosis signal transduction [49]
	P53*	Bad response	Reduce DAC-induced apoptosis [50]
Cell signaling	DP subset	Low pretreatment levels: better response	Involved in Stat3/5 signaling profiles in $CD34^+$ cells and consist with leukemia propagating cell phenotypes [51]

* High expression vs. low expression; # genetic mutation vs. wild type; DP subset: a $CD34^+$ G-CSF-inducible Stat3/5 double-positive subpopulation

Clinical parameters

Numerous clinical parameters have prognostic implications. A study found that males benefit less from HMAs, presenting lower overall survival (OS) and response rate [52]. Diverse enzymatic activities between males and females might account for this difference. Males seem to have relatively higher cytidine deaminase (CDA) activity or expression, which is an enzyme responsible for the metabolism and clearance of cytidine analogues [7]. Advanced age is also a risk factor [8]. Moreover, a relatively poor performance status (ECOG > 1) is associated with a low response rate [9]. Bone marrow examination and some hematologic parameters are also of value in predicting the response to HMAs. For example, higher than normal white blood count (WBC) [9, 10], low hemoglobin level, and low platelet count [11] before HMA therapy are associated with poor response and low survival. In particular, if the platelet count of a patient rises to twice the baseline level after the first AZA cycle, the patient is more likely to respond to AZA [12]. In contrast, BM blasts > 15% and aberrant immunophenotype of myeloid progenitors predict poor responses to AZA in MDS [13, 14]. Also, late-stage MDS patients with expression of CD25 antigen on $CD34^+$ cells had poor OS following treatment with AZA [15]. As for cytogenetics, complex karyotypes or poor cytogenetics predict a bad response [8, 9, 11, 12]. In acute erythroleukemia (AEL) patients treated with AZA, high-risk cytogenetics is also associated with decreased survival [16]. Based on the ECOG score, WBC before AZA onset and cytogenetics, Ramos et al. designed the European ALMA score (E-ALMA), which was successfully used as a predictor of AZA response in 710 elderly AML patients [9, 53]. The revised International Prognostic Scoring System (IPSS-R) is also important in predicting cytogenetic as well as clinical responses to HMAs [38, 54]. Poor karyotypes are generally associated with adverse outcomes, but aberrations in chromosome 7 appear to be an exception. Several studies have shown that patients with chromosome 7 abnormalities had satisfactory responses to AZA and DAC [17, 18, 55]. As many genes with vital physiological significance are located on chromosome 7, the striking findings of these studies appear to contradict conventional situations. Therefore, more studies are needed to clarify whether the findings about chromosome 7 abnormalities are incidental or not. Although adverse karyotype remains a poor prognostic factor, several studies reported the overall survival for the HMAs group was superior to the conventional chemotherapy group in high-risk MDS and older AML patients with adverse karyotype [6, 56, 57]. So, HMAs are generally recommended in these patients. In addition, higher levels of fetal hemoglobin (HbF) [19], normal lactate dehydrogenase (LDH) level [20], and high vitamin D (VitD) [21] prior to DAC or AZA therapy have been reported to be associated with longer survival. The response to AZA of untreated AML patients was better than that of those with prior chemotherapy [8, 10, 13]. Among patients with early post-transplant relapse, those who received more intensive induction chemotherapy (i.e., GCLAM or FLAG-IDA) before hematopoietic stem cell transplantation (HSCT) were more likely to respond to AZA than those who received conventional induction chemotherapy ("7 + 3" regimen) [58]. The dose and duration of AZA therapy also affect the outcome. Although the optimal dose and cycles of AZA are uncertain, studies have shown that patients treated with AZA at a dose of 75 mg/m^2/day have a higher probability to achieve a positive response than those treated with a dose of 100 mg/day [10, 22]. As for DAC, one study showed that MDS patients treated with a dose of 100 mg/m^2/course were more likely to respond to treatment than those treated with 60–75 mg/m^2/course and 135 mg/m^2/course [59]. Prolonged AZA treatment was suggested based on that some patients achieve a response only after six cycles. Furthermore, some patients with consistent stable disease could benefit from AZA even without achieving response [10].

Gene mutations

Mutation of methylation modifier genes

Aberrant DNA methylation can promote the initiation and development of hematopoietic malignancies. Mutations of methylation modifier genes contribute partly to these aberrations. However, the exact relationship between these mutations and various DNA methylation profiles is uncertain [27, 60, 61]. DNA methyltransferase 3A (DNMT3A), ten-eleven-translocation 2 (TET2), and isocitrate dehydrogenases 1/2 (IDH1/2) are the most frequently studied methylation modifier genes [62] (Fig. 1). Increasing evidence suggests that mutations in these genes are linked to the response to HMAs. However, it remains controversial how these mutations affect treatment outcome.

DNMT3A encodes a DNA methyltransferase that catalyzes the methylation of C5 position of CpG dinucleotides. Mutations of DNMT3A often result in poor prognosis [63]. However, patients with DNMT3A mutations can benefit from HMAs. In an analysis on a cohort of 46 AML cases treated with DAC, six of eight patients with DNMT3A mutations achieved complete response (CR, 75%) in contrast to 13/28 patients (34%) without DNMT3A mutations [23]. Similarly, Traina et al. found that DNMT3A mutations are linked to a higher response rate and prolonged progression-free survival (PFS) in MDS patients treated with demethylation therapy

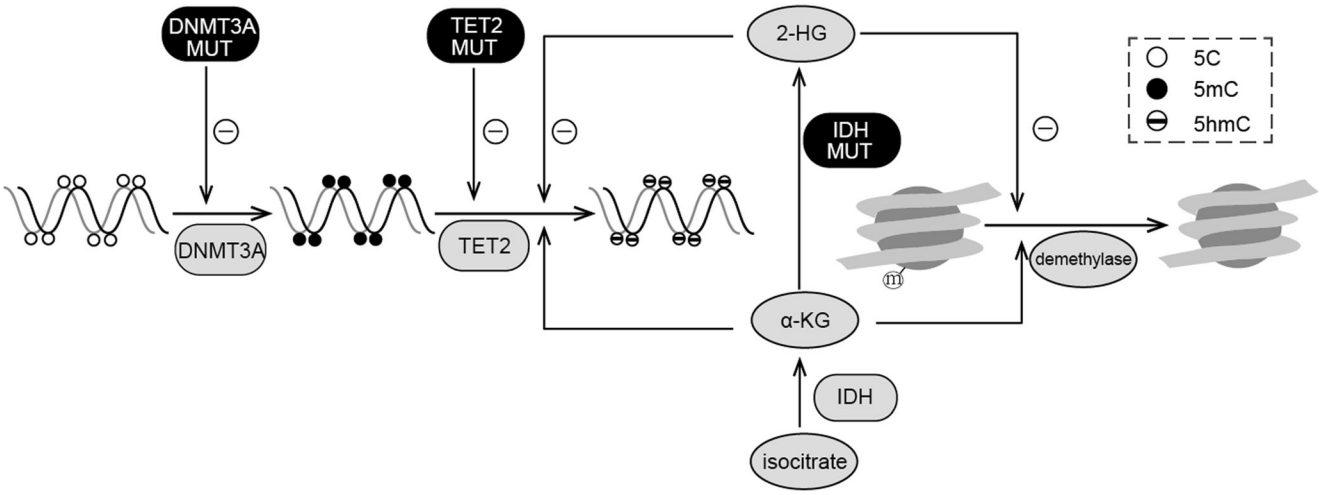

Fig. 1 Effects of DNMT3A, TET2, and IDH1/2 mutations on aberrant DNA methylation

[60]. However, no such relationship was found in a different study [64]. DNMT3A mutations can lead to epigenetic disorders, which might disturb the stability of the genome, enabling a response to HMAs. However, more studies are needed to further understand the underlying mechanisms.

TET2 encodes a hydroxylase that catalyzes the hydroxylation of 5-methylcytosine (5-mC), converting it to 5-hydroxymethylcytosine (5-hmC). Mutations in TET2 can impair the activity of this hydroxylase and lead to low levels of 5-hmC, with surprisingly widespread hypomethylation at differentially methylated CpG dinucleotides [61]. A multi-center study revealed that AML/MDS patients harboring TET2 mutations had a higher response rate to AZA [24]. Moreover, the increase in response rate was even more prominent when TET2 mutation was combined with wild-type ASXL1 [65]. In another study, 46% (5/11) and 24% (5/21) of high-risk MDS patients with mutant TET2 and wild-type TET2 responded to AZA, respectively [28]. Though the aforementioned studies show better responses to demethylation therapy in TET2 mutant patients, other studies found the relationship between TET2 mutations and survival to be unclear [28, 60].

IDH1/2 exert their effects by catalyzing the decarboxylation of isocitrate into alpha-ketoglutarate (α-KG), which is essential for DNA demethylation through TET2 and histone demethylation. In the presence of IDH mutations, α-KG is converted into 2-hydroxyglutaratea (2-HG), which is an analogue and competitor of α-KG. Through the accumulation of 2-HG, IDH mutations can lead to DNA hypermethylation [66]. Interestingly, this result appears contradictory to what is discussed above [61]. In most cases, mutations in IDH1/2 were linked with a poor prognosis, especially those in IDH1 [67]. However, a higher response to HMAs compared to wild type was recently found in patients with IDH mutations. By reviewing the clinical data and IDH mutations of 42 AML patients treated with HMAs, Emadi et al. identified a relationship between IDH mutations and a higher response rate: 71.4% (5/7) of patients with IDH mutations compared to 22.9% (8/35) without IDH mutations ($P = 0.01$) [25]. A similar finding was revealed in a meta-analysis, suggesting the possible predictor value of IDH mutations [68]. However, no such correlation was found in two other studies [60, 64], suggesting unknown mechanisms underlying these findings. DNMT3A mutations are often accompanied by either TET2 mutations or IDH1/2 mutations, but not by both, implying an intricate interaction among these mutations [66].

Mutations in TP53

Tumor suppressor gene TP53 encodes the protein p53, an indispensable transcription factor in regulating cell cycle and apoptosis [69]. Mutations in TP53 have been consistently linked to complex karyotypes and poor outcomes in hematopoietic malignancies [70–72]. However, it remains controversial whether TP53 mutations influence the response to HMAs of these patients. In a clinical trial of 10-day courses of DAC, Welch et al. found a higher response rate among patients with TP53 mutations compared with those with wild-type TP53 ($P < 0.001$) [26]. A similar result was obtained from the analysis of a cohort of 109 MDS patients, in which, however, the high response rate did not improve survival [70]. In another study, the shorter duration of response, rather than the response rate to HMAs in MDS patients, was associated with TP53 mutations [71]. Other studies found the poor prognosis-related trait of TP53 mutations, but no significant differences in response rates between patients with mutated and wild-type TP53 [72–74]. In addition, TP53 mutations were associated with poor response to AZA in patients who relapsed post-transplantation [75]. It is possible that TP53 mutations are related with better HMA response, but the effect might be compromised by the accompanying complex karyotype. More in-depth and extensive studies are warranted to provide new insights into this question.

DNA methylation

Global DNA methylation

Aberrant DNA methylation plays an indispensable role in oncogenesis, including hematopoietic malignancies. Different from normal cells, cancer cells often undergo genome-wide hypomethylation together with hypermethylation of promoter-associated CpG islands [27]. The association between DNA methylation and prognosis is complicated [76]. A relatively high level of DNA methylation is often associated with poor prognosis [77, 78]. Treatment with HMAs was found to reduce the level of methylation [79]. However, how DNA methylation patterns influence the clinical response to HMAs remains unclear. Shen et al. analyzed samples from 317 MDS patients for the methylation of 10 candidate genes. No association was found between the methylation level of these genes and clinical responses to HMAs. However, the DAC responders, when compared with non-responders, displayed a significant decrease in methylation after therapy [78]. Zhang et al. also found that a conspicuous drop in global methylation after DAC therapy was correlated with higher CR rates, as well as longer PFS [77]. Therefore, it is the decrease in methylation, rather than the baseline level, that might predict the outcomes of HMA therapy.

Methylation of documented genes

Numerous genes, including oncogenes, tumor suppressor genes, signal molecule genes, apoptosis/anti-apoptosis genes, and transcription factor genes, are methylation targets in hematopoietic malignancies [62]. To date, only a few of these genes have been investigated regarding the relationship between their methylation status and the outcome of demethylation therapy.

BCL2 like 10 (BCL2L10) gene belongs to the BCL2 family with both pro-apoptotic and anti-apoptotic functions. BCL2L10 is frequently hypermethylated in several cancers, such as acute leukemia (AL), and the hypermethylation might promote the transition from MDS to AML [3, 28]. As a hypomethylating target of DAC, BCL2L10 expression is upregulated in HL60 cells by DAC [3]. In a study containing 27 higher-risk MDS patients treated with AZA, subjects bearing over 50% BCL2L10 methylation were less likely to achieve response or have long survival [28]. On the contrary, high protein expression of BCL2L10 was associated with AZA resistance in SKM1-R cells [80]. High percentage of BCL2L10 positive bone marrow cells could predict resistance to AZA in MDS patients at a cut-off of 50% [80], which was validated in a prospective study recently [81]. These different results might be caused by the various functions of BCL2L10 at different expression levels [81], and much remains to be uncovered between BCL2L10 methylation and protein expression. The predictor value of BCL2L10 methylation and the change in BCL2L10 methylation level during HMA treatment need to be verified in future studies.

Cyclin-dependent kinase inhibitor 2B (CDKN2B) encodes p15INK4b, which regulates cellular arrest in the GI phase by inhibiting cyclin-dependent kinase 4 (CDK4), CDK 6, and CDK4/6 complexes [29]. CDKN2B promoter methylation occurs frequently in AML and high-risk MDS [29]. In addition, CDKN2B promoter methylation is often associated with poor prognosis and is increased during MDS progression and evolution to AML [29, 82]. It could be interesting to explore whether CDKN2B methylation predicts responses to HMAs because it was reported to have no relationship with the expression level of DNA methyltransferase genes [83]. CDKN2B promoter methylation is often decreased following treatment with HMAs. However, whether the demethylation is correlated with treatment response remains controversial [17, 84]. Raj et al. found that lower baseline CDKN2B promoter methylation was associated with response ($P = 0.07$), whereas when patients harbored baseline methylation > 24%, no response was achieved [17]. However, it was reported that a 60-year-old secondary AML patient with CDKN2B promoter methylation > 24% reached a dramatically favorable response [85], suggesting that the correlation found by Raj et al. has exceptions. Together, these studies provide evidence that under most circumstances HMAs might not be efficient enough in the presence of a high level of CDKN2B promoter methylation.

Gene expression

Pi-PLCβ1

Phosphoinositide phospholipase Cβ1 (PI-PLCβ1) is a pivotal enzyme involved in the nuclear phosphoinositide (PI) signaling pathway, which plays important roles in cell proliferation, growth and differentiation [86]. PI-PLCβ1 exerts its role by regulating the cell cycle as a checkpoint in the GI phase, targeting CDK3, and influencing the development of hematologic malignancies through genetic and epigenetic changes [30, 87]. Recent studies suggest that PI-PLCβ1 could be a potential target of HMAs. AZA could reduce the methylation level of PI-PLCβ1 and increase the expression of proteins in responders with MDS [88], which might become obvious after three cycles of treatment [30]. As a downstream target of PI-PLCβ1 signaling, the expression of CKD3 significantly increases during therapy with AZA, with or without valproic acid [87, 89]. Furthermore, the recruitment of relevant transcription factors, such as Sp1, CEBPA, and MZF-1, changes greatly in responders to AZA therapy [87]. Taken together, it is reasonable to hypothesize that AZA might epigenetically activate the PI-PLCβ1-dependent signaling pathway.

Therefore, PI-PLCβ1 might serve as a dynamic indicator of the effect of HMAs, and the significant changes in PI-PLCβ1 expression after early AZA cycles might become a prognostic factor for MDS patients.

MLL5

MLL5, a member of the mixed lineage leukemia (MLL) family, belongs to the trithorax group and plays critical roles in regulating homeotic gene (HOX) expression [31]. MLL5 is essential in regulating the cell cycle and maintaining genomic integrity, particularly during hematopoiesis and hematopoietic differentiation [32]. An analysis of 509 subjects with AML demonstrated that patients with high MLL5 expression tended to have a longer survival [90]. Meanwhile, loss of MLL5 in murine HSC resulted in pleiotropic hematopoietic defects and a dramatic sensitivity to DAC-induced differentiation [91]. For humans, high expression of MLL5 in AML patients treated with DAC could predict beneficial outcomes. Additionally, high MLL5 expression was linked to higher global DNA methylation and could raise the sensitivity to DAC in leukemia cells [92]. Therefore, high MLL5 expression might lead to a better response and survival with HMA therapy.

MYC

MYC proto-oncogene (MYC) encodes a transcription factor involved in cell cycle regulation, cellular transformation, gene expression, and oncogenesis. MYC is overexpressed in high-risk MDS and AML in contrast to low-risk MDS, and could be used as both a predictive tool and therapeutic target [33, 93]. There is convincing evidence that MYC contributes, to some degree, to drug resistance in AML. Overexpression of MYC was found in drug-resistant leukemia cells, and the MYC inhibitor 10058-F4 could restore the sensitivity to cytotoxic drugs [94]. In a group of 21 patients under treatment with AZA, the response rates were 12.5% (1/8) and 61.5% (8/13) in subgroups with MYC overexpression and low expression, respectively ($P = 0.03$), highlighting a predictive value of MYC for response to AZA [95]. This is also consistent with findings on miR-29b [39], which was reported to be negatively regulated by MYC [96]. Therefore, overexpression of MYC might serve as an unfavorable predictor.

PD-1

Programmed death 1 (PD-1) is an immune inhibitor. PD-1 plays an important role as a regulator of T-cell activation, tolerance, and autoimmunity by interacting with its ligand, PD-L1 [97]. The PD-1/PD-L1 pathway was associated with resistance to conventional chemotherapeutic agents and has been targeted in cancer immunotherapy [98]. Previous studies suggested that HMAs decrease the methylation level of the PD-1 promoter in leukemia cells, which was accompanied by an increase in PD-1 expression. The effect was more visible in resistant patients, leading to worse survival [34]. Resistant patients also showed a higher level of PD-1 methylation in T cells, compared with healthy controls, before treatment [99]. On the contrary, a moderate rather than a large increase in PD-1/STAT1 rate was reported to benefit survival in low-risk MDS [35]. Taken together, the methylation level of PD-1 at baseline might be considered when using HMAs because the demethylation and increased expression of PD-1 might contribute to HMA resistance. A combination of PD-1/PD-L1 inhibitors and HMAs might be a promising therapy in HMA-resistant patients [99, 100]. The relationship between the immune system and HMAs is very complex. We could potentially target the immune system to overcome HMA resistance and vice versa.

NKD2

Naked family 2 (NKD2) is a negative regulator of the Wnt/β-catenin signaling pathway [36]. NKD2 is often methylated in many cancers and hypermethylation can lead to decreased NKD2 expression, which was found to be associated with poor prognosis [101]. Similarly, in AML, low NKD2 expression was associated with shorter OS [102]. HMAs could reduce the hypermethylation of the NKD2 promoter in leukemia cell lines and restore its expression [102]. However, more in vivo evidence is required to verify the association of NKD2 expression and HMA therapy.

BNIP3L

BCL2/adenovirus E1B 19 kDa interacting protein 3 (BNIP3) and BNIP3-like (BNIP3L), both acting as cellular pro-apoptotic proteins, are atypical members of the BCL2 family as they only contain the Bcl-2 homology 3 (BH3) domain (not BH1 to BH4) [37]. Aberrant methylation of BNIP3/BNIP3L, along with their aberrant expression, was observed in many tumors, e.g., hematopoietic tumors [103]. Lazarini et al. found that BNIP3L expression was decreased in MDS patient bone marrow cells, which appears to be an independent prognostic factor for worse OS under DAC therapy [104]. Intriguingly, DAC could increase the expression of BNIP3L in U937 cells, whereas loss of BNIP3L expression seemed to strengthen the apoptosis induced by DAC in U937 cells [104], suggesting a complex interaction between BNIP3L and DAC in apoptosis. Therefore, much remains to be understood about the role of BNIP3L as a potential predictor.

Apart from single gene expression, we can also take groups of genes into consideration. Research revealed that upregulated expression of a series of genes which are related to cell cycle progression was associated with AZA response [105].

Micro-RNAs

Micro-RNAs (miRNAs) are a family of short non-coding RNAs that regulate gene expression at post-transcriptional level. They are involved in most vital physiological processes, usually through degrading mRNAs or down-regulating the translation of proteins [106]. Aberrant expression and methylation of specific miRNAs are associated with hematological malignancies [107]. In addition, miRNAs might exert a crucial effect on resistance to chemotherapeutic drugs [108]. Therefore, the capacity of miRNAs as predictors of response to HMAs should be exploited. Solly et al. found seven miRNAs, five of which were considered anti-DNMT1, differentially expressed between AZA resistance and sensitivity in SKM1 cells. Subsequently, they found that the lower expression of miRNA-126* (one of the two mature products of miR-126 precursor derived from the 3′ arm) in MDS could predict lower response rates and poor outcome [38]. In a single-center phase II study with a cohort of 53 patients, subjects with higher pre-treatment miRNA-29b levels showed higher response rates [39]. However, the results were opposite to miR-29c. Butrym et al. showed that a lower expression of miR-29c in AML patients before treatment was associated with better response to AZA [43]. The underlying mechanisms remain unknown, and the opposite effects are intriguing considering that both miRNAs are reported to target DNA methyltransferases [109]. Higher miR-21 level was also found to correlate with response to HMAs in MDS patients [40], which is similar to the study of miR-181 [41]. In a study of 95 AML cases in which some patients were treated with AZA, the correlation between higher expression of miR-331 and lower possibility to achieve CR was significant [42]. However, the correlation between the miR-331 expression level and the response to AZA remains to be determined.

Protein expression

Proteins involved in HMA transport and metabolism

The mechanisms underlying the resistance to DAC and AZA remain unknown, with complex pharmacological properties and metabolic characteristics considered to be partially responsible (Fig. 2). DAC is effective primarily due to its incorporation in DNA, whereas AZA mainly targets the RNA [110]. The uptake of AZA and DAC into the cell is mediated by the human nucleoside transporters (hNTs), which are classified into two different families, namely, the human equilibrative nucleoside transporters (hENTs) and the human concentrative nucleoside transporters (hCNTs). Recent studies suggested that AZA is transported by both hENTs and hCNTs, while DAC is transported almost exclusively by hENT1 or hENT2 [111]. Once inside the cell, DAC is phosphorylated into its active forms initially by deoxycytidine kinase (DCK), whereas AZA is phosphorylated by uridine–cytidine kinase (UCK) [110]. CDA has the opposite effects by catalyzing the hydrolytic deamination of deoxycytidine and cytidine to deoxyuridine and uridine, respectively, thus decreasing the concentration of the active forms of DAC and AZA [7]. Any alterations in the transport and metabolism of these drugs might cause insufficient active forms and insufficient incorporation into DNA/RNA, resulting in resistance. An in vitro study using Madin–Darby canine kidney cells demonstrated that a significantly increased sensitivity to AZA was associated with hCNT1 expression [44]. Similar results were obtained for DAC. Higher hENT1 expression was strongly correlated with response to DAC and prolonged survival [45, 46].

Regarding enzymes catalyzing phosphorylation, subjects with higher UCK expression tend to have a good response to AZA ($P = 0.07$) [47], whereas those with decreased DCK expression carry the risk of secondary resistance to DAC in MDS [46]. An in vitro study found that the transfection of wild-type DCK into resistant cell lines restored DAC sensitivity [5]. Given the importance of CDA in the metabolism of DAC and AZA, CDA expression levels and catalytic activity were measured in 90 patients under treatment with HMAs. Higher CDA expression/activity was found to contribute to reduced exposure to HMAs, leading to poor prognosis [7]. Considering the opposite influences that DCK/UCK and CDA exert on the metabolism of cytidine analogues, the ratio of the two different parameters was analyzed by Qin et al. The authors found that a high CDA/DCK ratio likely conduced to primary resistance in MDS patients undergoing DAC therapy ($P = 0.027$) [48]. Taken together, these results clarify the fundamental function of the genes involved in the transport and metabolism of AZA/DAC and support the roles of these genes as prognosis-associated biomarkers.

FAS

FAS is a receptor on the cell surface affecting the apoptosis signal transduction by binding to the FAS ligand (FASL). Considering that many drugs act through the FAS/FASL pathway, high expression of FAS receptor and FASL was suggested to be associated with a favorable prognosis [49]. In contrast, the absence or low expression of FAS-Associated Protein with Death Domain (FADD) in AML cells at diagnosis was linked with resistance to chemotherapy and poor outcomes [112]. In addition, functional FAS promoter polymorphisms that influence the expression of the FAS receptor were associated with increased risk of AML [113]. In a multi-sided study of 169 high-risk MDS and secondary AML patients [114], low FAS receptor expression before treatment, due to promoter hypermethylation, was linked to patient response to AZA [114]. AZA and DAC were found to reduce FAS

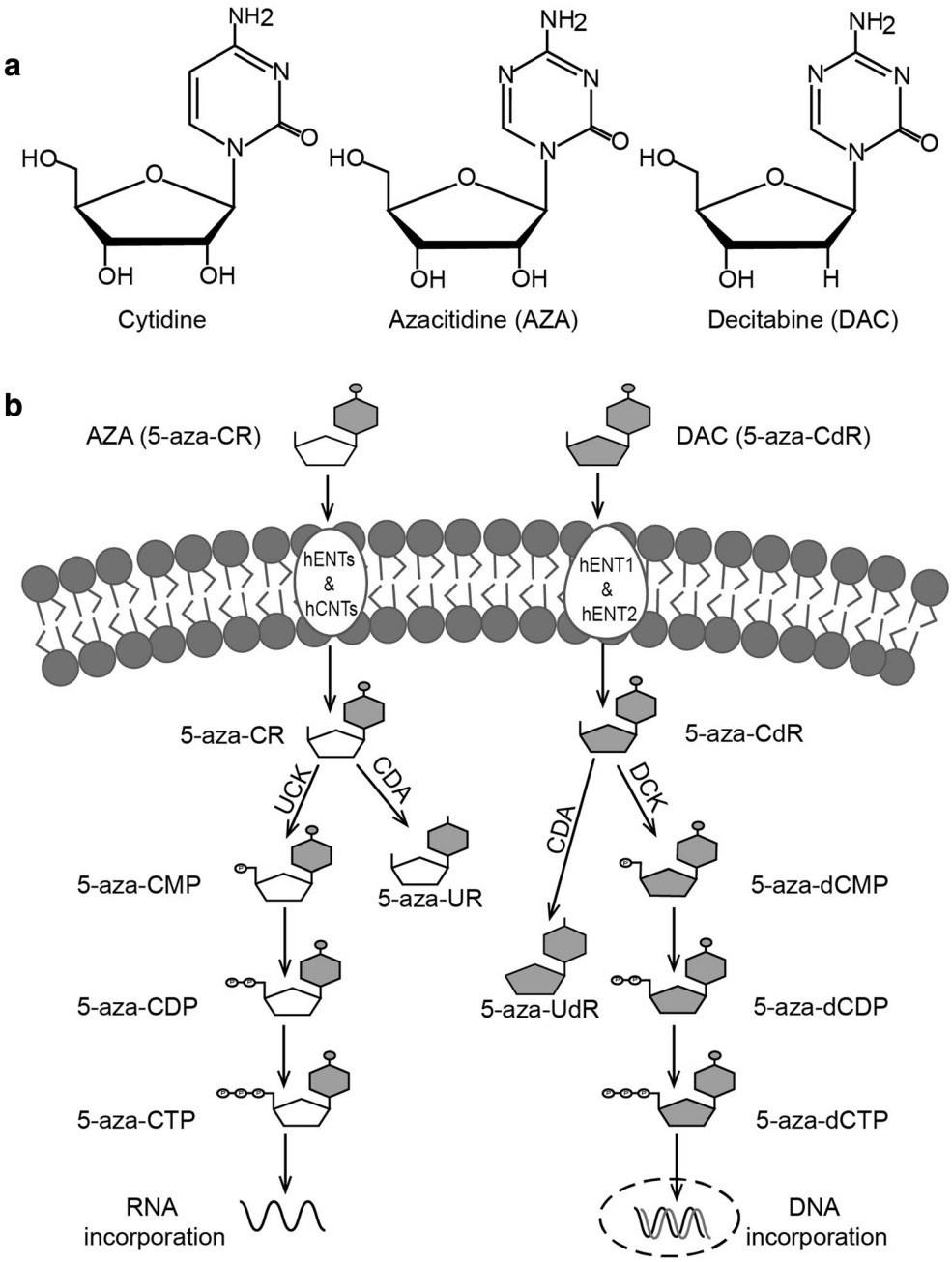

Fig. 2 **a** Structures of cytidine, AZA, and DAC. **b** Transport and metabolism of AZA (5-aza-CR) and DAC (5-aza-CdR)

promoter hypermethylation and restore its expression [114, 115]. Hence, FAS receptor expression appears to be a potential indicator of the response to HMAs.

P53

The p53 protein, encoded by the TP53 gene, is an extensively studied tumor suppressor in humans. It plays essential roles in cellular processes such as apoptosis and genomic stability. High expression of p53 is often used as an alternative readout for TP53 mutations, for which the exact mechanisms are unclear [116]. High protein expression of p53 was rarely linked with a favorable response or long survival under HMA therapy. Mostly, treatment response was low, or the correlation was insignificant [117, 118]. This result is quite different from that of TP53 mutations [26]. In contrast, loss of p53 expression in mice was reported to enhance the apoptosis induced by DAC [50]. However, the relationship between p53 and the response to HMAs is not fully known. It seems that p53 expression has an effect on DAC-induced apoptosis rather than on demethylation [50], and DAC might influence the methylation of p53 pathway regulators, thus leading to p53 expression changes.

In addition, Miltiades et al. found the Stat3/5 signaling biosignature in $CD34^{+}$ cells was associated with AZA

response and survival in high-risk MDS patients, which may serve as both a response biomarker and treatment target. Moreover, the team identified a $CD34^+$ G-CSF-inducible Stat3/5 double-positive subpopulation (DP subset) with the characteristics of leukemia propagating cell phenotypes and low pretreatment levels of DP subset predicted better AZA response [51].

Conclusions

It is important to identify predictors of the response and outcome of patients under HMA therapy. Many parameters, which are commonly associated with poor prognosis, tend to be associated with favorable responses to HMAs [23, 25, 26]. Recent studies provide evidence that these parameters might be important targets of HMA therapy. Though the responses do not always lead to a longer survival, HMA treatments might provide an opportunity for some patients to pursue other options such as HSCT. The clinical implications and reliability of these parameters must be further studied to determine whether these can be clinically used as predictors of responses to HMAs. This review discusses the potential predictors of responses to HMAs and provides new insights into clinical research and AML/MDS management. Clearly, larger-scale and more extensive studies are needed to better understand the mechanisms underlying HMA resistance and validate the predictive value of these parameters.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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