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REVIEW

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Novel approaches to the use of hypomethylating agents in myeloid malignancies

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ABSTRACT

Epigenetic dysregulation has been increasingly understood to be a key factor in the development of myeloid malignancies, often acting alongside genetic alterations to disrupt normal hematopoiesis. The inherent reversibility of epigenetic changes has provided an excellent opportunity for therapeutic intervention. Hypomethylating agents (HMA) preferentially alter gene expression in heavily methylated malignant myeloid cells by inhibiting DNA methyltransferases thereby altering gene expression. They have since become foundation therapies in myelodysplastic syndrome (MDS) and in older patients with acute myeloid leukemia (AML). However, complete responses to HMA monotherapy are limited and usually non-durable. In recent years, novel approaches have been sought to overcome resistance and expand the role of epigenetic therapies in MDS and AML as well as in less well-studied myeloid malignancies such as myeloproliferative neoplasms (MPNs) and MDS/MPN overlap syndromes. Combination regimens that synergistically pair HMAs or other epigenetically active therapeutics with agents targeting apoptosis, cellular metabolism, and immune evasion have also shown early promise in improving patient outcomes. Oral formulations of HMAs have made maintenance strategies more convenient and tolerable for patients, with demonstrated benefits in AML and ongoing investigations in MDS and post-transplant settings. This review will explore these novel therapeutic strategies in the treatment of myeloid malignancies.

PLAIN LANGUAGE SUMMARY

Blood cancers are driven not only by genetic mutations but also by epigenetic changes. These changes affect how genes are turned on or off without changing the DNA sequence itself. This article reviews a class of drugs called hypomethylating agents that remove chemical tags on DNA and allow cancer cells to mature more normally. Traditionally, these drugs had to be given by injection or infusion, but newer oral forms now offer greater convenience and in some cases, can help maintain remission for longer periods than before. Researchers are also studying why some patients eventually develop resistance to these therapies, and the current research being done in developing new drugs that can work in combination with hypomethylating agents to improve outcomes for people living with these challenging blood cancers.

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Cancer epigenetics; myeloid neoplasms; AML; MDS; HMAs; drug combinations

1. Introduction

Myeloid malignancies encompass a broad spectrum of hematologic neoplasms that includes acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPNs), and overlap syndromes such as MDS/MPN. While their clinical features vary, these disorders are marked by a common pathobiology involving clonal expansion of hematopoietic stem and progenitor cells, driven by a combination of genetic and epigenetic alterations that lead to aberrant differentiation, dysregulated hematopoiesis, and in the case of MDS and MPNs, an inherent risk of progression to AML [1]. Classical models in cancer research, such as the original two-hit hypothesis for somatic mutations have been insufficient in explaining the characteristics of decreased differentiation in leukemia and lack of widespread genomic instability in many myeloid neoplasms. Increasing evidence instead highlights the profound epigenetic dysregulation common to this heterogenous group of disorders [2,3]. In normal hematopoiesis, the differentiation and self-renewal of hematopoietic stem and progenitor cells are tightly regulated through epigenetic mechanisms involving DNA methylation, histone acetylation, histone methylation, and the action of noncoding RNAs. Recurrent mutations in key epigenetic regulators – including *DNMT3A* [4], *TET2* [5], *ASXL1* [6], *IDH1/2* [4], and *EZH2* [7] – are highly prevalent across the spectrum of myeloid neoplasms and typically found early in the driver clone [7].

The recognition of epigenetic changes as both a central and reversible hallmark of myeloid malignancies has made targeting them an attractive therapeutic option. The development of DNA hypomethylating agents (HMAs) such as azacitidine and decitabine marked a pivotal advance, with their 2004 and 2006 approvals demonstrating that epigenetic modulation could improve survival in MDS and older AML populations [8–10]. However, several limitations persist: 1) Only approximately 50% of patients achieve objective responses and only 15–30% of patients achieve remission [8]; 2) Even in responders, median response durations remain approximately 12–15 months as founder clones remain [11]; and 3) No reliable predictive biomarkers exist

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Article highlights

HMAs in Myeloid Malignancies
- Despite the widespread use of HMAs in AML and MDS, no consistent biomarker for response or survival benefit has been identified.
- HMA-based regimens show modest activity in accelerated-phase and blast-phase MPNs, and combination strategies with ruxolitinib and venetoclax are under active investigation.

Epigenetic-Based Combination Therapy
- Resistance to HMA monotherapy is common and multifactorial, and may involve impaired drug uptake and efflux, altered apoptosis, and persistence of drug-resistant leukemic stem cells.
- Combination therapies synergistically pair HMAs with agents targeting apoptosis, leukemic cell metabolism, and immunomodulation. The combination of AZA+Ven has been the most successful regimen thus far in older adults with AML and sAML though recent data is disappointing in MDS.

Oral HMAs and Maintenance Therapy
- Patients with AML in remission who are not candidates for alloHCT experience high rates of relapse, with subsequent median survival of less than 6 months.
- Oral HMAs allow for prolonged epigenetic suppression in outpatient settings with improved patient convenience and adherence.
- The QUAZAR AML-001 established oral AZA as the first agent to improve both RFS and OS in AML after induction chemotherapy.
- Ongoing trials are assessing the utility of oral HMAs in MDS, the post-transplant setting, and in combination with targeted agents, and questions remain regarding optimal treatment duration and dosing schedules.

despite known mutation profiles. These shortcomings highlight the need for novel strategies that build upon first-generation epigenetic therapies. In this review, we focus on three evolving domains in the use of epigenetic therapies for myeloid malignancies: the role of HMAs in MPNs and MDS/MPN overlap syndromes, combinatorial approaches with other targeted or immune therapies, and emerging maintenance strategies using oral HMAs.

2. Methodology

A comprehensive literature search was conducted with a focus on HMAs and emerging epigenetic modulators in myeloid malignancies. Literature searches were performed in PubMed, Web of Science, Scopus, and major oncology/hematology conference abstracts from 2005 to May 2025. Search terms included combinations of keywords and Medical Subject Headings terms such as "hypomethylating agents," "azacitidine," "decitabine," "oral azacitidine," "oral decitabine/cedazuridine," "myelodysplastic syndromes," "myeloproliferative neoplasms," "chronic myelomonocytic leukemia," and "MDS/MPN overlap." The ClinicalTrials.gov registry was additionally reviewed to identify ongoing or recently completed trials presenting preclinical or clinical data in the above disease settings

Filters were applied to only include peer-reviewed original research articles, reviews, and clinical studies published in English. Data from the selected sources were synthesized into a narrative framework. While comprehensive, this review is limited by potential publication bias, the exclusion of non-English articles, and the possibility that recent studies may not have been indexed in the selected databases.

3. HMAs in the spectrum of myeloid diseases

3.1. HMAs in AML and MDS

Following their FDA approval in the early 2000s, HMAs such as azacitidine (AZA) and decitabine (DEC) have been a mainstay in treatment for patients with AML and MDS. In higher-risk MDS (HR-MDS), azacitidine monotherapy has demonstrated superior overall survival (OS), improved rates of transfusion independence (TI), and delayed progression to AML compared to supportive or conventional care in multiple randomized controlled trials [8]. DEC, while not showing a definitive OS advantage in clinical trials for HR-MDS, has been associated with improvements in leukemia-free survival, hematologic response rates, and quality of life measures [12]. In lower-risk MDS, both AZA and DEC have been shown to increase red blood cell transfusion independence and reduce rates of leukemic progression. Recent data also suggests that earlier intervention with low-dose HMAs may offer clinical benefit in prolonging durations of response [13], and the results from ongoing trials (NCT02269280) are awaited to clarify their role in this setting.

For older or medically unfit older patients with AML, HMA-based regimens are now standard. The DACO-016 trial demonstrated that DEC monotherapy modestly improved overall survival compared to low-dose cytarabine or supportive care in older AML patients (7.7 vs 5.0 months) [14]. Similarly, the AZA-AML-001 trial demonstrated improved median OS in patients with high-blast AML not candidates for standard induction treated with azacitidine compared to conventional care (10.4 vs 6.5 months) [10]. More recently, the VIALE-A trial established a new standard of care in older adults, with the addition of venetoclax to azacitidine significantly improving survival compared to azacitidine alone (CR: 66% vs 28%, mOS: 14.7 months vs 9.6 months; $p < 0.001$) in patients > 75 with comorbidities precluding intensive chemotherapy [15]. Subsequent analysis has found molecular profiling to strongly predict response to this regimen, with studies suggesting mutations in *NPM1* and *IDH1/2* having a favorable prognosis, while *TP53* mutations continue to be associated with shorter durations of remissions consistent with historical controls [16]. Toxicity of this regimen also remains a key limiting factor, with febrile neutropenia (42% vs 19%) and infections of any grade (85% vs 67%) more common with the addition of venetoclax.

Despite the widespread use of HMAs in MDS and AML and the high frequency of mutations in epigenetic regulators like *DNMT3A* or *TET2* in these diseases, reliable predictive biomarkers have not been consistently identified. While certain clinical features such as older age, poor performance status, and high transfusion requirements are associated with primary resistance or short-lived response, molecular predictors have been inconsistent [17,18]. *TET2* mutations, particularly in the absence of *ASXL1* mutations, have shown the most consistent association with higher response rates to azacitidine. Other mutations, including *DNMT3A*, *IDH1/2*, *RUNX1*, and *FLT3-ITD* have yielded mixed or no clear associations with response or survival. Epigenetic features such as methylation patterns in tumor suppressor genes of global hypomethylation throughout treatment have also produced inconsistent findings, with

some small studies suggesting predictive roles for CDKN2B/p15^{INK4B} or LINE-1 hypomethylation [19,20]. Recent genome-wide methylation profiling has identified several differentially methylated regions outside of promoters as potential biomarkers and require further validation [21].

In both AML and HR-MDS, long-term outcomes of HR-MDS and AML patients treated with an HMA remain suboptimal, with 5-year survival rates between 4–8%. Prognosis is especially poor following HMA failure, with median OS dropping below 6 months in high-risk MDS and as low as 1.3–2 months in AML.

3.2. HMAs in MPNs

While HMAs have not been formally approved for use in the typical Philadelphia-negative MPNs and in most MDS/MPN disorders, data from retrospective studies and subgroup analyses of broader clinical trials have suggested clinical benefit for targeting epigenetic regulators, particularly in high-risk or advanced disease states. The typical Philadelphia-negative MPNs are polycythemia vera (PV), essential thrombocytopenia (ET), and primary myelofibrosis (PMF) or post-PV or post-ET myelofibrosis, all of which are characterized by clonal hematopoiesis, myeloid proliferation, and the potential for progression into ineffective hematopoiesis, MDS, or AML. The classic MPNs are most commonly associated with activating driver mutations in *JAK2*, *CALR*, and *MPL*, leading to constitutive activation of the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway [22]. In recent years, high-throughput sequencing has revealed frequent co-occurring mutations in epigenetic regulators (e.g., *TET2, ASXL1, DNMT3A, IDH1/2*) [23], and transcriptional control genes (*SRSF2, SF3B1, U2AF*) [24]. These mutations have been associated with clonal dominance, inferior survival, and a higher risk of leukemic transformation. For example, in a large European cohort of 483 patients with PMF, mutations in *ASXL1*, *SRSF2*, and *EZH2* were each independently associated with significantly shorter overall survival (HR: 2.21, 2.6, 1.91, respectively), while *ASXL1 SRSF2* and *IDH1/2* were linked with inferior leukemia-free survival. Collectively, the presence of any high-risk mutation predicted a significantly shorter median survival (81 months vs 148 months; $p < 0.0001$) and increased risk of leukemic transformation (HR 2.96; $p < 0.0001$) [25].

Prospective trials of HMAs in MPNs remain limited, with most data derived from patients with secondary AML (sAML) arising from MDS or phenotypes with overlapping MDS features. In patients with accelerated phase (MPN-AP) or blast phase MPN (MPN-BP), HMA-based regimens have demonstrated modest clinical activity and improved survival compared to historical chemotherapy-treated cohorts. In a retrospective series of 54 patients with MPN-BP (PV: $n = 21$, ET: $n = 21$, PMF: $n = 7$, unclassified MPN: $n = 5$) with progression to MDS or AML, AZA yielded an ORR of 52% and median OS of 11 months [26]—outcomes that compare favorably to historical controls (3–4 months OS in untreated patients) [27].

Beyond advanced and blast-phase disease, HMAs have also been evaluated in MF, particularly in combination with JAK inhibition or in ruxolitinib-refractory settings. As monotherapy, AZA has yielded limited clinical response for patients with PMF or sMF, with response rates around 20–30% [28]. Additionally, HMAs have shown activity as salvage therapy after allogeneic transplant, with one study reporting 58% of patients with restoration of donor chimerism after 2 cycles of HMA, and 50% with molecular clearance of pre-transplant mutations [29], though these findings require confirmation in larger cohorts.

3.3. HMAs in MDS/MPN overlap syndromes

Epigenetic dysregulation is especially pronounced in MDS/MPN overlap syndromes. These syndromes represent a heterogenous group of clonal myeloid neoplasms exhibiting both dysplastic and proliferative features, including CMML, MDS/MPN-ring sideroblasts-thrombocytosis (MDS/MPN-RS-T), BCR-ABL1 negative atypical chronic myeloid leukemia (aCML), MDS/MPN-unclassifiable (MDS/MPN-U), and juvenile myelomonocytic leukemia (JMML). In CMML, > 90% of patients harbor a mutation in one or more epigenetic regulators. Additional Sex Combs Like 1 (*ASXL1)* mutations are especially common and portend a poor prognosis; conversely, patients with wild type (WT) *ASXL1* and *TET2* mutations have demonstrated improved response rates to HMAs and a survival advantage [30]. These mutations further appear to influence post-transplant outcomes. In a study of 52 CMML patients who underwent HCT, patients with ≥ 4 mutated epigenetic regulators were found to be at a significantly increased risk for relapse (HR 5.4: $p = 0.003$) and inferior survival [31].

Although AZA and DEC have been FDA-approved for CMML, their approval was granted based on the small groups of dysplastic CMML (MD-CMML) patients included in broader MDS trials (AZA-001 and CALGB 9221) [8,32]. Subsequent analyses have provided greater granularity, with ORRs ranging from 40–50% and median OS of 12–22 months depending on clinical phenotype [33,34]. In a prospective phase II study of DAC in CMML ($n = 43$), response rates appeared to be lower in myeloproliferative CMML (MP-CMML) compared to MD-CMML, with CR 14% (vs 21% in MD-CMML) and ORR 39% (vs 64% in MD-CMML) [35]. In the European DACOTA trial that randomized 170 patients with higher-risk MP-CMML to decitabine or hydroxyurea, treatment with decitabine resulted in higher ORR rates (63% vs 35%; $p = 0.0004$) and a delayed progression to AML (cause-specific HR: 0.62; $p = 0.005$), though the study was underpowered to determine a clear survival benefit [18]. Data also suggests that low dose decitabine may produce superior response rates compared to low dose AZA. In a recent phase II trial, low dose DAC demonstrated a better ORR (ORR 70% vs 49% p – 0.03) and cytogenetic responses (61% vs 25% $p = 0.02$) compared to low dose AZA, though with no difference in event free survival [36].

HMAs overall appear to offer meaningful clinical benefit in subsets of patients with MDS/MPN overlap syndromes and advanced-phase MPNs. However, responses are often incomplete and non-durable.

3.4. Oral HMAs

With advances in induction chemotherapy and the introduction of effective lower-intensity regimens such as HMA/VEN for older patients with AML, 70–80% of individuals under age 60 with newly diagnosed AML and 40–50% of those older than 60

achieve CR following initial therapy [37,38]. However, long-term disease-free survival remains limited, with approximately 30% of patients under age 60 and fewer than 15% of patients over 60 maintaining durable remissions [39]. Despite standard consolidation therapy, patients with adverse-risk disease are at high risk of relapse, with median OS following relapse often less than 6 months. Given the poor outcomes associated with relapsed or refractory disease, multiple strategies have been explored to prolong remission duration following conventional induction chemotherapy or allo-HCT. While parenteral HMAs have been evaluated in this setting, their use has been limited by logistical barriers such as the need for frequent clinic visits and injections, toxicity, and disappointing clinical trials that failed to show a clear benefit in overall survival. In the single-arm CALGB 10,503 study, patients < 60 years old in first CR did not have any improvement in OS or event-free survival with 4–5 days of IV decitabine per 6-week cycle compared to historical controls [40]. Similarly, a phase III trial led by the HOVON group of subcutaneous azacitidine maintenance, patients aged > 60 years in first CR after induction chemotherapy displayed modest improvements in DFS in the therapy arm (12 month DFS 64% vs 42%), but no difference in OS and significant toxicity [41].

At present, there are two approved options for oral HMAs: oral azacitidine (CC-486) and oral decitabine/cedazuridine combination therapy (ASTX727) [42]. In a recent review examining the pharmacokinetics and pharmacodynamics of oral HMAs [43], investigators found AUC equivalence of oral DEC-C and IV decitabine. The phase III ASCERTAIN trial in higher-risk MDS/CMML confirmed pharmacokinetic bioequivalence of DEC-C and IV decitabine and a safety profile consistent with IV decitabine, leading to the regulatory approval of DEC-C for MDS/CMML [44]. Oral DEC-C later demonstrated similar clinical activity to DEC-IV in patients with AML ineligible for intensive induction chemotherapy, leading to its subsequent registration for this indication [45]. Oral azacitidine, on the other hand, displays a different profile in absorption, metabolism, and systemic exposure and is not bioequivalent to IV or subcutaneous azacitidine. Other investigational oral HMAs, such as ASTX030 (oral azacitidine with cedazuridine) are also under development (NCT04256317). These oral HMAs represent a significant advancement due to their improved convenience and potential for prolonged drug exposure to sustain epigenetic reprogramming [46]. In the pivotal phase III Quazar AML-001 trial, oral azacitidine significantly prolonged both relapse-free survival (RFS) and OS as maintenance therapy in AML [47]. In this study, oral azacitidine in a 14 day regimen per 28-day cycle significantly extended mOS (24.7 vs 14.8 months; $p < 0.001$) and RFS (10.2 vs 4.8 months; $p < 0.001$) compared to placebo. Subgroup analysis suggests that older patients, those in CR, those who did not receive consolidation therapy, and those with MRD positivity may benefit more from therapy [48]. Importantly, the safety profile of oral azacitidine was manageable, with GI symptoms (CC-486: 41%, placebo 24%) and thrombocytopenia (CC-486: 22%, placebo: 21%) being the most common adverse events. Notably, there was a lack of eligibility requirements regarding the range of induction and consolidation requirements prior to the trial, with at least 20% of the patients included not receiving any consolidation. In addition, the trial did not define a fixed duration of maintenance therapy and some patients with morphological progression on trial had a dose increment of the drug. Nonetheless, in the following post hoc analyses of the trial, treatment with oral azacitidine was found to improve OS regardless of post-induction MRD status and increased both the conversion to MRD negativity (37% vs 19%) and duration of MRD negativity (11 mo vs 5 mo) [48]. Oral azacitidine was further found to prolong RFS and OS for patients in first remission after IC independent of the number of induction and consolidation courses received before beginning maintenance [49]. Efforts to build upon these results are ongoing, with particular interest in combining oral azacitidine with other targeted therapies. Of note is the phase III VIALE-M trial (NCT04102020) evaluating the combination of oral azacitidine and venetoclax as maintenance therapy enrolling patients with newly diagnosed AML in first CR/CRi following intensive induction and consolidation. A Phase 1b multi-arm trial (NCT05010772) is also investigating ASTX727 in patients with AML in first remission (CR/CRi) previously ineligible for allogeneic transplant, administered either as monotherapy or in combination with venetoclax, gilteritinib, enasidenib, or ivosidenib [50].

Although CC-486 is currently FDA-approved only for maintenance therapy in transplant-ineligible patients with AML in first CR, early trials in the post allo-HCT setting have been explored with promising results. In a phase I/II dose-escalation trial of CC-486 after alloSCT in 31 patients with AML or MDS [51], patients received either 200 or 300 mg for 7 days in a 28 day cycle, or 150 mg or 200 mg for 14 days in a 28 day cycle. With a median follow up of 19 months, treatment was associated with a relatively low (21%) rate of relapse, median OS was not reached in any dosing cohort and 1-year survival rates were above 80% in each cohort. RPFS rates were also higher in the combined 14-day dosing cohort compared to the 7-day cohort (72% vs 54%), supporting an extended dosing regimen in the transplant setting as well. The ongoing phase III AMADEUS trial (NCT04173533) is now underway, evaluating the efficacy of a 14-day regimen of maintenance oral azacitidine in a 28-day cycle in patients with AML or high-risk MDS post-HCT compared to placebo.

In contrast to AML, where CR is typically the prerequisite for maintenance, patients with MDS more often experience disease stabilization rather than deep remissions. The role of maintenance therapy in lower-risk disease is less well defined as treatment goals prioritize cytopenia improvement over deep remission. Nevertheless, oral azacitidine has demonstrated efficacy in the treatment of MDS. In the phase 1/2 study AZA-MDS-003 [52], patients with IPSS low or intermediate-risk MDS with transfusion-dependent anemia and thrombocytopenia were randomized to receive either oral azacitidine (300 mg for 14 or 21 days per 28 day cycle) or placebo. Oral azacitidine was found to significantly improve 56-day RBC-TI rates (31% vs 11%, $p = 0.0002$) and platelet hematologic improvement (24.3% vs 6.5%, $p = 0.0003$). At a median follow up of 13.3 months, oral azacitidine was additionally found to decrease the rate of progression to AML (7.5% vs 16.7%, $p = 0.04$) compared to placebo, though the trial was underpowered to identify if oral-azacitidine prolonged RBC-TI duration. Unfortunately, the incidence of early death due to infection was higher in the oral-azacitidine arm, primarily among patients with pretreatment neutropenia in the oral-azacitidine arm. The phase 3

ASTREON trial (NCT05469737) is currently underway, investigating oral azacitidine (200 or 300 mg for 14 days per 28 day cycle) as treatment in lower risk MDS [53].

In higher-risk MDS where patients may achieve disease control with HMAs, the concept of maintenance therapy is more analogous to that of AML. In addition to the aforementioned AMADEUS trial, a phase II study (NCT04806906) is evaluating 14 days of 300 mg oral azacitidine as maintenance therapy in patients with higher risk MDS who achieved disease control (CR/CRi, PR, or SD with HI) after > 6 cycles of subcutaneous-azacitidine. In an interim analysis of 11 enrolled patients at median follow up of 167 days, 4 patients had progression to AML after a median of 9 cycles, 2 discontinued therapy due to patient preference following a grade 3 GI event ($n = 1$) and reduced compliance ($n = 1$), 4 patients continued on therapy in CR ($n = 3$) or PR ($n = 1$), and the status of one patient was not reported. These early findings suggest that oral azacitidine may be a feasible maintenance approach in HR-MDS and further follow-up will be critical to assess its long-term efficacy and tolerability. In the post-transplant setting, a phase 1b trial (NCT04980404) is investigating ASTX727 as post-transplant maintenance therapy in patients with MDS or CMML.

Overall, oral HMAs have demonstrated a clear survival benefit in post-CR transplant-ineligible AML and safety and efficacy for the treatment of higher risk MDS, though ongoing studies will determine its role in post-transplant. Despite this progress, several questions remain regarding optimal treatment duration, combination partners, and patient selection. Moving forward, tailoring maintenance strategies based on genetic risk will be essential to improving long-term outcomes and personalizing epigenetic maintenance therapies.

4. Combinatorial approaches to overcome HMA resistance

Although various potential pathways toward HMA-resistance have been explored, none seems to definitively explain why only approximately half of patients with MDS respond to therapy and why most with MDS or AML who do respond will relapse. Notably, relapses in MDS patients responding to DEC appear to occur despite ongoing hypomethylation by long interspersed nuclear element-1 (LINE-1) analysis [54]. Thus, pathways that are not specific to the demethylating function of the HMAs have been (Table 1) or are being explored (Table 2) as targets for combinatorial approaches to optimize the efficacy of HMAs.

HMAs are nucleoside analogs of cytidine that incorporate into RNA (azacitidine) and DNA (decitabine) in an S-phase-dependent manner and result in depletion of DNA-methyltransferase 1 (DNMT1). Primary resistance, defined as lack of any response to treatment in the first 4–6 cycles of therapy, may thus be related to quiescence of hematopoietic stem/progenitor cells (HSPCs). Gene expression studies demonstrate upregulation of ITGA5 transcription, which maintains HSPCs in a quiescent state, in CD34+ bone marrow cells of AZA non-responders [11]. Co-culture of HSPCs with AZA and an inhibitor of ITGA5 resulted in restoration of differentiation. Cell cycle stimulation has been tested in conjunction with HMAs without significant improvement in overall response to therapy compared to historical trials. In a phase I trial of older patients with AML undergoing standard therapy with decitabine, the CXCR4 antagonist plerixafor was found to enhance mobilization of leukemia stem cells resulting in a modest response rate of 14% in previously treated patients and 46% in untreated patients [55]. Twenty-eight patients with MDS were treated in a phase I trial combining plerixafor with granulocyte-colony stimulating factor (G-CSF) and azacitidine, resulting in an overall response rate (ORR) of 53%, with better responses among patients demonstrating stem cell mobilization and no responses in those who did not receive G-CSF [56].

Common mechanisms of chemotherapy resistance include alterations in membrane-associated drug transporters responsible for inflow and efflux of drug. Altered levels of these transporters do not, however, appear to definitively explain HMA resistance in humans [57]. Cytidine deaminase (CDA) is the enzyme which deaminates AZA and DEC, promotes rapid clearance of the drugs and is responsible for the very short half-life of HMAs. Elevated levels appear to correlate with lower overall response rates in men [58]. However, efforts to consistently correlate CDA levels with resistance across studies have been inconclusive. Current strategies to block CDA, such as with cedurazidine which has been used in conjunction with an oral form of decitabine, have resulted in equivalent serum concentrations and demethylation activity as compared to parenteral forms but not in superior outcomes [59]. Moreover, guadecitabine, a dinucleotide of decitabine and deoxyguanosine that resists deamination and has a longer half-life than DEC has not demonstrated superior outcomes compared to AZA or DEC in large randomized controlled trials in MDS and AML [60,61].

Dysregulation of apoptosis proteins contributes to malignant transformation and chemotherapy resistance in a variety of hematologic neoplasms. Preclinical studies using AML cell lines made resistant by chronic HMA exposure demonstrate increased expression of B-cell lymphoma 2(BCL-2) family proteins as well as Nuclear Factor kappa-light-chain-enhancer of activated B cells (NFkB), an inhibitor of apoptosis [62,63]. These data provide a scientific justification for what has been the most effective combinatorial approach to date, the addition of inhibitors of the anti-apoptotic proteins to HMA in myeloid malignancies.

Lastly, myeloblasts and leukemia-initiating stem cells appear to have several mechanisms by which they evade the immune system including upregulation of T-cell ligands that induce exhaustion, downregulation of MHC molecules and increased expression of CD47 which blocks macrophage clearance [64]. Unfortunately, trials utilizing CD47 blockade in combination with HMA therapy have not produced clinical benefit. The addition of immune checkpoint inhibitors (ICI) to HMA has had mixed results in clinical trials, but recent identification of potential predictors of response provides justification for further investigation [65].

4.1. HMA's in combination with agents targeting apoptosis

By far the most promising improvement upon HMA monotherapy in patients with AML unfit for chemotherapy over the last decade has been the addition of the oral BCL-2 inhibitor venetoclax (VEN) to AZA or DEC. Based on the VIALE-A trial, AZA in combination with VEN was effective across disease subtypes (de novo and secondary

Table 1. Selected completed epigenetic-based combination trials in myeloid malignancies.

Epigenetic agent	Combination Partner	Trial Phase	Participants	Inclusion criteria	Outcomes	Trial #/Name
Azacitidine (DNMTi)	Entinostat (HDACi)	II	149	MDS, CMML, AML	HN: 32% (5-AZA alone) versus 27% (combination); median OS: 18 months (5-AZA alone) versus 13 months (combination)	NCT00313586 (E1905)
Azacitidine (DNMTi)	Pracinostat (HDACi)	III	406	ND unfit AML	Median OS: 9.95 months (combination and AZA groups). OR: 38.4% (combination) vs 35.5% (AZA)	NCT03151408 (PRIMULA)
Azacitidine (DNMTi)	Ivosidenib (IDH1i)	III	146	ND unfit IDH1mut AML	Median EFS: 22.9 months (combination) vs 4.1 months (AZA). Median OS: 24 months (combination) vs 7.9 months (AZA). CR: 47% (combination) vs 15% (AZA)	NCT03173248 (AGILE)
Azacitidine (DNMTi)	Enasidenib (IDH2i)	I/II	101	ND IDH2mut AML	ORR: 74% (combination) vs 36% (AZA).	NCT02677922 (AG221-AML-005)
Azacitidine (DNMTi)	Venetoclax (BCL2i)	III	431	ND unfit AML	Median OS: 14.7 months (combination) vs 9.6 months (AZA). CR/CRi: 66.4% (combination) vs 28.3% (AZA)	NCT02993523 (VIALE-A)
Azacitidine (DNMTi)	Venetoclax (BCL2i) Magrolimab (anti-CD47)	III	378	ND unfit AML	Median OS: 10.7 months (magrolimab) vs 14.1 months (control). CR: 41.3% (magrolimab) vs 46.0% (control)	NCT05079230 (ENHANCE-3)
Azacitidine (DNMTi)	PLX51107 (BETi)	I	37	HR RR-MDS, RR-AML	ORR: 22%, median OS: 5.7 months	NCT04022785
Azacitidine (DNMTi)	Sabatolimab (anti-TIM-3)	III	530	Higher risk-MDS or CMML-2	Median OS: 22.31 months (combination) vs 18.83 months (AZA). CR+PR+HI: 42.6% (combination) vs 34.7% (AZA)	NCT04266301 (STIMULUS-MDS2)
Azacitidine (DNMTi)	Gilteritinib (FLT3i)	III	123	Unfit FLT3mut AML	Median OS: 9.8 months (combination) vs 8.9 months (AZA). CRc: 4.5 months (combination) vs 0.03 months (AZA)	NCT02752035
Azacitidine (DNMTi)	Tamibarotene (RARA agonist)	III	246	HR-MDS	CR: 23.8% (combination) vs 18.8% (AZA)	NCT04797780 (SELECT-MDS-1)
Azacitidine (DNMTi)	IO-202 (Anti-LILRB4)	I	21	CMML	CR: 57.1%, ORR: 78.6%	NCT04372433
Azacitidine (DNMTi)	Plerixafor G-CSF	I	28	MDS	ORR: 53%; Median OS: 452 days	NCT01065129
ASTX727 (DNMTi)	Venetoclax (BCL2i)	II	62	ND unfit AML, RR-AML	1) Untreated patients: ORR: 64%; Median OS: 11.5 months. 2) Pretreated patients: ORR: 46%; Median OS: 7.2 months	NCT04746235
Decitabine (DNMTi)	Valproic Acid ATRA	I/II	53	RR-AML, HR-MDS	ORR: 42%; Median response duration: 26 weeks	NCT00326170
Tranylcypromine (LSD1i)	ATRA LDAC	I	25	Unfit RR-AML, HR-MDS	ORR: 48%; Median OS: 2 months	NCT02717884 (TRANSATRA)
Panobinostat (HDACi)	Idarubicin Cytarabine	I/II	38	ND AML	CR: 64%, Median OS: 17 months	NCT00840346
ASTX727 (DNMTi)	Telaglenastat (Glut-i)	I/II	16	HR-MDS	mCR/HI: 62.5%, Median OS: 10.2 months	NCT03047993
Guadecitabine (DNMTi)	Atezolizumab (anti-PD-1)	I/II	33	RR MDS	ORR: 33%. Median OS: 15.1 months	NCT02397720
Azacitidine (DNMTi)	Ruxolitinib (JAKi)	II	46	Int-High risk MF	ORR: 72%; Median OS: 46 months	NCT01787487

Abbreviations: 5-AZA, 5-azacytidine; AML, acute myeloid leukemia; ATRA, all-trans retinoic acid; CMML, chronic myelomonocytic leukemia; CR, complete remission; EFS, event-free survival; Glut-i: Glutaminase-inhibitor; HI, hematologic improvement; HN, hematologic normalization; HR-MDS, high-risk myelodysplastic syndrome; JAKi, Januse kinase inhibitor; LDAC, low-dose cytarabine; mCR, morphologic complete remission; MDS, myelodysplastic syndrome; mut, mutated; NCT, ClinicalTrials.gov identifier; ND, newly diagnosed; OR, objective response; ORR, overall response rate; OS, overall survival; RARA, retinoic acid receptor alpha gene; RR, relapsed/refractory.

Table 2. Selected ongoing epigenetic-based combination trials in myeloid malignancies.

Epigenetic Agent	Combination Partner	Trial Phase	Disease Context	Status	Trial #/Name
Azacitidine (DNMTi)	Sonrotoclax (BCL2i)	I/II	RR AML	Preliminary response: Median OS: 11.8 months. CR/CRh: 47%.	NCT04771130
Azacitidine (DNMTi)	Lisaftoclax (BCL2i)	III	ND unfit AML	Ongoing	NCT06389292
Azacitidine (DNMTi)	Venetoclax (BCL2i) + Revumenib (Menin-i)	III	NPM1mut/KMT2A rearrangement ND AML	Ongoing	NCT06652438
Azacitidine (DNMTi)	Ruxolitinib (JAKi)	II	MDS/MPN	Ongoing	NCT05817955
ASTX727 (DNMTi)	Venetoclax (BCL2i) + Ivosidenib (IDH1i) or Enasidenib (IDH2i)	I/II	RR AML, ND unfit AML	Ongoing	NCT04774393
Azacitidine (DNMTi)	Enasidenib (IDH2i)	II	ND IDH2mut MDS	Preliminary response: ORR: 74%, Median OS: 26 months	NCT03383575
Azacitidine (DNMTi)	Olutasidenib (IDH1i)	II	HR MDS/CMML, AP-MPN	Ongoing	NCT06597734
Azacitidine (DNMTi)	Alrizomadlin (MDM2i)	I/II	RR AML, CMML, HR-MDS	Ongoing	NCT04358393
Azacitidine (DNMTi)	Luspatercept	III	HR-MDS	Ongoing	NCT06927232
Azacitidine (DNMTi)	timdarpacept (CD47 blockade)	III	ND CMML	Ongoing	NCT06647862
Azacitidine (DNMTi)	Nivolumab (Anti-PD1) + Relatlimab (Anti-LAG3)	II	ND AML, RR AML	Ongoing	NCT04913922 (AARON)
ASTX727 (DNMTi)	Cirtuvivint (CLKi+DYRKi)	I	RR AML, RR MDS	Ongoing	NCT06484062
ASTX727 (DNMTi)	SNDX-5613 (Menin-i) + Venetoclax (BCL2i)	I/II	ND AML, RR AML, MPAL	Preliminary response: ORR: 88%, MRD-negativity among responders: 74%, 6-month OS: 74%	NCT05360160 (SAVE)
ASTX727 (DNMTi)	Venetoclax (BCL2i) + Lintuzumab-Ac225 (CD33 Antibody)	I/II	ND AML	Ongoing	NCT06802523
ASTX727 (DNMTi)	Iadademstat (LSD1i)	II	AP/BP-MPN	Ongoing	NCT06661915
Azacitidine (DNMTi)	Venetoclax (BCL2i) + Iadademstat (LSD1i)	I	ND AML	Ongoing	NCT06514261
ASTX727 (DNMTi)	Venetoclax (BCL2i) + Gilteritinib (FLT3i)	I/II	ND/RR FLT3mut AML	Ongoing	NCT05010122

Abbreviations: AML, acute myeloid leukemia; AP/BP, accelerated phase or blast phase; BETi, bromodomain and extraterminal domain inhibitor; BCL2i, BCL-2 inhibitor; CLKi, CDC-like kinase inhibitor; CMML, chronic myelomonocytic leukemia; CR, complete remission; CRh, complete remission with partial hematologic recovery; DNMTi, DNA methyltransferase inhibitor; DYRKi, dual-specificity tyrosine phosphorylation-regulated kinase inhibitor; ET, essential thrombocythemia; FLT3i, FMS-like tyrosine kinase 3 inhibitor; HR-MDS, high-risk myelodysplastic syndrome; IDH1i, isocitrate dehydrogenase 1 inhibitor; IDH2i, isocitrate dehydrogenase 2 inhibitor; JAKi, Janus kinase inhibitor; LAG3, lymphocyte activation gene 3; LSD1i, lysine-specific demethylase 1 inhibitor; MDM2i, mouse double minute 2 homolog inhibitor; MDS, myelodysplastic syndrome; Menin-i, menin inhibitor; MF, myelofibrosis; MPAL, mixed phenotype acute leukemia; MPN, myeloproliferative neoplasm; MRD, measurable residual disease; mut, mutated; ND, newly diagnosed; ORR, overall response rate; OS, overall survival; PD-1, programmed cell death protein 1; R/R, relapsed/refractory.

AML) as well as in patients with high-risk cytogenetics and molecular mutations [15]. Patients with mutations in isocitrate dehydrogenases 1 and 2 (*IDH1* and *IDH2*) had a survival benefit when treated with the combination as opposed to AZA monotherapy, whereas those with p53 mutations did not. This is now considered standard of care as first line therapy for elderly patients with AML and for most patients with secondary AML, though patients with post-MPN AML were not included in the study. Toxicity, particularly myelosuppression, has been the biggest obstacle to continuous administration and tolerance. In response to this, several groups have tested regimens with shorter exposure to VEN. In a retrospective comparison of newly diagnosed AML patients treated with standard AZA + VEN for 7 days against patients treated with the standard dose HMA + continuous VEN, response rates and OS were similar with reduced platelet transfusions and 8-week mortality (6% vs 16%, $p = 0.03$) in the shorter exposure group [66].

A recently reported phase 1b clinical trial enrolled patients with previously untreated high-risk MDS to receive AZA + VEN, administered at a reduced duration of 14 days [67]. The overall response rate of 80% and median OS of 26 months were promising, and many practitioners began incorporating VEN in the treatment of MDS. However, the phase 3 VERONA trial found no survival benefit to the regimen when compared to monotherapy with AZA [68]. Similarly, SELECT-MDS-1 (NCT04797780) combined AZA with tamibarotene, a selective RARA agonist that promotes differentiation and apoptosis, failed to demonstrate a survival benefit in patients with newly diagnosed HR-MDS.

An ongoing phase 1 study (NCT04771130) is evaluating a more selective and potent BCL-2 inhibitor, sonrotoclax, in combination with AZA in relapsed and refractory AML. Another second generation BCL-2 inhibitor, lisaftoclax, appears promising in combination with AZA in untreated and relapsed/refractory MDS and AML, including previously VEN-exposed patients. An ongoing phase 3 clinical trial is underway in untreated HR-MDS (NCT06641414) as well as in AML patients unfit for chemotherapy (NCT06389292).

4.2. HMAs in combination with agents targeting leukemic cell metabolism

Mutations in *IDH1* and *IDH2* occur in 5–15% of MDS/AML and result in the overproduction of 2-hydroxyglutarate, which promotes leukemic progression by disrupting histone demethylases and DNA hydroxylases. Inhibitors of these mutants thus have an epigenetic as well as metabolic effect on malignant

cells. Ivosidonib is an IDH1 inhibitor that is FDA-approved in combination with AZA for newly diagnosed, older patients with AML and as monotherapy for relapsed/refractory MDS and AML. Enasidenib is an IDH2 inhibitor approved as monotherapy in previously treated AML.

Malignant cells rely on glutamine for energy; the glutaminase inhibitor telaglenastat in combination with AZA demonstrated safety and efficacy in a phase 1b/2 trial for advanced MDS [69]. It is not clear whether this drug will undergo further clinical development in myeloid malignancies.

4.3. HMAs in combination with agents targeting immune cells

Despite promising efficacy in early phase trials, immunotherapeutics have failed to deliver in MDS. The ENHANCE trial (NCT04313881), evaluating AZA plus magrolimab – a macrophage checkpoint inhibitor targeting CD47—was terminated after an interim analysis showed no survival benefit. Similarly, STIMULUS-MDS2 (NCT04266301), which combined azacitidine with sabatolimab, a TIM-3 immune checkpoint inhibitor, also yielded negative results.

Several early phase clinical trials have been conducted combining currently approved immune checkpoint inhibitors (ICI) with HMAs. Though some show promise in terms of efficacy, particularly for HMA-resistant MDS patients, reported toxicities vary widely [70–72]. Thus, predictive biomarkers are needed to identify the patient population that is most likely to benefit. In a phase I/II trial combining guadecitabine with atezolizumab, a PD-L1 inhibitor, the co-occurrence of *ASXL1* mutations in T lymphocytes correlated with a significant survival benefit in relapsed and refractory MDS patients [73]. ASXL1 mutations were also associated with improved outcomes in patients with AML treated with nivolumab, a PD-1 inhibitor, and AZA, though the T cells were not investigated [72]. In a murine model, the disruption of ASXL1 conferred stem-like properties on T lymphocytes as well as enhanced sensitivity to ICI, with a proliferative burst that was effective in suppressing cancer and infection [74].

4.4. HMAs in combination with JAK-STAT pathway inhibition

Combinatorial approaches to the treatment of MPNs and MDS/MPN overlap syndromes, particularly with inhibitors of the JAK-STAT pathway and/or VEN, may be a reasonable strategy to enhance the efficacy of HMAs in these disorders. The addition of JAK/STAT inhibition to HMA for the treatment of MF has yielded more encouraging results than HMA monotherapy. In the long term follow up of a phase II trial (NCT01787487) of ruxolitinib and AZA of primarily intermediate-high risk MF, median OS was 46 months with median EFS of 33 months and median duration of response of 43 months [75] In a phase II trial of 25 patients with MPN-AP/BP treated with the combination of ruxolitinib and DEC, ORR rates were 44% with a median OS of 9.5 months [76]. In a recent meta-analysis investigating HMA-based therapies in MPN-AP/BP, ORR rates ranged from 30–47% (HMA alone: 30%, HMA+VEN: 47%, HMA + ruxolitinib: 45%), though no significant difference in survival was found between the regimens [77]. However, a small pilot study of triple therapy with AZA, VEN, and ruxolitinib in MPN-BP reported an impressive 80% ORR and 40% CRR, with manageable toxicity and encouraging early survival data with mOS of 13.4 months at median follow-up of 10 months [78]. These promising results suggest that the combination of HMAs with JAK and/or BCL2 inhibition may synergistically target distinct biologic pathways and have led to ongoing phase II trials (NCT01787487, NCT04282187) further evaluating these combinations.

For other MDS/MPN overlap syndromes, data is limited, though recent studies on combinatorial approaches show promise. In a phase II study of AZA in combination with ruxolitinib in patients with MDS/MPN (MDS/MPN-U: 14, CMML: 17, aCML: 4), ORR rates were 57% with 64% of patients with baseline splenomegaly achieving spleen response [79]. At a median follow-up of 5 years, the median OS was 31.8 months overall and 36.4 months among responders, with a median time to AML transformation of 13.2 months in 23% of patients (NCT01787487) [80]. Several ongoing clinical trials are evaluating novel combinations that may improve outcomes in these disorders, including HMAs with VEN (NCT05600894) and JAK inhibitors (NCT04061421, NCT05817955).

5. Conclusions

Efforts to improve upon the clinical efficacy of HMAs in the treatment of myeloid malignancies have been challenging. Therapeutic strategies to overcome HMA resistance are outlined in Table 3. While the addition of VEN and the IDH mutation inhibitors to AZA have demonstrated benefit in AML, several other agents have performed poorly in Phase II trials despite early signs of efficacy, highlighting the heterogeneity of myeloid malignancies. HR-MDS, particularly after HMA failure remains an unmet, for which translational research is critical to developing HMA-based strategies that target disease-specific biomarkers.

6. Future perspectives

Given the heterogeneity, at the molecular and phenotypic level, of myeloid malignancies, optimization of epigenetic therapies must be tailored to patients based on cell-intrinsic and extrinsic biomarkers. The recent ability to apply advanced epigenomic profiling to clinical samples will help map tumor epigenetic states in response to therapy and enable a precision medicine approach to maximize the clinical application of epigenetic drugs alone or in combination with other anticancer treatments.

While the early success of epigenetic agents has been in MDS and subsets of AML, HMA-based combination strategies may benefit less well-studied disease groups such as MDS/MPN overlap syndromes and AP/BP-MPNs. In parallel, evidence supports the role of maintenance oral epigenetic drugs in sustaining epigenetic suppression and maintaining remission. Close collaboration between clinicians and translational researchers will be essential to better understand mechanisms of response to epigenetic therapies, and to develop and validate these biomarkers to guide trial design.

Table 3. Combinatorial approaches to treatment of myeloid malignancies based on HMA resistance mechanism [81].

DISEASE	ANTI-APOPTOSIS	METABOLIC DYSREGULATION	IMMUNE RESISTANCE	JAK-STAT UPREGULATION
MDS	BCL-2 INHIBITION (Venetoclax) with azacitidine	IDH1 inhibitor (Ivosidonib) with azacitidine	TIM-3 inhibitor (sabatolimab) with azacitidine	
			PD-1 or PDL-1 inhibition with HMA	
		Glutaminase inhibitor (telagnastat) with azacitidine	CD47 inhibitor (magrolimab) with azacitidine	
MF, MDS/MPN OVERLAP or BP-MPN	BCL-2 INHIBITION (Venetoclax) with azacitidine	IDH1 inhibitor (ivosidenib) with azacitidine and ruxolitinib[81]		JAK inhibitor (ruxolitinib) with HMA
				JAK inhibitor (ruxolitinib) with HMA and venetoclax
		IDH2 inhibitor (enasidenib) + azacitidine + ruxolitinib[81]		
AML	BCL-2 INHIBITION (Venetoclax) with azacitidine	IDH1 inhibitor (Ivosidonib) with azacitidine IDH2 inhibitor (enasidenib)		
	BCL-2 Inhibitors (sonrotoclax, lisaftoclax) with azacitidine			

Abbreviations: HMA, hypomethylating agent; BCL-2, b-cell lymphoma 2; JAK-STAT, Janus kinase-signal transducer and activator of transcription; IDH1/2, isocitrate dehydrogenases 1 and 2. RED BOXES: phase 3 clinical trial(s) failed to show a benefit. YELLOW BOXES: clinical trials in progress and/or published reports of possible benefit. PALE YELLOW BOXES: promising published reports with small sample size. GREEN BOXES: phase 3 clinical trial(s) demonstrating safety and efficacy and/or FDA-approved.

Author contributions

Casey O'Connell: Conception, study design, execution, and interpretation. Justin Cheng: Conception, study design, execution, and interpretation. All authors reviewed and approved the final manuscript.

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