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Immunomodulatory Effects of Hypomethylating Agents in Myelodysplastic Syndromes: Mechanisms and Therapeutic Implications

Yi Liu [1, †], Jiayi Zhao [2, †], Ting Wei [1], Guanlun Gao [1], Qingshan Li [1, *]

[1] Department of Hematology, Guangzhou Red Cross Hospital, Guangzhou Red Cross Hospital of Jinan University, Guangzhou, Guangdong, 510220, China
[2] Department of Hematology, The First People's Hospital of Zhaoqing, Zhaoqing, Guangdong, 526000, China

[*] **Corresponding author**: Qingshan Li (Email: drliqingshang@126.com)

[†] These authors contributed equally to this work.

Abstract: Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic disorders characterized by ineffective hematopoiesis, peripheral cytopenias, and an increased risk of progression to acute myeloid leukemia. Increasing evidence indicates that immune dysregulation and chronic inflammatory signaling play critical roles in the pathogenesis and progression of MDS. Hypomethylating agents (HMAs), particularly azacitidine and decitabine, remain the backbone of treatment for patients with higher-risk disease. Beyond their canonical epigenetic effects, HMAs exert broad immunomodulatory activities that influence both innate and adaptive immunity. These agents can activate endogenous retroviral elements, induce viral mimicry, and enhance antigen presentation, thereby improving immune recognition of malignant cells. In addition, HMAs modulate the function of multiple immune cell populations, including natural killer cells, T lymphocytes, dendritic cells, macrophages, and myeloid-derived suppressor cells, ultimately reshaping the bone marrow immune microenvironment. However, clinical responses to HMAs remain heterogeneous, and treatment resistance frequently develops. A better understanding of the immunological basis of HMA activity may facilitate the development of more effective therapeutic strategies, particularly rational combination approaches with immunotherapy. This review summarizes current knowledge of immune dysregulation in MDS and discusses the immunomodulatory mechanisms and therapeutic implications of HMAs.

Keywords: Myelodysplastic Syndromes; Immune Modulation; Hypomethylating Agents.

1. Introduction

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of clonal myeloid neoplasms characterized by ineffective hematopoiesis, peripheral cytopenias, and a variable risk of transformation to acute myeloid leukemia (AML). With the development of refined prognostic models, including the Molecular International Prognostic Scoring System (IPSS-M), patients with MDS can be stratified more precisely according to clinical, cytogenetic, and molecular risk features, which in turn facilitates treatment selection [1, 2]. In general, therapeutic goals differ by risk category: management of lower-risk MDS focuses on improving hematopoiesis, reducing transfusion dependence, and preserving quality of life, whereas treatment of higher-risk disease aims to delay leukemic transformation and prolong survival [3].

Current treatment options for MDS include allogeneic hematopoietic stem cell transplantation (HSCT), hypomethylating agents (HMAs), immunomodulatory agents, immunosuppressive therapy, chemotherapy, and supportive care. Among these approaches, HMAs—particularly azacitidine and decitabine—have become a cornerstone of therapy for patients with higher-risk MDS who are not candidates for allogeneic transplantation [4, 5].

This review highlights the importance of immune dysregulation in MDS pathogenesis and provides a comprehensive description of the immunomodulatory role of demethylating agents in MDS along with its possible mechanisms. Additionally, the review also discusses the efficacy of HMAs in combination with various immune checkpoint inhibitors, aiming to contribute to the development of more effective and rational MDS treatment strategies.

2. Mechanism of Hypomethylating Agents

Hypomethylating agents (HMAs) currently used in clinical practice mainly include decitabine (DAC), azacitidine (AZA), and the fixed-dose oral combination of decitabine with cedazuridine (oral DAC/CED). These agents do not act solely through epigenetic reprogramming; rather, their antitumor activity involves multiple interconnected processes, including inhibition of DNA methyltransferases, reactivation of epigenetically silenced genes, induction of DNA damage at higher exposure levels, and regulation of antitumor immune responses.

As cytosine analogs, HMAs are incorporated into newly synthesized DNA during S phase. After incorporation, they form irreversible complexes with DNMT1, leading to depletion of this maintenance methyltransferase and progressive loss of CpG methylation. This hypomethylating effect may restore transcription of genes that are aberrantly silenced in malignant cells, including tumor suppressor genes involved in cell-cycle control and apoptosis [6].In MDS, mutations affecting epigenetic regulators such as DNMT3A and TET2 contribute to disordered methylation landscapes [7, 8]. By partially correcting these abnormalities, HMAs may reactivate genes such as p15INK4B, p16INK4a, TP53, and DAPK1, thereby suppressing malignant cell survival [9]. In

addition, decitabine has been reported to promote FOXO1 activation in MDS-L cells, which may further facilitate apoptosis [10].

The biological effects of HMAs are dose related. At relatively low concentrations, their activity is dominated by epigenetic modulation, whereas at higher concentrations they may directly trigger DNA damage responses, resulting in inhibition of proliferation, cell-cycle arrest, and apoptosis. Importantly, growing evidence indicates that the therapeutic effect of HMAs cannot be explained only by direct cytotoxicity. These agents also alter tumor immunogenicity, for example by increasing the expression of tumor-associated antigens and improving immune recognition of malignant cells. It has also been reported that HMAs may partially reverse the dysfunctional state of exhausted T cells and restore their proliferative capacity, providing a rationale for combining HMAs with immunotherapeutic approaches [11].

From a clinical perspective, HMAs have become a major treatment option for patients with higher-risk MDS, especially for those who are not eligible for allogeneic hematopoietic stem cell transplantation. Although these drugs usually do not eradicate the malignant clone, they can reduce transfusion requirements, delay progression to AML, and prolong survival in a proportion of patients. However, treatment responses remain heterogeneous, and resistance frequently emerges during the disease course.

In recent years, increasing attention has been paid to the role of immune dysregulation in MDS pathogenesis. Accumulating studies suggest that clonal hematopoiesis in MDS develops within a bone marrow microenvironment characterized by chronic inflammatory activation, abnormal innate immune signaling, and disturbed interactions between malignant cells and immune cells [12]. The immune landscape differs across disease stages: lower-risk MDS is more often associated with inflammatory injury and excessive apoptosis of hematopoietic progenitors, whereas higher-risk MDS is more frequently characterized by clonal dominance, immune evasion, and an immunosuppressive marrow milieu. In addition, approximately 20%–30% of patients with MDS reportedly present with concomitant autoimmune manifestations, further underscoring the close relationship between MDS and immune imbalance [13].

Taken together, HMAs should be viewed not only as epigenetic drugs but also as agents capable of reshaping the immune microenvironment. On this basis, the following sections focus on immune abnormalities in MDS and summarize how HMAs may modify these abnormalities, with the aim of providing a broader framework for understanding their therapeutic value.

3. Immune Dysregulation and Immunomodulatory Effects of HMAs in MDS

3.1. Innate Immune Dysregulation in MDS

Aberrant innate immune activation is now recognized as an important feature of MDS biology. Under physiological conditions, innate immune cells sense pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) through pattern recognition receptors (PRRs), thereby initiating inflammatory signaling and regulating hematopoietic homeostasis [14]. Among these receptors, Toll-like receptors (TLRs) are particularly relevant in MDS because they connect inflammatory stimulation to abnormal hematopoietic output [15].

Persistent activation of TLR-related pathways has been documented in the bone marrow of patients with MDS. In $CD34^+$ hematopoietic progenitor cells from these patients, multiple components of innate immune signaling are upregulated compared with normal controls [16]. Sustained inflammatory signaling may interfere with differentiation and maturation of hematopoietic cells, thereby contributing to ineffective hematopoiesis, one of the hallmarks of the disease.

Genetic abnormalities in MDS may further amplify these inflammatory circuits. More than half of patients have been reported to harbor mutations or dysregulated expression of genes linked to innate immune regulation [17]. A representative example is del(5q) MDS, in which loss of miR-146a relieves repression of TRAF6, an essential mediator downstream of TLR signaling. Experimental studies have shown that enhanced TRAF6 activity in hematopoietic stem and progenitor cells impairs normal hematopoiesis, supporting a mechanistic link between innate immune dysregulation and disease development [18].

In addition to pathway-level abnormalities, several innate immune cell populations are functionally altered in MDS. Defects involving natural killer (NK) cells, myeloid-derived suppressor cells (MDSCs), and macrophages may collectively weaken immune surveillance, promote immune escape, and support clonal evolution. HMAs may partly counteract these changes by epigenetically reprogramming immune cells and modifying the marrow microenvironment.

3.1.1. Natural Killer Cells

NK cells are major effector cells of the innate immune system and play a critical role in early antitumor defense. They eliminate malignant cells through cytolytic granule release, death receptor-mediated killing, and secretion of cytokines such as interferon-γ and tumor necrosis factor-α. In MDS, however, this surveillance function is often impaired. Studies have shown that NK cells from patients with MDS exhibit reduced cytotoxicity, abnormal receptor profiles, and defective cytokine production, and these abnormalities tend to be more evident in higher-risk disease, where they are associated with poorer clinical outcome [19].

HMAs may influence NK-cell activity through epigenetic regulation. Expression of killer cell immunoglobulin-like receptors (KIRs), which are important for recognition and response to target cells, is partly controlled by promoter methylation. By altering methylation status, HMAs may modify the pattern of KIR expression and thereby affect NK-cell reactivity. For example, azacitidine has been shown to increase the proportion of KIR-positive NK cells both in vitro and in patients with MDS, suggesting that demethylation may enhance the capacity of NK cells to recognize malignant targets [20].

The effect of HMAs on NK cells is not uniformly stimulatory and appears to depend on dose and context. Low-dose decitabine has been reported to alter the expression of both activating and inhibitory NK-cell receptors, whereas excessive drug exposure may impair NK-cell proliferation and survival by suppressing transcriptional activity [21]. These findings suggest that moderate HMA exposure may support immune activation, while overly intensive dosing could have the opposite effect.

Genetic background may further influence this interaction. TET2 mutations, which are common in myeloid malignancies, have been associated with reduced NK-cell cytotoxicity, likely through disruption of normal DNA demethylation

processes [22]. Some studies indicate that HMA treatment may partially restore NK-cell function in this setting. Overall, currently available evidence supports the view that HMAs can, under appropriate conditions, enhance NK-cell-mediated immune surveillance in MDS.

3.1.2. Myeloid-derived Suppressor Cells

MDSCs are a heterogeneous group of immature myeloid cells with potent immunosuppressive activity. Their accumulation has been described in multiple malignancies and is increasingly recognized as an important contributor to immune escape in MDS. Compared with healthy individuals, patients with MDS, particularly those with higher-risk disease, often show expansion of MDSCs in the bone marrow microenvironment. These cells commonly display increased expression of signaling molecules associated with suppressive function, including STAT3 and CCR2 [23].

MDSCs inhibit antitumor immunity through several complementary mechanisms. They suppress NK-cell function via direct cell-cell contact and through secretion of inhibitory mediators such as TGF-β and reactive oxygen species. These factors reduce the expression of activating receptors including NKG2D, NKp46, and NKp44, thereby weakening NK-cell activation and cytotoxicity. MDSCs can also decrease perforin production and blunt responsiveness to IL-2, ultimately limiting the proliferative and effector capacity of NK cells. Coculture studies further support these observations by showing reduced receptor expression, decreased cytokine release, and increased apoptosis in NK cells exposed to MDSCs [24].

HMAs may mitigate this immunosuppressive network. Decitabine has been reported to reduce the abundance of MDSCs and to enhance antitumor immune responses in patients with MDS [25]. Similar findings have been described in experimental tumor models, in which decitabine restrained MDSC expansion and improved the efficacy of T-cell-based immunotherapy [26]. These data suggest that part of the clinical benefit of HMAs may derive from attenuation of MDSC-mediated suppression rather than from direct action on malignant cells alone.

Given their central role in shaping an immunosuppressive microenvironment, MDSCs represent a plausible therapeutic target in MDS. Therefore, modulation of MDSC number and function may be an important component of the immunoregulatory activity of HMAs [27].

3.1.3. Macrophages

Macrophages are highly plastic cells within the bone marrow microenvironment and exert context-dependent effects on tumor progression. In MDS and AML, tumor-associated macrophages are an important stromal and immune component of the marrow niche. According to their functional characteristics, macrophages are often simplified into two major polarization states: the classically activated M1 phenotype, which is generally associated with pro-inflammatory and antitumor functions, and the alternatively activated M2 phenotype, which tends to support immune suppression, tissue remodeling, and tumor progression.

Available evidence suggests that macrophages in MDS are skewed toward an M2-like phenotype. Such cells typically produce higher levels of immunosuppressive cytokines, including IL-10 and TGF-β, while showing reduced expression of cytokines associated with effective antitumor immunity. This polarization pattern favors establishment of an immune-tolerant microenvironment and may facilitate malignant clone persistence.

Macrophage-mediated tumor control is also regulated by the balance between pro-phagocytic and anti-phagocytic signals. Malignant cells can escape phagocytosis by expressing “don’t eat me” molecules such as CD47, whereas molecules such as calreticulin serve as “eat me” signals that promote engulfment. Increased CD47 expression has been described in high-risk MDS, which may help diseased cells evade macrophage clearance.

HMAs may affect macrophage biology at more than one level. Experimental studies suggest that decitabine can influence macrophage differentiation and polarization [28]. Azacitidine has also been reported to enhance the expression of calreticulin, thereby potentially increasing macrophage-mediated phagocytosis of malignant cells. These observations support the idea that HMAs may contribute to remodeling of the marrow immune milieu by shifting macrophage function toward a state less permissive for tumor persistence.

3.2. Adaptive Immune Dysregulation in MDS

In addition to innate immune abnormalities, adaptive immune responses are profoundly altered in MDS. Dysregulation of T lymphocytes, B lymphocytes, and immune checkpoint pathways contributes to immune escape and disease progression.

3.2.1. T lymphocytes

T lymphocytes are central components of adaptive immunity and play a major role in antitumor immune control. In patients with MDS, however, the composition and function of T-cell subsets are frequently disturbed. Compared with healthy individuals, MDS patients often show a shift in helper T-cell balance, typically manifested by increased proportions of Th2 cells and regulatory T cells (Tregs), together with reduced Th1- and Th17-related responses. In some studies, Th22 cells were also found to be elevated, particularly in patients with more advanced disease stages.

Among these alterations, expansion of Tregs is of particular interest because it is closely related to immune suppression. Increased numbers of Tregs have been detected in both bone marrow and peripheral blood in MDS, and this change has been associated with disease progression as well as poorer prognosis. Functionally, Tregs inhibit antitumor immunity mainly through secretion of suppressive cytokines such as IL-10 and TGF-β, which restrain effector T-cell activity and may favor survival and expansion of malignant clones. For this reason, Treg abundance has been proposed as a potential indicator of disease progression and clinical outcome in MDS [29].

3.2.2. B Lymphocytes

Abnormal B-cell development is another manifestation of immune dysregulation in MDS. One commonly reported finding is a marked reduction, and in some cases near absence, of B-cell progenitors within the bone marrow microenvironment [30]. This suggests that lymphoid differentiation is impaired alongside the better-recognized myeloid abnormalities of the disease.

In addition to reduced progenitor numbers, bone marrow B cells in MDS exhibit increased apoptosis when compared with those from healthy controls and from patients with acute myeloid leukemia [31]. Clinical observations further indicate that the proportion of $CD34^+$ B-cell precursors may have prognostic relevance. For example, patients with a relatively preserved proportion of these precursors have been reported to show longer overall survival than those with lower levels. Since MDS predominantly affects older adults, age-related

decline in hematopoietic and immune function may also contribute to defective B-cell development, although this is unlikely to be the only explanation [32].

3.2.3. Immune Checkpoint Molecules

Immune checkpoint pathways are essential for maintaining peripheral tolerance under physiological conditions, but in malignant disease they may be co-opted to suppress effective antitumor immunity. In MDS, several inhibitory checkpoint molecules, including PD-1, PD-L1, PD-L2, and CTLA-4, have been reported to be upregulated [30].

Increased expression of these molecules can attenuate T-cell activation and facilitate immune escape of malignant clones. This has led to growing interest in checkpoint blockade as a therapeutic strategy in MDS, particularly in the setting of combination treatment. Although clinical outcomes have not been uniformly satisfactory, the abnormal activation of checkpoint pathways supports their continued investigation as part of immune-based therapeutic approaches in this disease.

3.3. Immunomodulatory Effects of Hypomethylating Agents in MDS

HMAs are a core component of current MDS treatment and exert effects that extend beyond reversal of aberrant DNA methylation. In addition to reactivating silenced genes and inducing differentiation or apoptosis of malignant cells, these agents can alter the immune context of the bone marrow microenvironment and thereby influence antitumor responses. Their activity therefore reflects a combination of direct epigenetic effects on tumor cells and indirect immunoregulatory effects on multiple immune cell populations.

Evidence from experimental studies indicates that HMAs may enhance antitumor immunity at several levels. For example, azacitidine has been reported to suppress proliferation of regulatory T cells and to alter cytokine secretion patterns, suggesting that it can partially relieve immune suppression in MDS. In parallel, HMA treatment has been associated with increased infiltration of $CD8^+$ cytotoxic T cells and enhancement of T-cell-mediated antitumor responses [33]. Low-dose decitabine has also been shown to promote activation and proliferation of $CD4^+$ T cells through the NF-κB pathway [34]. Mechanistically, this effect has been linked to upregulation of β-TrCP, subsequent degradation of IκBα, enhanced NF-κB transcriptional activity, and increased IFN-γ production, ultimately strengthening T-cell effector function.

HMAs may also influence the balance among helper T-cell subsets. In vitro findings suggest that azacitidine can favor Th1-related responses while reducing Th17 cells. However, in vivo observations are not entirely consistent, with some studies showing mild increases in Treg populations together with decreases in Th17 cells. These discrepancies indicate that the immunological effects of HMAs are context dependent and may involve broader regulation of the Th17–Treg axis rather than a simple unidirectional shift [35].

Another important aspect of HMA-mediated immune regulation is enhancement of tumor immunogenicity. HMAs can induce expression of cancer-testis antigens, such as MAGE-A1, MAGE-A3, and Sp17, which are normally silent in most somatic tissues but may become expressed in malignant cells after demethylation. Upregulation of these antigens may increase recognition of tumor cells by cytotoxic T lymphocytes and thereby reinforce immune-mediated tumor clearance [36]. Clinical studies have further shown that azacitidine can augment T-cell responses against tumor cells expressing these antigens in patients with MDS [37].

At the same time, HMAs may activate compensatory inhibitory pathways. Increased expression of PD-1, PD-L1, and CTLA-4 has been observed during treatment, raising the possibility that checkpoint upregulation may contribute to adaptive resistance. This provides an important biological rationale for combining HMAs with immune checkpoint inhibitors. Taken together, available evidence suggests that HMAs exert broad immunomodulatory effects involving both adaptive and innate immune compartments. By modifying immune-cell function, altering cytokine networks, and increasing tumor immunogenicity, these agents may help restore immune surveillance in MDS.

4. Combination of Hypomethylating Agents and Immunotherapy

4.1. Immune Checkpoint Inhibitors in Combination with HMAs

Immune checkpoints comprise receptor–ligand systems that fine-tune immune activation and prevent excessive immune injury. In the setting of malignancy, however, these pathways are often co-opted by tumor cells to dampen antitumor responses and facilitate immune escape [38]. Inhibitors targeting checkpoint pathways have therefore become an important component of modern cancer immunotherapy. By interrupting inhibitory signaling, these agents can restore T-cell effector function and strengthen immune-mediated killing of tumor cells [39, 40].

Although checkpoint blockade has changed the treatment landscape of several solid tumors, its benefit in myeloid malignancies, including MDS, has been less consistent. Even so, increasing evidence indicates that immune evasion contributes to disease development and progression in MDS. Malignant clones may suppress antitumor immunity through enhanced expression of inhibitory checkpoint molecules, thereby weakening T-cell responses and promoting disease persistence. Among currently recognized checkpoint pathways, CTLA-4 and PD-1 were the first to be translated successfully into anticancer therapeutic targets and remain the most extensively studied in this field [41].

HMAs, especially azacitidine and decitabine, continue to serve as first-line treatment for many patients with higher-risk MDS. In addition to their effects on DNA methylation, these agents can modify immune recognition of tumor cells by increasing antigen expression, improving antigen presentation, and activating immune-related transcriptional programs. At the same time, HMAs may also increase expression of inhibitory molecules such as PD-1, PD-L1, and CTLA-4, a change that may partly underlie adaptive resistance during treatment [30].

This dual effect supports the concept of combining HMAs with checkpoint inhibitors. On one hand, HMAs may render malignant cells more visible to the immune system and facilitate T-cell priming. On the other hand, checkpoint blockade may release the inhibitory brakes that limit effective T-cell activity. For this reason, combination therapy has been proposed as a potentially more effective way to amplify antitumor immunity in MDS.

4.1.1. Interaction between HMAs and PD-1/PD-L1 Inhibitors

PD-1 (CD279) is an inhibitory receptor mainly expressed on activated T cells and belongs to the CD28/B7 superfamily. Engagement of PD-1 by PD-L1 or PD-L2 attenuates T-cell receptor signaling, reduces cytokine secretion, and promotes functional exhaustion of T cells. As a result, effector T-cell responses are weakened and tumor-directed immunity is diminished[41].

In myeloid malignancies such as MDS and AML, the PD-1/PD-L1 axis has been implicated as an important mechanism of immune escape. PD-L1 can be expressed by malignant cells as well as hematopoietic progenitor cells, thereby limiting T-cell activation and supporting immune suppression. Its expression is often relatively low at initial diagnosis but may increase as disease advances, suggesting that checkpoint activation is linked to both disease evolution and progressive impairment of antitumor immunity [42].

HMAs can further influence this pathway through epigenetic remodeling. Demethylation of regulatory regions within checkpoint-related genes may increase transcription of PD-1, PD-L1, PD-L2, and CTLA-4. Yang et al. observed significant upregulation of these molecules in patients with MDS during HMA treatment, implying that activation of checkpoint pathways may participate in acquired resistance to these agents. In addition, elevated PD-L1 expression on hematopoietic stem and progenitor cells has been reported in MDS, particularly in cases carrying TP53 mutations and showing increased risk of leukemic transformation [43]. These findings strengthen the biological rationale for therapeutic interruption of PD-1/PD-L1 signaling in this disease.

Clinical evaluation of HMA plus PD-1/PD-L1 blockade has yielded variable results. In a randomized phase II/III trial (NCT03092674), azacitidine combined with nivolumab was tested in relapsed AML and previously untreated MDS. The regimen showed activity comparable to azacitidine alone, but early mortality related to treatment-associated adverse events was higher in the combination group [44]. A separate phase II study (NCT01953692) assessing azacitidine with pembrolizumab in MDS reported acceptable tolerability and a manageable safety profile, supporting continued exploration of this approach[45].

Results have likewise been inconsistent in studies of PD-L1 blockade. In a randomized phase II trial (NCT02775903), azacitidine plus durvalumab proved feasible but did not produce a clear improvement over azacitidine monotherapy [46]. Similarly, a trial investigating atezolizumab in combination with azacitidine (NCT02508870) did not demonstrate a favorable balance between efficacy and toxicity in MDS [47].

Taken together, current clinical evidence does not yet confirm a stable survival advantage for HMA plus PD-1/PD-L1 inhibition, despite encouraging mechanistic data. Future work should focus on patient selection, biomarker development, and refinement of treatment timing and dosing.

4.1.2. Interaction between HMAs and CTLA-4 Inhibitors

CTLA-4 (CD152) is another major inhibitory receptor involved in regulation of T-cell responses. It is expressed on activated $CD4^+$ and $CD8^+$ T cells and shares ligands, namely CD80 and CD86, with the costimulatory receptor CD28 [48]. Unlike CD28, which supports T-cell activation, CTLA-4 transmits suppressive signals during the priming phase of the immune response. Because CTLA-4 binds CD80/CD86 with higher affinity than CD28, it competitively limits the costimulatory signaling required for full T-cell activation [49]. Through this mechanism, CTLA-4 contributes to peripheral tolerance but may also be exploited by malignant cells to evade immune elimination [50].

There is also evidence that HMAs may affect CTLA-4 expression through epigenetic regulation. Previous work has suggested that decitabine can increase CTLA-4 expression in leukemia cells and immune cells, raising the possibility that HMA exposure may create a biological context in which CTLA-4 blockade becomes more relevant. This provides a theoretical basis for combining HMAs with anti-CTLA-4 therapy.

Ipilimumab, a monoclonal antibody directed against CTLA-4, has shown antitumor activity in several solid malignancies and has also been explored in hematologic cancers. Early clinical studies of decitabine plus ipilimumab in relapsed or refractory MDS and AML have suggested an acceptable safety profile together with preliminary evidence of clinical activity [51]. These findings indicate that CTLA-4 inhibition may represent a feasible adjunct to HMA therapy in selected patients.

Beyond direct disease control, such regimens may also have value as bridging treatment for patients proceeding to allogeneic hematopoietic stem cell transplantation. Nevertheless, immune-related adverse events remain an important limitation of CTLA-4 blockade. Accordingly, future studies should aim to define the patients most likely to benefit, optimize combination schedules, and reduce treatment-related immune toxicity.

5. Role of Hypomethylating Agents in Allogeneic Hematopoietic Stem Cell Transplantation for MDS

The use of HMAs, particularly azacitidine and decitabine, has been extensively explored in the setting of allogeneic hematopoietic stem cell transplantation (allo-HSCT) for MDS. Before transplantation, these agents are often administered as pretransplant therapy to control disease burden and to reduce the likelihood of relapse after transplantation. After allo-HSCT, HMAs may be given alone or together with donor lymphocyte infusion (DLI) as post-transplant intervention to delay or prevent recurrence. This strategy may strengthen the graft-versus-leukemia effect while causing only a limited increase in graft-versus-host complications. Although hematologic toxicity can occur, post-transplant HMA treatment is generally considered tolerable and has not been associated with a marked rise in treatment-related mortality. Some studies have also suggested benefits in survival and post-transplant quality of life.

A multicenter prospective phase II study evaluated the feasibility of azacitidine administration before transplantation in 70 patients with MDS, 19 with AML, and 8 with chronic myelomonocytic leukemia. Most participants were ultimately able to proceed to transplantation after azacitidine treatment. Because unrelated donors are often the major donor source in clinical practice, azacitidine may serve as a useful interim therapy during the interval between diagnosis and donor identification. For patients with high-risk MDS, this approach may represent a more suitable pretransplant option than intensive chemotherapy [52].

Following transplantation, low-dose azacitidine has also been investigated as maintenance therapy. Available data

suggest that this approach may lower relapse risk in AML and MDS and may also prolong event-free survival and overall survival [53].

A recently reported retrospective analysis further indicated that post-transplant azacitidine maintenance, with or without DLI, was associated with a lower incidence of relapse in patients with AML or MDS [54]. Even among patients with particularly unfavorable prognostic features, this strategy appeared to reduce recurrence. In addition, overall survival was improved relative to historical controls, and the regimen was generally well tolerated without a clear increase in GVHD. These findings support continued evaluation of azacitidine-based maintenance, especially in combination with DLI, as a post-transplant strategy.

At present, allo-HSCT remains the only treatment with curative potential for patients with higher-risk MDS and can substantially prolong survival [55, 56]. However, graft-versus-host disease continues to be one of the main factors limiting transplant benefit. The pathogenesis of GVHD is complex and involves multiple inflammatory mediators and immune cell subsets, among which donor-derived T cells act as the principal effector population.

o reduce this complication, investigators have attempted to incorporate HMAs into GVHD prevention strategies. In a murine GVHD model, Wang et al. found that azacitidine combined with the histone H3K27 methyltransferase inhibitor 3-deazaneplanocin A (DZNep) more effectively suppressed Th1/Tc1 responses, increased the Th2/Tc2 ratio, and promoted regulatory T-cell differentiation than either agent used alone [57]. These findings suggest that the combined regimen may attenuate GVHD by reshaping donor T-cell differentiation and modifying cytokine- and chemokine-related immune signaling. In parallel, earlier studies have shown that azacitidine may facilitate FOXP3 demethylation and promote expansion or stabilization of Tregs, which provides another possible explanation for its protective effect against GVHD. A phase I/II trial evaluating azacitidine before matched unrelated donor allo-HCT also supported a role for this agent in lowering GVHD risk[58].

Decitabine-based strategies have likewise shown potential in this setting. A retrospective study suggested that incorporation of low-dose decitabine into a modified conditioning regimen could improve post-transplant outcomes in AML and MDS, in part by reducing GVHD severity [59]. In addition, another clinical study found that lenalidomide combined with azacitidine after allogeneic transplantation displayed antileukemic activity and might also help decrease GVHD in patients with AML or MDS [60]. Taken together, these observations indicate that HMAs may contribute to transplantation not only through disease control, but also through immune regulation after graft infusion.

6. Conclusion and Future Perspectives

The pathobiology of MDS involves not only genetic and epigenetic abnormalities, but also profound disturbances of the immune microenvironment. In this context, HMAs should not be viewed solely as demethylating drugs, because their therapeutic effects also involve regulation of immune-cell function and tumor–immune interactions. Nevertheless, the immune consequences of HMA exposure are not entirely favorable. Along with enhancing tumor immunogenicity, these agents may also activate inhibitory checkpoint pathways, which could contribute to limited or heterogeneous treatment benefit. This may help explain why combinations of HMAs with immunotherapy remain biologically attractive but have not yet produced consistently satisfactory clinical outcomes.

Further work is required to elucidate the mechanisms that determine sensitivity or resistance to HMA-based treatment, improve biomarker-guided patient stratification, and optimize rational combination regimens. Better integration of epigenetic and immunologic insights may provide a stronger basis for improving therapy in MDS.

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