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The evolution of epigenetic therapy in myelodysplastic syndromes and acute myeloid leukemia

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A B S T R A C T

Mutations in the group of epigenetic modifiers are the largest group of mutated genes in Myelodysplastic Syndromes (MDS) and are very frequently found in Acute Myeloid Leukemia (AML). Our advancements in the understanding of epigenetics in these diseases have helped develop groundbreaking therapeutics that have changed the treatment landscape of MDS and AML, significantly improving outcomes. In this review we describe the most common epigenetic aberrations in MDS and AML, and current treatments that target mutations in epigenetic modifiers, as well as novel treatment combinations, from standard therapies to investigational treatments.

Introduction

Acute Myeloid Leukemia (AML) and the myelodysplastic syndromes (MDS) are a heterogenous group of malignant hematopoietic stem cell (HSC) disorders, MDS is characterized by disordered growth and differentiation of hematopoietic progenitors leading to cytopenias, and a variable risk of transformation to AML [1]. AML is an aggressive hematological cancer that is characterized by malignant self-renewal and block in myeloid differentiation [2]. Epigenetic modifications comprise a class of changes in gene expression that are inheritable by cell division but not caused by changes in the DNA sequence itself, which include DNA methylation, histone modification and chromatin remodeling [3]. Mutations in the group of epigenetic modifiers are the largest group of mutated genes in MDS [4]. Similarly, close to 70% of recurring mutations in AML target regulators of gene expression [2,5]. Epigenetic processes play a pivotal role in hematopoiesis and cell differentiation in healthy hematopoietic stem cells [6]. Dysregulation in epigenetic patterns and mutations in epigenetic modifiers that disrupt normal hematopoiesis, contribute to the development of different types of leukemias [6,7]. Moreover, there is an increasing amount of evidence showing how epigenetic changes are independent factors of disease progression, relapse, and are possibly AML and MDS main drivers of disease [5,8].

The aim of this review is to describe the most common epigenetic aberrations in AML and MDS, and current treatments that target mutations in epigenetic modifiers, as well as novel treatment combinations, from standard therapy to cutting edge investigational treatments.

Epigenetic Mutations in MDS and AML

Epigenetic mutations are the largest group of mutated genes in MDS and are found very frequently in AML [2,4,5]. The frequency of each recurrent mutation is highly variable, with many possible combinations in a single patient. The most common epigenetic mutations involve genes in 2 functional groups: DNA methylation and histone modification [9]. Epigenetic mutations in DNA Methylation genes include: Ten-Eleven-Translocation 2 (*TET2*), Isocitrate Dehydrogenase 1 and 2 (*IDH1/2*), and DNA Methyltransferase 3A (*DNMT3A*). Mutations that involve histone modification are Additional Sex Combs Like 1 (*ASXL1*) and Enhancer of Zeste Homolog 2 (*EZH2*).

TET2 mutations occur in 22-35% of patients with MDS and 13-27% of patients with AML [4,10,11]. *TET2* promotes DNA demethylation, and encodes a protein: dioxygenase, that catalyzes the conversion of 5-methylcytosine to 5-hydroxymethylcytosine (5hmC), promoting the demethylation of cytosines. *TET2* mutations are more frequent in low-risk MDS, they are loss of function mutations that include frame shift, generated stop codons, in-frame deletion, and amino acid substitutions which lead to DNA hypermethylation and subsequent dysregulated gene expression in hematopoietic stem cells [12-14].

IDH1/2 are more frequently mutated in AML than in MDS, affecting around 20% of AML patients and only 2-5% of patients with MDS [12,13,15]. *IDH1* and *IDH2* are enzymes of the citric acid cycle, and they catalyze the conversion of isocitrate to α-ketoglutarate in the mitochondria in the case of *IDH2* and in the cytosol in *IDH1*, the mutated enzymes produce R-2-hydroxyglutarate(R-2HG),

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instead of α-Ketoglutarate [16]. α-Ketoglutarate is a cofactor for more than 80 enzymes, including *TET2*, several histone demethylases, and prolyl hydroxylases [17]. Several studies showed that R-2HG is a competitive inhibitor of α-ketoglutarate-dependent enzymes. Mutant *IDH1/2* block *TET2*, resulting in decreased 5hmC, DNA hypermethylation, and block in cellular differentiation [18]. Furthermore, *IDH1/2* mutations have broad epigenetic consequences, including DNA hypermethylation, chromatin modifications, and the activation of the HIF1a pathway and tumor hypoxia resistance [19].

Mutations in *DNMT3A* are one of the most common mutations in AML observed in about 20% of these patients, but only seen in 10% of patients with MDS [12,13,20,21]. *DNMT3A* encodes an enzyme that catalyzes the methylation of DNA, by transferring methyl groups to specific CpG structures in DNA [22]. Additionally HSCs with *DNMT3A* mutations appear to have a proliferative advantage compared to wild-type HSCs and predispose HSCs to malignant transformation [23].

EZH2 mutations are seen in 5% to 6% of MDS patients, but observed less frequently in AML patients [5,12,13]. *EZH2* along with other proteins (*EED, SUZ12,* and *RBBP4*) form the Polycomb Repressive Complex 2 (*PRC2*), in which *EZH2* forms the catalytic region of the complex. The *PRC2* is a methyltransferase that contributes to the epigenetic silencing of numerous genes [24]. *EZH2* mutations and 7q loss, where *EZH2* is located, cause premature chain termination of *EZH2* leading to direct interruption of histone methyltransferase activity [25].

ASXL1 mutations are seen in 15% to20% of MDS patients and are infrequent in AML.[19] *ASXL1* encodes for a chromatin-binding protein, that is thought to disrupt chromatin in localized areas leading to enhanced transcription of some genes, while repressing the transcription of others. *ASXL1* belongs to the Enhancer of Trithorax and Polycomb (*ETP*) genes that can activate and repress *HOX* genes [26]. Mutations in *ASXL1* lead to changes in the Plant Homeodomain (*PHD*) of the gene, which is its main functional domain [27]. Truncation of the C-terminus of the *PHD* domain is shown to induce MDS in vivo via inhibition of *PRC2* [28]. *ASXL1* mutations are more frequent in high-risk MDS patients [13].

Epigenetic modifications are frequently reversible, therefore several treatments targeting epigenetic mechanisms, such as DNA methylation have been successfully developed, and many more are currently being explored.

Azacitidine and decitabine in MDS and AML (DNA- Hypomethylating agents)

The main class of drugs used as DNA-Hypomethylating agents (HMAs) are the nucleoside analogues azacitidine (5-azacytosine) and decitabine (2'-deoxy-5-azacytidine). These agents are cytosine analogues (azanucleosides), that freely incorporate into DNA in the place of cytosine (and RNA in the case of azacitidine) in replicating cells where they irreversibly bind and deplete DNA methyltransferases (DNMTs), which are enzymes responsible for catalyzing the methylation of DNA [29,30]. The primary mechanism by which HMAs are thought to exert their efficacy is by reactivation of tumor-suppressor genes that have been silenced by aberrant DNA methylation [30]. Apart from causing hypomethylation, it has been shown that HMAs have cytotoxic effects on DNA and RNA [31].

These drugs have been extensively assessed in clinical trials and were the first class of epigenetic drugs to be approved for the management of higher-risk MDS and AML with blast count <30%. First, azacitidine received approval in 2004 for the treatment of higher-risk MDS and AML with 20% to30% blast count, after a successful clinical trial from CALGB showed a 60% response in MDS patients compared to supportive care; while only 7% of patients achieved complete remission (CR), and 16% showed a partial response, 37% had improvement in blood counts [32]. Although CR rates are relatively low, further trials, including the AZA-001 trial have shown overall survival (OS) benefit, and improved quality of life in MDS, with a median improvement of approximately 9 months over conventional care regimens [33]. Also, for AML a subset-analysis showed that azacitidine prolongs OS in elderly AML-patients with 20% to30% BM-blasts [34].

Subsequently, decitabine was approved in 2006 after showing effectiveness over best supportive care in elderly patients with intermediate and high-risk MDS ineligible for intensive chemotherapy [35].

HMAs are now standard of care for patients with higher-risk MDS and for elderly or unfit AML patients that are not eligible for induction chemotherapy. In addition, while therapy in lower-risk MDS has usually been directed toward treatment of cytopenias with growth-factor support or lenalidomide in the setting of del(5q) MDS, HMAs have been shown to be as effective in lower-risk disease, with recent studies suggesting a role of early intervention using these agents, albeit with regimens that have lesser intensity [36,37].

Despite these results, the efficacy of monotherapy with HMAs has several setbacks: the response rates are 10% to 50% in patients with AML and 40% to 60% in patients with MDS (including hematologic improvement). The responses are transient, less than 1 year for AML and loss of response within 2 years for MDS. They require 3-4 months to achieve a best response and have a median OS of less than a year for AML. Also, for MDS once response to these agents is lost, the median OS for high-risk patients is close to 4 months [32,33,38-40]. Development of new therapeutic strategies to prevent and overcome failure to HMAs is of utmost importance. Especially, since there are no standard-of-care options for patients with MDS that fail to respond to HMAs.

Resistance to azacitidine and decitabine

Resistance to azacitidine and decitabine as aforementioned is a universal and intractable problem which accounts for limited success and durability of treatment in MDS and AML. Resistance can be divided into 2 broad categories: primary resistance, in which patients fail to respond to HMAs after at least 4 to 6 cycles of therapy, or when the MDS progresses to higher-risk categories or transforms to AML without having responded to therapy; or acquired resistance, when there is loss of response, progression to a higher-risk category or transformation to AML in a patient who had an initial response to therapy [41].

There are some molecular biomarkers associated with response to HMAs. As some patients respond to demethylating agents well while others don't, it was hypothesized that patients with mutations that induce DNA hypermethylation would have a greater response to HMAs. Although there is some evidence that patients with *TET2* mutations derive greater benefit, particularly in the absence of *ASXL1* mutations [42,43], these findings have not been translated into patients with other hypermethylating mutations such as *IDH1* and *IDH2*[44]. The expression of miR29b has also been shown to have a role in response to HMAs. miR29b causes reduction of the expression of DNMTs, resulting in global DNA hypomethylation and re-expression of hypermethylated, silenced genes in AML. The overexpression of miR29b in myeloblasts has been associated with clinical response to decitabine [45,46]. The therapeutic efficacy of HMAs is dependent on cellular uptake, therefore any alterations in transport, metabolic activation and increased degradation may result in resistance [47].

HMAs enter cells using human nucleoside transporters (hENTs), such as hENT1 and hENT2. Once they are inside the cell, decitabine undergoes a first phosphorylation by deoxycytidine kinase (dCK), which in the case of azacitidine this phosphorylation is performed

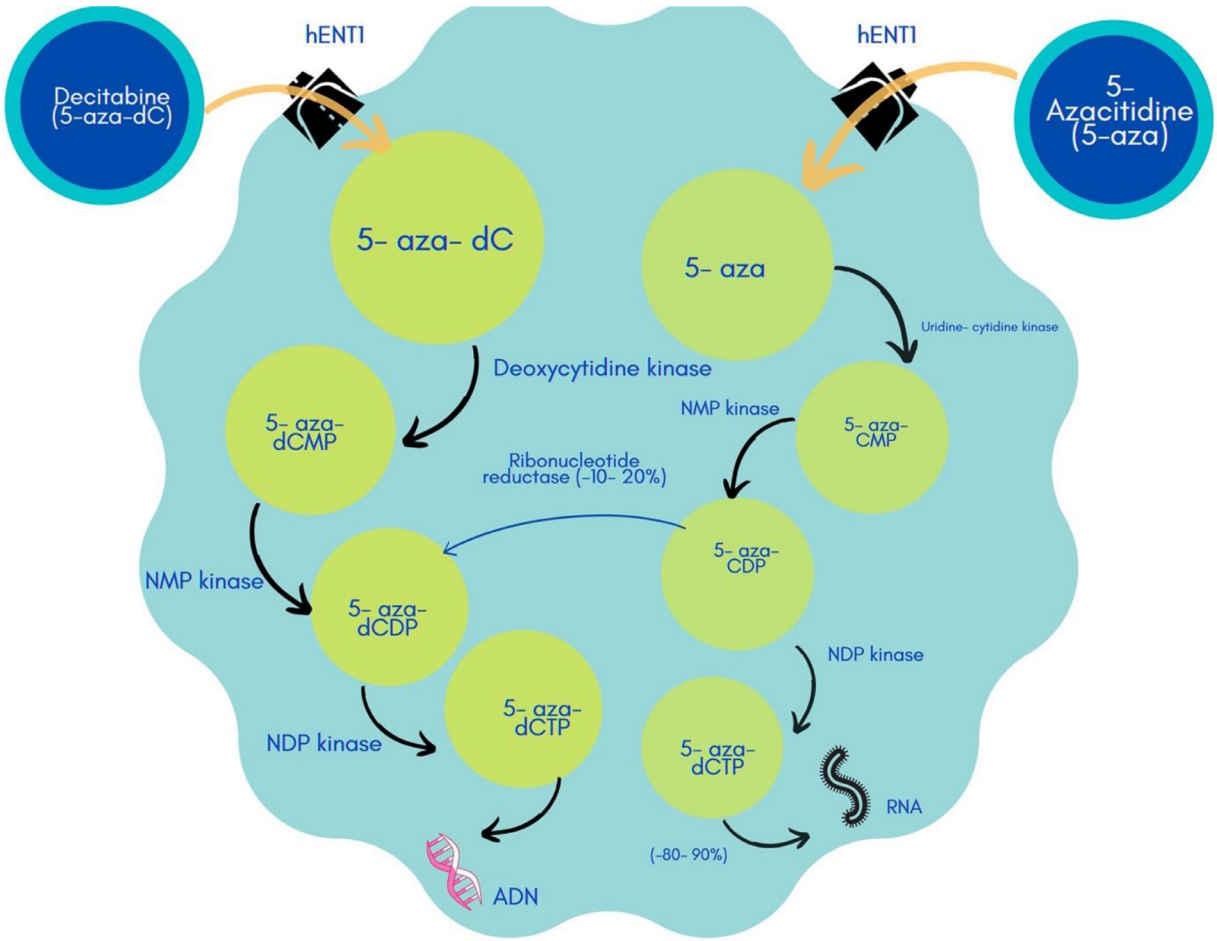

Fig. 1. Intracellular intake pathways of azacitidine and decitabine. Azacitidine and decitabine enter cells via nucleoside transporters, such as hENT1. Once they are inside the cell, decitabine undergoes a first phosphorylation by deoxycytidine kinase (dCK), which in the case of azacitidine this phosphorylation is performed by the enzyme uridine-cytidine kinase (uCK). Mono-phosphate and di-phosphate forms of the nucleosides are subsequently phosphorylated into active tri-phosphate forms. Decitabine is exclusively incorporated into DNA, while the majority (80%-90%) of 5-azacitidine is incorporated into RNA. 10% to 20% of 5-azacitidine dinucleotides is reduced by ribonucleotide reductase to deoxyribonucleotides (5-aza-dCDP) which are further phosphorylated and incorporated into DNA. DP: nucleoside diphosphate; NMP: nucleoside monophosphate.

by the enzyme uridine-cytidine kinase (uCK). HMAs are subsequently phosphorylated to their active forms by other enzymes and incorporated into DNA, and RNA in the case of azacitidine, where they induce demethylation (Fig. 1). HMAs metabolites might also be substrates for catabolizing enzymes such as cytidine deaminase (CDA), which catalyze their inactivation, thereby decreasing the amount of active forms of HMAs that can be formed [48]. Resistance to azacitidine and decitabine arise from adaptive responses of the pyrimidine metabolism networks that can then be tapped into to improve responses [49].

Acquired mutations in dCK with loss or decreased dCK activity were initially found in cultured human cell lines resistant to decitabine. This finding was further confirmed in vivo, in a subset of patients with MDS, where decreased levels of dCK and increased levels of CDA were suggested to be markers of primary resistance. Decitabine non-responders had a higher CDA/dCK ratio compared to responders. Additionally, it has been shown that patients with decreased levels of hENT1 and hENT2 have resistance to HMAs [48,50]. Similarly low levels of uCK due to mutations in the uCK2 gene have been found in resistant azacitidine cell lines [51], and it correlates with poor clinical outcomes in vivo [52].

Apart from pharmacokinetics, multiple other mechanisms of resistance have been explored, including:

- Primary azacitidine resistance secondary to down regulation of cell-cycle-related genes of HSCs mediated by Integrin Alfa-5 (ITGA5) signaling. The blockade of ITGA5 signaling by an inhibitor in combination with azacitidine has been shown to improve hematopoiesis [53].
- Upregulation of innate immunity signaling via Toll-Like Receptor (TLR) signaling and Nuclear-Factor Kappa B (NF-κB) activation, where overexpression of TLR2 in HSCs has been seen in patients with MDS, particularly after failure of response to HMAs, and inhibition of TLR2 signaling restores colony-formation capacity [54,55].
- Adaptive immunity molecules, such as immune-checkpoint regulators, programmed cell-death 1 (PD-1) and programmed cell-death ligand 1 (PD-L1) expression in HSCs has been associated with apoptosis and ineffective hematopoiesis and linked to resistance to HMAs [56].
- Resistance to azacitidine has also been significantly correlated with the amount of AML or MDS cells that express BCL-2-like protein 10 (BCL2L10) [57].

Other lines of substantiation for secondary resistance to HMAs come from the inability of HMAs to eliminate leukemic stem cells that later grow and lead to relapse and eventual drug resistance [58].

It is important to mention that despite the fact that *TP53* mutated AML and MDS, especially with high allele burden (>40%), has been proven to have poorer outcomes, and inferior survival [59], whether *TP53* mutations predict a higher response rate to HMA therapy remains unclear, with contradictory results reported. A study from 2016 reported high response rates to decitabine in *TP53*-mutant AML and MDS [60]. Later studies have shown that *TP53* doesn't affect response to HMAs [61]. *TP53* wild-type and mutant *TP53* have comparable ORR and CR rates in MDS (30%-50% and ~20% respectively), however, *TP53* mutant patients do have shorter response duration and inferior OS (6-12 months) compared to wild-type patients [61,62]. This difference in OS is not affected by type of HMA [62] or affected by an increased day regimen dosage [63], hence new strategies need to be urgently explored for *TP53*-mutant AML and MDS.

Despite the efforts in trying to elucidate the underlying mechanisms of resistance to HMAs, most of them remain unclear and are likely associated with a diversity of biological processes and dependent on specific bone-marrow-cell populations. In order to overcome this resistance, several new agents are currently under development, and successful combinations of HMAs with other molecules have had very promising results.

Novel HMA Formulations

Significant efforts for creating an oral HMA were made, in the hope that it would provide patient convenience, and potentially enhance adherence to treatment. Neither decitabine nor azacitidine were readily bioavailable in oral form due to rapid clearance by cytidine deaminase (CDA) present in the gut and the liver. A CDA inhibitor E7727, later named cedazuridine showed promise in preclinical models, leading to a successful phase 1 study that showed that the dose level of oral decitabine 30 mg and 40 mg plus cedazuridine 100 mg produced mean day-5 decitabine AUCs equivalent to 20mg/m2 of IV decitabine [64]. Successively, a phase 2 trial of a fixed dose combination tablet with cedazuridine 100 mg/decitabine 35 mg vs standard decitabine 20 mg/m^2 IV showed Oral/IV AUC ratios of 97.6%, with 21% CR, 60% ORR, and a similar safety profile in patients with intermediate and high-risk MDS [65]. The positive results of a phase 3 trial with this combination were recently presented at the American Society of Hematology (ASH) 2019 Congress, leading to the United States Food and Drug Administration (FDA) approval of oral decitabine for intermediate and high-risk MDS, as well as for CMML in July of 2020. This trial showed a similar demethylating activity, and similar safety profile for oral decitabine compared to IV decitabine, as well as oral/IV AUC ratio of 98.9 and a preliminary response analysis showed CR in 11.9%, and ORR in 64% of patients (including hematological improvement), the mature results are awaited [66].

Similarly, oral azacitidine has been trialed in lower-risk MDS. Unfortunately, despite positive results presented at the 2020 European Hematology Association Congress showing significant RBC transfusion independence, 30.8% vs 11.1% compared to placebo, the study was terminated early due to a higher incidence of deaths in the active therapy arm [67]. Despite the outcome in MDS, oral azacitidine (CC-486), later named onureg, has yielded positive results as a maintenance therapy in AML in a phase 3 randomized study that included patients aged ≥55 years in first remission following induction chemotherapy, where 472 patients received either oral azacitidine or placebo. At a median follow-up of 41.2 months, median OS was 24.7 months vs 14.8 months favoring the treatment group, and RFS was also significantly prolonged, both benefits were demonstrated regardless of baseline cytogenetic risk [68]. These results led to the FDA approval of Onureg, in September 2020, for the continued treatment of AML patients who have achieved first complete remission after intensive induction chemotherapy who are not able to complete intensive curative therapy [NCT01757535]. Moreover, a combination pill of azacitidine plus cedazuridine has shown promise in murine models and is expected to enter clinical trials [69].

Combination with Venetoclax

B-Cell Lymphoma 2 (*BCL-2*), a member of the *BCL-2* family of genes, is an integral part of the intrinsic mitochondrial apoptotic pathway, and it is a pro-survival gene. *BCL-2* has been shown to be up-regulated in AML being a pivotal negative regulator of apoptosis, playing an important role in AML transformation, survival, and resistance [70,71].

Venetoclax is a potent and highly selective oral *BCL2* inhibitor. Several studies have assessed its activity, either alone or in combination with HMAs in MDS and AML patients. Venetoclax has shown response as a single agent in patients with relapsed or refractory (R/R) AML, demonstrating 19% of ORR in heavily pretreated patients [72]. The outcomes of venetoclax combination regimens in R/R AML, MDS and blastic plasmocytoid dendritic-cell neoplasm were reported in a study that included 43 patients. In combination with venetoclax, the majority of patients received either decitabine (53%) or azacitidine (19%); including 21 patients (68%) who had already received HMAs. The ORR was 21%, median OS was 3 months, and prolonged cytopenias were the most common complication [73]. Currently there is an ongoing phase I clinical trial evaluating venetoclax alone and in combination with azacitdine in high-risk MDS after HMA failure [NCT02966782].

In 2018, the FDA granted approval for azacitidine or decitabine in combination with venetoclax, for elderly AML patients who are not candidates for high-intensity chemotherapy. This was based on the results of a phase 1 study that included 145 patients who received different doses of oral venetoclax in combination with decitabine or azacitidine, which showed a combined CR and complete remission with incomplete count recovery (CRi) of 67% in patients ≥65 years old with a median duration of CR+CRi of 11.3 months and a median OS of 17.5 months. In addition, a CR+CRi of 60% was noted in patients with poor risk cytogenetics and 65% CR+CRi in patients ≥75 years old. Furthermore, the venetoclax 400 mg cohort, showed CR+CRi rate of 73%, with a median duration of CR+CRi that was not reached for venetoclax + azacitdine. *TP53* mutated patients had a CR+CRi of 47% with a median duration of 5.6 months and 7.2 months OS. This combination was well tolerated without the side-effect of tumor-lysis syndrome [74]. Subsequently, a phase 3 study of venetoclax 400 mg combined with azacitidine in adults with untreated AML ineligible for induction therapy demonstrated practice changing results, with a median OS of 14.7 months in the azacitidine-venetoclax group compared to 9.6 months in the control group, as well as CR+CRi rate of 66.4% vs 28.3% in the control group and CR rate of 36.7% [75].

One of the setbacks of venetoclax combination regimens, is that *TP53* is a driver of venetoclax resistance. Preclinical studies have shown that *TP53* mutation impedes *BCL2* expression, decreasing the target of venetoclax directly and leading to drug resistance [76]. Additionally, expression of *MCL-1* and *BCL-XL* has been found in venetoclax resistant cells lines, furthermore these resistant-cell lines revealed modulation of sensitivity to *mTOR*, *MEK*, and *FLT3* pathways, and inhibitors of these specific signaling pathways, were found to synergistically induce apoptosis in AML cells and possibly prevent emergence of venetoclax resistance [77]. A study analyzing 81 AML patients receiving venetoclax-based combination regimens showed that primary and acquired resistance to venetoclax-based combinations was most commonly characterized by acquisition or enrichment of clones activating signaling pathways such as *FLT3*, *RAS* or biallelically perturbing *TP53*. Moreover, in functional studies, *FLT3* internal tandem duplication gain or *TP53* loss conferred cross-

resistance to both venetoclax and cytotoxic-based therapies [78]. Genes involved in mitochondrial organization and function, such as *CLBP*, have also been found to be upregulated in venetoclax-resistant AML, and its ablation sensitizes AML to venetoclax, leading to a possible future target to circumvent venetoclax resistance in a p53-independent manner [79].

Combination with APR-246 in p53 mutant MDS

The tumor-suppressor gene *TP53* encodes the p53 protein, which regulates cell cycle and apoptosis. *TP53* mutations and p53 overexpression in MDS and AML are independent, negative prognostic factors that have been associated with aggressive disease clinical course, poor OS, and resistance to conventional therapies [80].

APR-246 (APR) is a compound that induces apoptosis in human tumor cells through restoration of the transcriptional transactivation function to mutant p53 [81]. APR is a prodrug that forms an active moiety that covalently binds to thiol groups of the core domain of mutated p53 protein, thereby resulting in a structural change that restores its active conformation [82].

APR has shown to be effective inhibiting the proliferation of *TP53*-mutated myeloid cell lines, as well as in murine models on its own or in combination with azacitidine [83].

The favorable results of a phase 1b/2 study combining APR and azacitidine were reported at ASH 2019 [NCT03072043]. The study included HMA-naïve TP53-mutated higher risk MDS, and AML patients with $\leq$ 30% blasts. 55 patients were enrolled, of 45 evaluated patients, the ORR was 87% and the CR was 53% with a median follow up of 10.5 months. Median time to response was 2.1 months with a median duration of response of 6.5 months. CR rate for MDS was 61%, and 50% for AML. Median OS in responding patients was 12.8 months vs 3.9 months in nonresponders. The ORR, CR rate, and OS are very promising and this study additionally found that patients that had isolated TP53 mutation and $>$10% p53 positive bone marrow mononuclear cells by immunohistochemistry had higher CR rates [84], making APR a potential drug targeted for *TP53* mutant disease, which is currently a disease with very poor outcomes. The preliminary results of a phase 2 study from the Groupe Francophone Des Myélodysplasies (GFM) was also presented at ASH and later at EHA25 with similar positive results [85,86].

APR plus azacitidine is a well-tolerated combination with high response rates in TP53-mutant MDS/AML. The data of the phase 3 randomized trial of APR-246 in combination with azacitidine vs azacitidine monotherapy [NCT03745716**]** are eagerly awaited.

Combinations with anti-CD47 antibody

CD47 is a transmembrane protein that functions as an antiphagocytic signal, enabling CD47 expressing cells to evade phagocytic elimination mainly by macrophages, this inhibition of phagocytosis occurs by CD47 binding to its receptor Signal Regulatory Protein Alpha on macrophages leading to tyrosine phosphatase activation and inhibition of myosin accumulation at the phagocytic synapse site, preventing phagocytosis [87]. CD47 has been shown to be overexpressed in multiple malignancies and was first described in AML cell lines [88]. Pre-clinical data has shown that blockade of CD47 with an anti-CD47 antibody induces phagocytosis of leukemic cells in vitro and eradicates human AML and MDS cells in vivo [89,90].

Interestingly anti-CD47 antibodies do not eliminate normal cells, even though these cells also express CD47. When normal cells become damaged they induce expression of pro-phagocytic signals that lead to their removal by phagocytosis [91]. It's hypothesized that as a protective mechanism, malignant cells upregulate CD47 to counterbalance pro-phagocytic signals, therefore the anti-CD47 antibody blocks the CD47 signal and unmasks and exposes an unopposed pro-phagocytic signal leading to phagocytosis [92].

Magrolimab, an anti-CD47 IgG4 antibody, was initially evaluated in a multicenter phase 1 trial that enrolled 15 patients with R/R AML [NCT02678338], where 5 dose cohorts were studied, initial results reported at EHA Congress 2018 showed no maximum tolerated dose or dose limiting toxicity with dosing up to 30mg/kg. Anemia was the most common adverse effect in 93% of patients, no therapy discontinuation due to AEs or drug-related deaths were reported, 73% of patients achieved stable disease with no objective responses. 40% of patients had a reduction in bone marrow blast count (mean decrease of 27%) [93]. Overall magrolimab is well tolerated, with evidence of activity that was insufficient for further development as a single agent.

Magrolimab was then investigated in a phase 1b trial in combination with azacitidine in untreated AML patients ineligible for induction chemotherapy and higher-risk MDS patients [NCT03248479]. The results were presented at ASCO 2020. The study included 68 patients, 39 with MDS and 29 with AML, in which 68% had poor risk cytogenetics and 27% were *TP53*-mutant. The combination was well-tolerated with safety similar to azacitidine alone. Anemia was mitigated with an initial priming dose of magrolimab. ORR was 91% with CR of 42% in MDS, and 64% ORR with CR+CRi of 56% in patients with AML. Responses deepened over time, with 56% CR at 6 months in MDS patients. The median time to response was 1.9 months. These are very positive results and the median treatment response is faster than with azacitidine alone. Complete cytogenetic responses and minimal residual disease negativity were observed in both groups. The median overall survival had not been reached in either group at the time of analysis, and a subgroup analysis showed that patients with a *TP53* mutation had ORR of 75%. Additionally, the combination dramatically reduced the *TP53* mutational burden during treatment [94].

These data reflect that the combination magrolimab + azacitidine has high response rates in AML and MDS patients, and it could be another potential option for *TP53*-mutant disease.

An expansion single arm MDS cohort is ongoing. ENHANCE, which is a randomized phase 3 trial with MDS patients is planned [NCT04313881].

IDH1/2 Inhibitors

Ivosidenib and enasidenib are oral, targeted, small-molecule inhibitors of mutant *IDH1* and mutant *IDH2*, respectively. In preclinical models both ivosidenib and enasidenib treatment decreased intracellular levels of 2-hydroxyglutarate and induced differentiation in models of *IDH1*/*IDH2*-mutated tumors [95,96].

Enasidenib was approved by the FDA in August 2017 for the treatment of adult patients with R/R AML with an *IDH2* mutation based on the successful results of a phase 1/2 trial. In this trial, patients with R/R AML treated with enasidenib had an ORR of 40.3%, CR of 19.3%, with a median response duration of 5.8 months. Median overall survival among R and/or R patients was 9.3 months, and 19.7 months for the patients who achieved CR [97]. Similarly, ivosidenib was approved by the FDA in July 2018, for treatment in the R/R setting, after the results of a phase 1 trial that included *IDH1*-mutant R/R AML patients, treated with ivosidenib monotherapy. This trial showed an ORR of 41.6% and a CR+CRi of 30.4%, with median duration of response of 8.2 months for the patients that achieved CR+CRi and 6.5 months for the patients that achieved an overall response [98].

More recently, in 2019, ivosidenib received FDA approval as first-line treatment in *IDH1*-mutant AML for the treatment of elderly and ineligible patients for induction chemotherapy, based on the results of a phase 1 trial where 34 patients with newly di-

agnosed AML ineligible for standard therapy received ivosidenib. The trial showed a CR+CRi rate of 42.4%, CR rate of 30.3%, median durations of CR+CRi and CR were not reached at 23.5 month follow-up, 61.5% of CR+CRi patients and 77.8% of CR patients were in remission at 1 year and the median overall survival was 12.6 months [99]. Enasidenib is not currently approved for the frontline treatment of AML in the United States, but favorable preliminary results have been recently reported [100].

Subsequently, the combination of ivosidenib with azacitidine was studied in a phase Ib trial with this combination, that included 23 newly diagnosed *IDH1*-mutant AML patients ineligible for intensive induction chemotherapy, the ORR was 78.3%, and the CR rate was 60.9%, with a median duration of response in responders that had not been reached at a median follow-up of 16 months. The 12-month survival estimate was 82.0% [101].

Additionally combination treatments for newly diagnosed *IDH1/2*-mutant AML with enasidenib or ivosidenib with induction and consolidation chemotherapy are underway and positive preliminary results have been presented at ASH 2018 [102].

Enasidenib has been studied in patients with *IDH2*-mutant MDS, in a phase 1/2 trial that included 17 MDS patients that were R/R or ineligible for standard chemotherapy. A total of 3 (18%) patients had relapsed after allogeneic stem-cell transplants, 13 (76%) had previously received therapy with HMAs, and 10 (59%) had received at least 2 previous therapies. ORR was 53%, with a median duration of response of 9.2 months. A total of 6 (46%) of 13 patients previously treated with HMAs responded. mOS was 16.9 months. This data is important since it shows that enasidenib can induce responses in IDH2-mutant MDS, including patients with previous HMA therapy [103].

There are currently several ongoing trials for *IDH1/IDH2*-mutant AML and MDS, including such as [NCT03173248], [NCT03471260], [NCT03683433], [NCT02719574] which will further elucidate the efficacy of targeting IDH1/2 in myeloid malignancies.

LSD1 inhibitors

Histone lysine specific demethylase 1 (LSD1) is a transcription co-repressor that works by demethylation of mono and demethylated H3K4 [104] and in conjunction with androgen and estrogen receptors causes demethylation of H3K9 [105,106]. LSD1 also regulates cell cycle and demethylation of non-histone proteins, such as p53, E2F1 and DNMT1 [107-109]. Additionally, LSD1 prevents ubiquination of factor-1a leading to an increase of tumor angiogenesis and growth [110].

It has been shown that inhibition of LSD1 is essential in maintaining the oncogenic potential of leukemic stem cells. In a preclinical model, knockdown of LSD1 led to a decrease in colony formation, increased differentiation and apoptosis of LSCs. LSD1 knockdown led to an increase in methylation of H3K4 and H3K9 with decreased expression of the MLL-AF9 gene. Knockdown LSD1 cells transplanted into mice were unable to establish leukemia and treatment with an LSD1 inhibitor caused anti-leukemic effects, anemia and thrombocytopenia in MLL-AF9 transgenic mice [111]. Preclinical models have also shown that LSD1 inhibitors combined with ATRA increase that percentage of cells that express CD11b, a marker of myeloid differentiation, and increase myeloid differentiation in human AML Lines. Additionally, ATRA plus and LSD1 inhibitor induced apoptosis in cells with p53 [112]. Given this promising pre-clinical activity various LSD1 inhibitors have been developed for clinical trials.

Tranylcypromine (TCP), which causes irreversible inhibition of LSD1, as previously stated has shown favorable results in combination with ATRA [112] but has shown in later studies that MLL-rearranged cells are less sensitive to this combination [113]. Initial results of a phase 1 trial presented at ASH 2018 with TCP in combination with ATRA that included patients with R/R AML and high-risk MDS, showed that the combination was safe with a maximum tolerated TCP dose of 20mg BID. Responses were seen in 2 of 15 patients, 1 CR (MDS) and 1 morphological free leukemia state (AML), 5 had stable disease for more than 3 months (2 AML, 1 CMML, 2 MDS), 3/4 MDS/CMML responders had hematologic improvement (HI) [NCT02273102] [114]. Other LSD1 inhibitor, ORY, a derivative of TCP, which is highly potent and selective for LSD1, has shown increase in global demethylation of H3K4 and induction of differentiation of macrophages and monocytes in vitro, additionally cell lines with MLL translocation were the most responsive to this agent, showing dose-dependent decrease of AML growth in MLL-AML xenograft murine models [115]. Initial results of a phase 1 trial with ORY in R/R AML patients were presented at ASH 2016, and showed a dose limiting toxicity in 2 patients (lobar pneumonia and febrile neutropenia), efficacy of the drug in 14 MLL-translocated AML and 6 M6 AML was reported, 5 patients (36%) had a response to therapy, 2 patients with t(9;11) had SD, 3 had PR, but no CR was observed. Evidence of morphologic blast differentiation in blood and bone marrow was observed in 9 patients [EUDRACT 2013-002447-29] [116]. A phase 1 trial

Table 1
Selected ongoing trials of epigenetic directed therapies.

Class	Agent	Phase	Population	Status	Identifier
HMA	Guadecitabine	3	R/R AML	Completed	NCT02920008
HMA	Guadecitabine	3	untreated AML	Completed	NCT02684162
HMA	Oral Azacitidine	3	AML in CR	Active	NCT01757535
HMA+BLC2 Inhibitor	Azacitidine+Venetoclax	1	R/R MDS	Active	NCT02966782
IDH1 Inhibitor+ BCL2 Inhibitor	Ivosidenib+Venetoclax	1/2	*IDH* mutant AML/MDS/MPN	Recruiting	NCT03471260
IDH1 Inhibitor+ HMA	Ivosidenib+Azacitidine	3	Untreated AML	Recruiting	NCT03173248
IDH2 Inhibitor+ HMA	Enasidenib+Azacitidine	2	R/R AML	Recruiting	NCT03683433
EZH2 Inhibitor	DS-3201b	1	AML/ALL	Recruiting	NCT03110354
HMA+APR-246	APR-246+Azacitidine	3	*TP53* mutant MDS	Active	NCT03745716
HMA+APR-246	APR-246+Azacitidine	1/2	*TP53* mutant AML/MDS/MPN	Active	NCT03072043
HMA+ Anti-CD47	Magrolimab+ Azacitidine	1	AML/MDS	Recruiting	NCT03248479
HMA+ Anti-CD47	Magrolimab+ Azacitidine	3	Untreated MDS	Not yet Recruiting	NCT04313881
LSD1 Inhibitor	TCP+ATRA	1	R/R AML/MDS	Completed	NCT02273102
LSD1 Inhibitor	IMG-7289+ATRA	1	AML/MDS	Completed	NCT02842827
LSD1 Inhibitor	TCP+ATRA+Cytarabine	1	AML/MDS	Recruiting	NCT02717884

Table 2
Selected trials of epigenetic directed therapies.

Trial Name	Class	Agent	Phase	Population	Results
AZA in MDS, CALGB [32]	HMA	Azacitidine vs Supportive Care	3	MDS	ORR: 60% CR: 7% PR: 16% HI: 37% in patients on Aza
AZA-001 [33]	HMA	Azacitidine vs Conventional Care	3	Higher-risk MDS	mOS: 24.5 mo for the AZA group mOS: 15 months for conventional care group (median follow-up: 21.1 mo)
AZA Prolongs OS in Elderly AML <30% blasts [34]	HMA	Azacitidine vs Conventional Care	3	Elderly patients with AML<30% blasts	mOS: 24.5 mo for the AZA group mOS: 16.0 months for CC group (median follow-up of 20.1 months) 2-year OS AZA group: 50% 2-year OS CC group: 16%
Decitabine vs Best Supportive Care in Elderly Intermediate or High-Risk MDS from MDS EORTC CLG and D-MDS [35]	HMA	Decitabine vs Supportive Care	3	Elderly patients with intermediate and High-risk MDS ineligible for intensive chemotherapy	mPFS: 6.6 mo for DACO group mPFS: 3 mo for SC group AML transformation at 1 year: 22% for DACO group vs 33% for SC group CR: 13% PR: 6% HI: 15% in DACO group
Hematologic response to 3 alternative dosing schedules of AZA in MDS [37]	HMA	Azacitidine	2	MDS (mostly lower-risk)	HI: 41% to 50% in lower-risk MDS RBC transfusion independence: 50-75% in lower-risk MDS
ASCERTAIN study [66]	HMA	ASTX727 (Oral cedazuridine/decitabine) vs IV Decitabine	3	Intermediate and High-risk MDS and CMML	Preliminary Results CR: 11.9%, ORR: 64% (including HI) mCR: 45.5% in ASTX727 arm
The QUAZAR AML-001 Maintenance Trial [68]	HMA	CC-486 (Oral Azacitidine) vs Placebo	3	AML in CR1 or CRi ineligible for HSCT	Preliminary Results mOS in CC-486: 24.7 mo mOS in placebo: 14.8 mo mRFS in CC-486: 10.2 mo mRFS in placebo: 4.8 mo (median follow-up of 41.2 mo)
Venetoclax with decitabine or AZA in treatment-naive, elderly patients with AML [74]	HMA+BCL2 inhibitor	Azacitidine or Decitabine + Venetoclax	1b	Elderly, untreated AML, ineligible for intensive chemotherapy	CR+CRi in ≥65 years: 67% mOS in ≥65 years: 17.5 mo CR+CRi in patients ≥75 years: 65% CR+CRi in Venetoclax 400 mg cohort: 73%, median duration of CR+CRi not reached for venetoclax + azacitidine
AZA and Venetoclax in Previously Untreated AML [75]	HMA+BCL2 inhibitor	Azactidine + Venetoclax (target 400mg) or placebo (control)	3	Untreated AML, ineligible for intensive chemotherapy	CR+CRi AZA+Venetoclax: 66.4% CR+CRi Control: 28.3% CR AZA+Venetoclax: 36.7% CR Control: 17.9% mOS AZA+Venetoclax:14.7 mo mOS Control: 9.6 mo
APR-246 and AZA in *TP53*-mutant MDS and Oligoblastic AML [84]	HMA+APR-246	APR-246+Azacitidine	1b/2	*TP53* mutant higher-risk MDS/MPN and AML<30% blasts	Preliminary Results ORR: 87%, CR: 53% (median follow up of 10.5 mo.) CR in MDS: 61% CR in AML: 50% mOS in responders: 12.8 mo mOS in non-responders: 3.9 mo.
Tolerability and efficacy of magrolimab combined with AZA in MDS and AML [94]	HMA+ Anti-CD47	Magrolimab+ Azacitidine	1b	Untreated intermediate and high-risk MDS and AML ineligible for intensive chemotherapy	Preliminary Results ORR in MDS: 91% CR in MDS: 42% ORR in AML: 64% CR+CRi in AML: 56% ORR in *TP53* mutant disease: 75%. mOS: not reached at time of analysis

of a different LSD1 inhibitor GSK2879552 in combination with ATRA in patients with RR AML [NCT02177812] was terminated for unknown reasons as well as a planned phase 1/2 study with the same molecule in patients with MDS [NCT02929498].

The results of several trials with LSD1 inhibitors in AML and MDS are anticipated including [NCT02273102] and [NCT02842827], and trial [NCT02717884] is currently ongoing. Mature data of this trials are awaited, but given these early unfavorable results, the future of LSD1 inhibitors as a monotherapy is uncertain, it's possible that LSD1 inhibitors may need to be trialed in combination with other drugs to optimize clinical activity.

Failed combinations with HDACIs

Histone deacetylases (HDACs) are a group of enzymes that remove acetyl groups from lysine in histones, reversing covalent modifications, thereby repressing DNA transcription. Aberrant expression and function of these regulators are common in MDS and AML. Treatment with HDAC Inhibitors (HDACIs) results in chromatin remodeling that permits re-expression of silenced tumor suppressor genes in cancer cells, which in turn, can potentially result in cellular differentiation, inhibition of proliferation and apoptosis [117].

Preclinical and clinical data showed synergistic activity of HDACIs with hypomethylating agents [118]. Unfortunately, multiple HDACIs have been explored in MDS and AML alone or in combination with HMAs with low response rates.

Numerous studies have explored the use of vorinostat in combination with azacitidine, and have shown ORR of 27–47% in MDS and AML patients, without response or survival benefits compared with azacitidine alone [119-121]. The notion that co-administration of HDACIs might inhibit cellular uptake of HMAs has been suggested but hasn't been extensively studied [121]. The possibility of pharmacodynamics antagonism also exists as HDAC inhibitors may lead to cell cycle arrest thereby preventing incorporation of azanucleotides in malignant cells [122]. In a randomized, placebo-controlled, phase 2 study of azacitidine alone or in combination with pracinostat, that included 102 patients with higher-risk MDS, pracinostat was associated with significantly lower CR rates (18% vs 33%), increased toxicity, and no improvement in PFS or OS [123].

Other HDACIs, such as panobinostat or entinostat, have also been trialed and have failed to improve response or survival outcomes compared with azacitidine monotherapy [124,125]. Therefore, the addition of HDACIs to HMAs is not effective, and may add toxicity and worse survival benefits in MDS and AML patients.

Conclusions

An increased understanding of the significance of epigenetics has led to several advancements in the field, with significant improvements in patient outcomes. Therefore, the continual introduction of novel agents is crucial to advance care. Unfortunately, many favorable preclinical results in epigenetic therapies have not translated into similar clinical responses, but the novel combinations of multiple agents with epigenetic therapies has yielded encouraging results. Current standard of care for higher-risk MDS patients not eligible for ASCT are HMAs, but they do not lead to a cure, thus the approval of HMA oral formulations will possibly improve patient compliance and quality of life. Moreover, the new combinations of HMAs with novel agents have yielded high response rates for higher-risk MDS patients, such as magrolimab and APR-246.

Furthermore, the outlook in induction ineligible AML patients with these novel combinations is also promising. The introduction of venetoclax in combination with HMAs has shown unprecedented results in this population, and the combination of APR-246 and magrolimab is expected to possibly increase our outcomes in *TP53* mutant disease. The mature data of many trials is eagerly awaited, the revolutionary epigenetic treatments in combination with novel agents has greatly advanced the field of AML and MDS treatment and a future with better patient outcomes and possible cures may be within our reach.

Tables 1 and 2.

Conflict of interest

Aditi Shastri has received research funding from Kymera Therapeutics and consultation fees from Guidepoint & GLG. There are no conflicts of interest with this study.

Amit Verma has received research funding from BMS, GSK, Incyte, Medpacto, Curis and Eli Lilly and is a scientific advisor for Stelexis, Novartis, Acceleron and Celgene and holds equity in Stelexis and Throws Exception. There are no conflicts of interest with this study.

CRediT authorship contribution statement

Jesus D. Gonzalez-Lugo: Methodology, Writing - original draft, Writing - review & editing. **Samarpana Chakraborty:** Writing - review & editing. **Amit Verma:** Methodology, Writing - review & editing. **Aditi Shastri:** Methodology, Writing - original draft, Writing - review & editing.

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