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Review

Current landscape of translational and clinical research in myelodysplastic syndromes/neoplasms (MDS): Proceedings from the 1st International Workshop on MDS (iwMDS) Of the International Consortium for MDS (icMDS)

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ABSTRACT

Biological events that contribute to the pathogenesis of myelodysplastic syndromes/neoplasms (MDS) are becoming increasingly characterized and are being translated into rationally designed therapeutic strategies. Herein, we provide updates from the first International Workshop on MDS (iwMDS) of the International Consortium for MDS (icMDS) detailing recent advances in understanding the genetic landscape of MDS, including germline predisposition, epigenetic and immune dysregulation, the complexities of clonal hematopoiesis progression to MDS, as well as novel animal models of the disease. Connected to this progress is the development of novel therapies targeting specific molecular alterations, the innate immune system, and immune checkpoint inhibitors. While some of these agents have entered clinical trials (e.g., splicing modulators, IRAK1/4 inhibitors, anti-CD47 and anti-TIM3 antibodies, and cellular therapies), none have been approved for MDS. Additional preclinical and clinical work is needed to develop a truly individualized approach to the care of MDS patients.

1. Introduction

Myelodysplastic syndromes/neoplasms (MDS) are clonal myeloid neoplasms that are characterized by dysplastic changes in one or more hematopoietic lineages in the peripheral blood and/or bone marrow leading to cytopenias and a variable risk of progression to acute myeloid

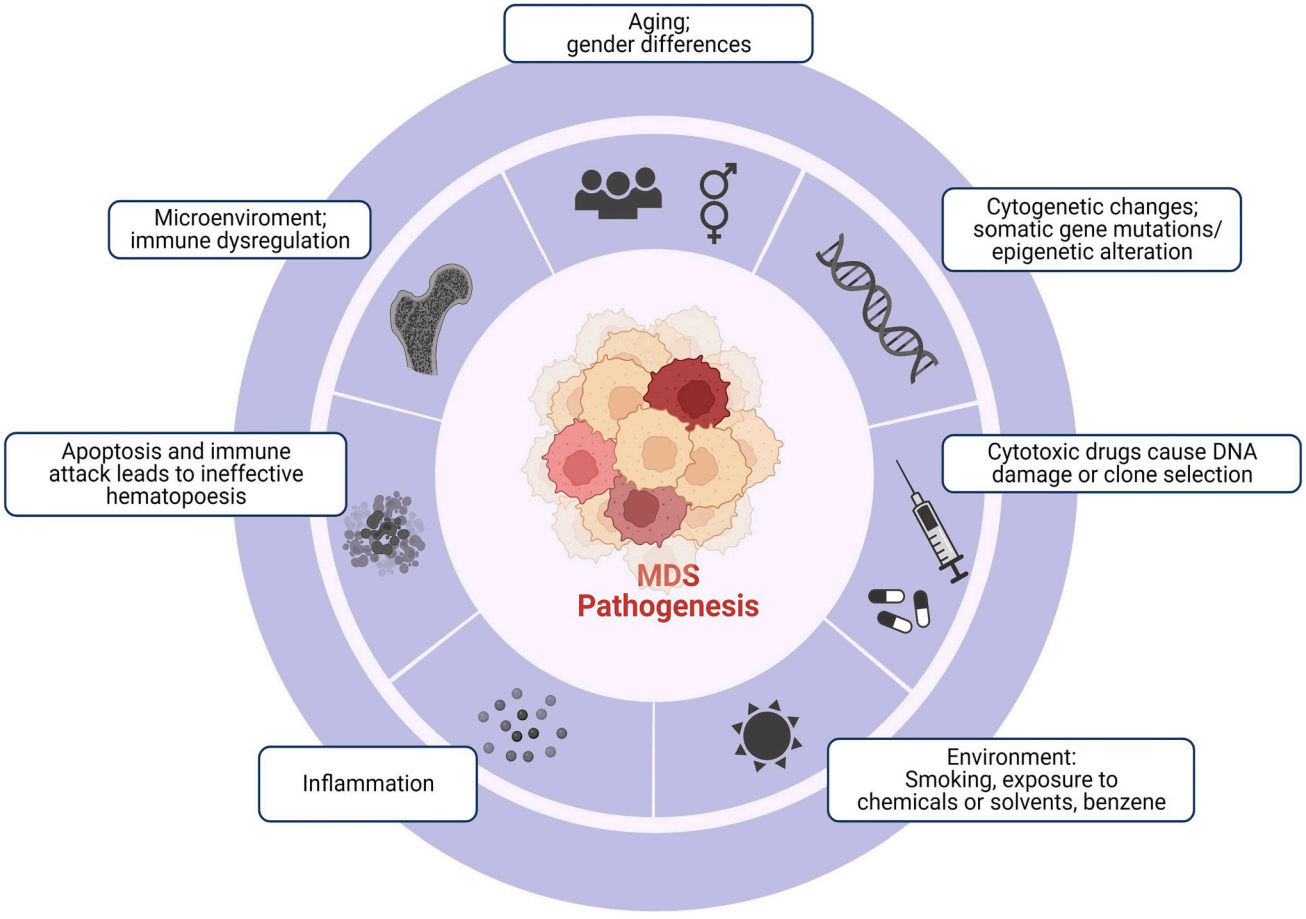

Fig. 1. Overview of MDS pathophysiology: MDS are clonal neoplasms that arise from mutations in hematopoietic stem cells. The pathogenesis of MDS include somatic mutations or cytogenetic changes, which occur in most patients; aging, exposure to cytotoxic chemotherapy and/or ionizing radiation or environmental toxins (e.g., tobacco, benzene) are associated with MDS development. Immune dysregulation, inflammation, and apoptosis are also associated with MDS development.

leukemia (AML) [1,2]. Over the last three decades several factors contributing to the pathogenesis of MDS including epigenetic and immune dysregulation, as well as recurrent genetic alterations have been increasingly characterized [3–7]. These advances are now being increasingly incorporated into MDS classification (e.g., *SF3B1*-mutant MDS, MDS/AML with *TP53* mutation) and risk stratification tools, such as the Molecular International Prognostic Scoring System (IPSS-M) [2,5,8–10]. In the future, it is hoped that novel therapeutic modalities will also become available to target specific aspects of MDS biology as part of an enhanced personalized care strategy for patients with this disorder. However, except for the approval of luspatercept, these breakthroughs in the understanding of MDS pathogenesis have so far only had modest consequences on MDS treatment. Fig. 1 provides an overview of the various pathophysiologic processes contributing to MDS.

In this review, we provide recent updates on the basic and translational science as well as novel investigational aspects of MDS biology and therapy, as discussed by members of the International Consortium of MDS (icMDS) during the inaugural international workshop on MDS (iwMDS) held in Miami, FL, USA in June 2022. This conference included a panel of international experts with expertise in preclinical and clinical MDS research encompassing basic/translational scientists, medical oncologists/hematologists, pathologists, and representatives from regulatory agencies. For a review on the current research priorities and clinical management of MDS, we would like to refer the reader to our companion manuscript as well as other recent publications [11,12].

2. Preclinical models of MDS

In contrast to other malignancies, human MDS cell lines are rare and patient-derived xenograft models are inherently limited by immunodeficiency of the host. Genetically modified mouse models are only applicable to a specific MDS subtype and limited by their failure to adequately capture the clonal complexity inherent to human disease [13].

As discussed in greater detail below, MDS pathophysiology is characterized by immune dysregulation [14]. Modelling physiologic human hematopoiesis in mice requires the expression of diverse human cytokines. These insights led to the development of multi-cytokine knock-in mouse models such as MISTRG permissive for the engraftment of patient-derived MDS hematopoietic stem and progenitor cells [15]. Recent validation studies have shown that the MISTRG mouse model is able to replicate the human MDS phenotype including progression to secondary AML [15]. An alternative approach to enhance engraftment is the co-transplantation of mesenchymal stromal cells leading to variable engraftment rates of 1–89%, which can potentially be increased by coadministration of humanized bone marrow matrix material [13,16–18]. Such mouse models can serve as a valuable tool to investigate novel combination therapies. Additional model optimization (e.g., increasing erythro- and thrombopoiesis by humanization of murine hepatocytes and novel engraftment protocols) is further expected to assist in the better understanding of the MDS immune milieu and the factors driving disease progression [19]. However, it is important to note that current xenograft models lack mature neutrophils and have no adaptive immune system due to the absence of B- and T-cells [13]. Additionally, peripheral blood cells in these models remain mostly of murine origin.

3. Genetic aspects of MDS pathogenesis and management

3.1. Moving towards a molecular definition of MDS

The enrichment of certain somatic mutations in MDS patients and their prognostic implications were initially described more than a decade ago, and our knowledge of the molecular landscape of MDS continues to expand with the wider use of more sensitive and broader sequencing techniques [6,7,20–22]. The strong association between genetic alterations and certain clinical and histopathologic characteristics has led to the introduction of several new molecularly defined MDS subtypes by both the 5th edition of the WHO classification of myeloid neoplasms and a similar publication by the International Consensus Classification (ICC) [1,2,23]. Table 1 provides an overview of key aspects of the 4th and 5th edition of the WHO and ICC classifications of MDS and related myeloid neoplasms. For example, MDS with mutated *SF3B1* has replaced the previous category of MDS with ring sideroblasts, which reduces the impact of interobserver variability on diagnosis, and also has therapeutic implications with the recent approval of luspatercept for the treatment of anemia in patients with transfusion-dependent MDS with ring sideroblasts, which is highly associated with the presence of *SF3B1* mutations [1,8,24,25].

It has also been shown that the specific characteristics of certain mutations have distinct prognostic implications. Bernard et al. have demonstrated that the adverse prognostic impact of *TP53* mutations in MDS patients is predominately related to patients with biallelic *TP53* loss [10]. Other adverse cytogenetic features such as a complex karyotype may also supersede the prognostic effect of blast count across the spectrum of MDS and AML [10,26,27]. The prognostic implications of *TP53* mutations are also reflected in various molecularly-inspired risk stratification tools such as the IPSS-M [5]. Accordingly, MDS with biallelic *TP53* inactivation has been introduced as a new MDS sub-entity in the WHO and ICC classifications [1,2,5,9,28]. However, conflicting data remain regarding the prognostic implications of *TP53* allelic state in myeloid neoplasms creating a significant challenge for clinical management of such patients [10,27,29]. Potential causes include differences in patient, disease, and treatment characteristics across studies; although some of this data may be confounded by differences in classification of the monoallelic and biallelic mutation state. Overall, the vast majority of *TP53* mutant patients with excess blasts (i.e., $\geq$5%), complex karyotype, higher variant allele frequency (VAF), and/or biallelic mutation have poor prognosis, likely representing >80% of patients but correct identification of patients with a more indolent course (e.g., monoallelic *TP53* mutations) can be clinically important given the differences in prognosis [5,30]. Table 2 provides an overview of recent studies on the prognostic impact of *TP53* mutations and allelic status in MDS.

The emphasis on molecular testing in the workup of MDS patients also raises logistical questions related to timely and universal access to molecular and cytogenetic testing results for all patients with MDS, especially in resource-limited settings. If NGS testing results are not available in a timely manner, the strong association of a complex monosomal karyotype as well as 17p abnormalities can potentially serve as a surrogate for the biallelic *TP53* mutation [26,31]. It remains to be seen how these novel, molecular risk stratification tools will be incorporated into clinical decision-making including timing of referral for allogeneic hematopoietic cell transplantation (allo-HCT). It is also important to note that serial molecular testing (e.g., at the time of progression to AML or disease relapse) can be helpful for both research and clinical purposes as the emergence of potentially targetable mutations such as *FLT3, IDH1,* and *IDH2* has been reported in a subset of patients [32,33].

3.2. Germline predisposition

While the median age at diagnosis of MDS patients in the United States is 77 years [34], characterization of pathogenic germline variants in genes such as *DDX41, GATA2, RUNX1, SAMD9L, SAMD9,* and those involved in DNA damage repair and telomere biology has led to identification of hereditary forms of MDS in a subset of patients [35,36]. It is important to note that these germline variants have variable penetrance and the risk of developing MDS therefore varies depending on the affected gene and even on the specific variant within a gene. For example, patients with mutations in *GATA2, SAMD9L,* or *SAMD9* tend to

Table 1
Comparison of WHO 2016, WHO 2022, and ICC 2022 classification schemes of MDS subtypes.

WHO 2016 [203]	WHO 2022[1]	ICC 2022[2]
MDS with low-blast count (<5% bone marrow blasts)		
MDS with single lineage dysplasia (MDS-SLD)	MDS with low blasts (MDS-LB) <5% BM and < 2% PB	MDS, not otherwise specified with single lineage dysplasia (MDS, NOS-SLD)
MDS with multi-lineage dysplasia (MDS-MLD)		MDS, not otherwise specified with multi-lineage dysplasia (MDS, NOS-MLD)
MDS with ring sideroblasts: • With single lineage dysplasia (MDS-RS-SLD) • With multi-lineage dysplasia (MDS-RS-MLD)	MDS with low blasts and mutated *SF3B1* or MDS with ring sideroblasts (if ≥15% RS and *SF3B1* wild-type)	MDS with mutated *SF3B1*
MDS with isolated del(5q)	MDS with low blasts and isolated 5q deletion (MDS-5q)	MDS with del(5q)
MDS unclassifiable	Not included	Not included
Not included	Not included	MDS, not otherwise specified without dysplasia (e.g., monosomy 7/del(7q))
Not included	MDS, hypoplastic (MDS-h)	Not included
MDS with increased blast count (≥5% bone marrow blasts)		
MDS excess blasts-1 (MDS-EB1; 5–9% bone marrow blasts)	MDS with increased blasts-1 (MDS-IB1; 5–9% bone marrow and/or 2–4% peripheral blood blasts)	MDS excess blasts (5–9% bone marrow and/or 2–9% peripheral blood blasts)
MDS excess blasts-2 (MDS-EB2; 10–19% bone marrow or peripheral blood blasts or Auer rods)	MDS with increased blasts-2 (MDS-IB2; 10–19% bone marrow or 5–19% peripheral blood blasts or Auer rods)	MDS/AML (10–19% bone marrow or peripheral blood blasts)
Not included	MDS with fibrosis (MDS-f; 5–19% bone marrow or 2–19% peripheral blood)	Not included
Genetically defined entities		
AML-defining genetics[1]	AML-defining genetics independent of bone marrow and peripheral blood blast count	AML-defining genetics with ≥10% bone marrow and peripheral blood blasts
Not included	MDS with biallelic *TP53* inactivation (Two or more *TP53* mutations, or 1 mutation with evidence of *TP53* copy number loss or cnLOH)	MDS with mutated *TP53* (Multi-hit *TP53* mutation, or *TP53* mutation (VAF >10%) and loss of 17p) and MDS/AML with mutated *TP53* (Any somatic TP53 mutation (VAF > 10%)
Clonal hematopoiesis		
Not included	Clonal hematopoiesis (CHIP, CCUS)[2]	Pre-malignant clonal cytopenias and CCUS[2]

1: AML-defining genetic abnormalities: Acute promyelocytic leukemia (APL) with t(15;17)(q24.1;q21.2)*/PML::RARA*; APL with other *RARA* rearrangements; AML with t(8;21)(q22;q22.1)*/RUNX1::RUNX1T1*; AML with inv.(16)(p13.1q22) or t(16;16)(p13.1;q22)*/CBFB::MYH11*; AML with t(9;11)(p21.3;q23.3)*/MLLT3::KMT2A*; AML with other *KMT2A* rearrangements; AML with t(6;9)(p22.3;q34.1)*/DEK::NUP214*; AML with inv.(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)*/GATA2; MECOM(EVI1)*; AML with other *MECOM* rearrangements; AML with other rare recurring translocations; AML with mutated *NPM1*; AML with in-frame bZIP *CEBPA* mutations (ICC only); AML with *RBM15::MRTFA* fusion (WHO only)*;* AML with *NUP98*-rearrangement (WHO only).
2: cytopenias are defined as follows: hemoglobin <13 g/dL in males and < 12 g/dL in females for anemia, absolute neutrophil count <1.8 × 10^9/L for leukopenia, and platelets <150 × 10^9/L for thrombocytopenia.

develop MDS in childhood or as young adults. Conversely, MDS onset in patients with germline pathogenic *DDX41* variants is much later in life and occurs at an age similar to that of non-familial MDS [37,38]. Individuals with a pathogenic germline *CEBPA* variant in the N-terminus have a near 100% lifetime risk of AML, whereas risk is lower in those with C-terminus variants [39]. With up to 20% of MDS patients diagnosed before age 40 years and 6% of patients older than 60 years harboring pathogenic germline mutations, it is incumbent upon providers to test for these mutations as the appropriate identification of such patients can have important implications not only for the affected patients themselves but also for their families and with regards to donor selection for allo-HCT and genetic counseling [40]. This is highlighted by recent data identifying that in a cohort of 404 MDS patients of any age who underwent an allo-HCT from a related donor, pathogenic or likely pathogenic germline variants were present in 7% of patients across all ages [40].

The identification of such germline variants is also essential for the correct classification of the specific subtype of myeloid neoplasms since the WHO and ICC include a separate category of myeloid neoplasms associated with germline predisposition [1,2]. While germline genetic testing is universally recommended for patients with certain solid tumors (e.g., ovarian cancer, pancreatic cancer) [41], there is limited guidance as to which patients with MDS should undergo germline genetic testing. The National Comprehensive Cancer Network (NCCN) has proposed clinical (e.g., age < 50 years, clinically suspected genetic predisposition syndrome, family history, multiple cancers in the same patient, >2 first degree relatives, hypocellular MDS, monosomy 7) and molecular criteria (e.g., potential pathogenic germline variant found on somatic mutation panels) for germline genetic testing [11]. However, the value, sensitivity, and specificity of those recommendations to identify this subset of MDS patients are unknown. Additionally, the method of testing for germline mutations predisposing to MDS is also subject of debate, and whole exome sequencing has been suggested in specific cases [42]. Regardless, NCCN guidelines highlight the importance of appropriate post-test counseling and active surveillance for individuals with a variant of unknown significance and those with a pathogenic variant who are asymptomatic [11].

3.3. Updates on Clonal Hematopoiesis and MDS precursor states

With the wider use of increasingly sensitive next-generation sequencing techniques, including error corrected sequencing, it is increasingly common to detect somatic mutations in genes associated with myeloid malignancies in patients without a hematologic malignancy. Clonal hematopoiesis of indeterminate potential (CHIP) is defined as the presence of a somatic mutation in a gene associated with a hematologic malignancy that is originating in the hematopoietic stem and progenitor cells and is detected at a VAF of ≥2% using routine NGS from the peripheral blood [43]. The VAF cut off of 2% was chosen given that it was the lower limit of detection for most clinical sequencing panels and supported by longitudinal data showing that these clones were most likely to be clinically consequential or to progress. Multiple studies have demonstrated that the prevalence of CH increases with age with prevalence rates of 5–15% in otherwise healthy individuals in their 70–80's and > 20% in octogenarians. The presence of CH has been associated with a variety of cardiovascular, pulmonary, and aging-related diseases as well as an increased risk of hematologic malignancies and all-cause mortality [44–48].

If CH is detected in the context of persistent and unexplained cytopenias, it is termed as clonal cytopenia of undetermined significance (CCUS) [1,49–51]. Compared to individuals with unexplained cytopenais and no somatic mutations, CCUS confers a substantially higher risk of evolution into a manifest hematologic malignancy (hazard ratio [HR]: 13.9, $p < 0.001$) [43,52,53]. Acknowledging the implications of CH, both the WHO and ICC classifications for the first time include specific definitions of both CHIP and CCUS, which is an important step to

Table 2
Summary of selected studies on the prognostic impact of TP53 allelic status in MDS patients.

Author (ref.)	Patient population	Treatment characteristics	Definition of TP53 allelic status	Outcomes
Bernard et al. [10]	3324 MDS patients (378 with *TP53* mutation)	Peri-diagnostic or pre-treatment samples	Monoallelic: single *TP53* mutation Multi-hit: Multiple *TP53* mutations, *TP53* mutation + chromosomal deletion or cnLOH at the *TP53* locus	AML transformation and OS worse for multi-hit vs mono-hit; OS similar for mono-hit vs wild-type
Grob et al. [27]	230 *TP53*-mutant AML ($n = 186$ patients) and MDS-EB patients ($n = 44$) treated on HOVON-SAKK trials between 2001	Diagnostic and CR bone marrow samples All patients treated with induction chemotherapy	Biallelic *TP53* mutations defined as ≥2 *TP53* gene variants regardless of VAF; *TP53* gene variant + cytogenetic aberration involving chromosome 17p; or *TP53* mutation with VAF >55%	No OS difference between AML and MDS-EB patients with *TP53* mutation; no OS difference between mono- vs biallelic *TP53* mutation with both worse than *TP53* wild-type
Weinberg et al. [26]	299 AML and MDS patients with complex karyotype; 247 with *TP53* mutation	Initial diagnosis or pre-treatment samples	Multi-hit *TP53* mutations defined as ≥2 *TP53* gene variants; *TP53* gene variant +17p loss by karyotype; or *TP53* mutation with VAF >60%	No OS difference between AML and MDS patients; OS independent of bone marrow blast percentage; no OS difference between mono- vs biallelic *TP53* mutation with both worse than *TP53* wild-type for AML patients; for MDS patients OS worse with multi- vs mono-hit
Stengel et al. [204]	1520 MDS and AML patients (180 with *TP53* mutations)	Not provided	*TP53* single hit defined as mutation, deletions or cnLOH; *TP53* double hit defined as *TP53* mutation + deletion or cnLOH or ≥ 2 *TP53* mutations	*TP53* double-hit mutations more common in MDS with ≥5% blasts compared with MDS with <5% blasts; OS worse for double-hit vs single-hit *TP53* mutation
Montoro Gomez et al. [205]	140 MDS patients with del(5q); 14% ($n = 20$) with *TP53* mutation	Not reported	*TP53* monoallelic: only 1 mutation present; *TP53* multi-hit: multiple mutations or mutation + deletion or cnLOH	Median OS similar for *TP53* wild type, monoallelic and multi-hit *TP53* mutations
Bahaj et al. [30]	7400 patients with myeloid malignancies (579 with biallelic, 192 probably monoallelic and 239 probably biallelic *TP53* mutations)	Not reported	*TP53* biallelic: single *TP53* hit and VAF >50%, or ≥ 2 *TP53* mutations + combined VAF > 50%, or *TP53* mutation + del(17p) Probably biallelic: *TP53* mutation with VAF > 23% Probably monoallelic: *TP53* mutation with VAF <23%	OS comparable for probably monoallelic *TP53* mutations and *TP53* wild-type and worse for probably biallelic; OS similar for AML and MDS patients among biallelic *TP53* mutations
Zeidan and Bewersdorf et al. [29]	61 *TP53*-mutant AML ($n = 37$) and MDS ($n = 24$) and 144 *TP53* wild-type patients	Patients enrolled in randomized FUSION trial of azacitidine +/− durvalumab [67,206]	Multi-hit *TP53* mutations: defined per ICC as ≥2 distinct *TP53* mutations (VAF ≥ 10%) or a single *TP53* mutation + cytogenetic deletion involving 17p, *TP53* mutation with VAF >50%, or *TP53* mutation + complex karyotype. Alternative definition per Grob et al. [27]	OS superior for *TP53* wild-type vs *TP53*-mutant. No difference for *TP53* mono- vs multi-hit using either definition

AML – acute myeloid leukemia; *cnLOH* – copy number neutral loss-of-heterozygosity; *MDS* – myelodysplastic syndrome; *OS* – overall survival; *VAF* – variant allele fraction.

harmonize definitions for clinical trials as well as population- and disease registry-based research [1,2].

A major area of ongoing research has focused on factors driving the progression of CH to an overt neoplasm. A better understanding of these processes could enable risk stratification of individuals with CHIP and CCUS and risk mitigation efforts to reduce the risk of cardiovascular complications and potentially delay – or even prevent – the progression to a hematologic malignancy. Progression risk appears to be driven by both clone-intrinsic and -extrinsic factors. For example, clonal growth rates were substantially different across mutations ranging from 5% for mutations in *DNMT3A* to over 50% for *SRSF2* mutations and the rates of clonal expansion varied across a patient's lifespan in a recent longitudinal cohort study [54]. Additionally, the risk of a specific adverse outcome depends, among others, on the clonal complexity, defined by the clone size, co-occurring mutations, nature of mutations, and associated mosaic chromosomal abnormalities/somatic copy number alterations [52,54–56]. Multiple studies have also highlighted the impact of extrinsic factors such as cytotoxic chemotherapy and radiation on clonal evolution, clonal expansion, and CHIP prevalence [57–61]. For example, patients with CHIP had an increased risk of all-cause mortality, therapy-related myeloid neoplasms, and cardiovascular events compared to patients without CHIP in a study of 401 patients with non-Hodgkin lymphoma who underwent an autologous HCT [60]. The high mortality of therapy-related myeloid malignancies raises the question of whether patients with high-risk CH should have an individualized approach when being considered for additional chemo- or radiation therapy, and if modalities that are least likely to modulate clonal selection pressures (e.g. monoclonal antibodies, tyrosine kinase inhibitors) should be preferred. An example of mutation specific clonal expansion was seen in therapy-related myeloid neoplasms (t-MN) in patients treated with PARP inhibitors (rucaparib), where t-MN risk directly correlated with the presence of *TP53* mutations, while other mutations such as *DNMT3A and ASXL1,* did not increase the risk [62].

Due to the high rate of progression to an overt myeloid neoplasm in some patients with CCUS, preventive therapeutic interventions could be of great clinical relevance. Clinical trials using the IDH1 inhibitor ivosidenib (NCT05030441) in individuals with CCUS and an *IDH1* mutation, high dose of ascorbic acid in *TET2* mutant CCUS (NCT03418038) or the anti-interleukin 1-β monoclonal antibody canakinumab (NCT05641831) are ongoing [63]. Additional ongoing trials test enasidenib (NCT05102370), metformin (NCT04741945), and atorvastatin/rosuvastatin (NCT05483010) in individuals with CCUS. It remains to be seen if these interventions can change the natural history of the disease (i.e., eliminate the CH clone or prolong development of an overt myeloid neoplasm) and whether the presence of CH should influence other medical decisions, for example, with regards to the management of cardiovascular risk factors or omission of adjuvant cytotoxic chemotherapy.

4. Immunologic aspects

Immune dysregulation has been identified as a key aspect in the pathogenesis of MDS affecting both the innate and adaptive immune system [3,4]. For example, upregulation of inhibitory immune checkpoint receptors such as PD-1/PD-L1 has been identified in MDS patients with *TP53* mutations [64,65]. However, in contrast to various solid

tumors, the use of anti-PD1/PD-L1 or anti-CTLA-4 antibodies in patients with MDS and AML either as monotherapy or in combination with azacitidine (AZA) only had limited efficacy [66–68]. Understanding the causes (e.g., differences in the tumor microenvironment) underlying the limited efficacy of immune checkpoint inhibitors in AML and MDS is essential to improve outcomes [69]. Preclinical data suggest that adding the BCL2 inhibitor venetoclax to anti-PD1 therapy can increase the number of PD-1^+ T effector memory cells and led to a survival advantage in syngeneic murine tumor models compared to monotherapy with either agent [70]. Additionally, several novel checkpoint inhibitors have been developed and are being evaluated in clinical trials including anti-TIM3, anti-LAG3, and anti-CD47 [71,72]. Fig. 2 provides an overview of the mechanism of action of these novel immunotherapy targets.

4.1. Anti-TIM3

T cell immunoglobulin and mucin domain-containing protein 3 (TIM3) is a co-inhibitory receptor that was first identified on interferon-γ (IFN-γ) producing $CD4^+$ and $CD8^+$ T-cells and is up-regulated on exhausted T-cells in chronic viral infections and cancer [73]. Initially, the role of TIM3 in the anti-cancer immune response was uncertain due to the absence of a defined inhibitory signaling motif [73]. However, subsequent studies have shown that interaction of TIM3 with galactin-9, which results in the oligomerization of TIM3 on the cell surface and release of BAT3, ultimately leads to the inhibition and death of T-cells [74]. Emerging data also show a key role of TIM3 in the anti-tumor immunity exerted by dendritic cells and inflammasome activation [75]. As such, deficiency of TIM3 on dendritic cells enhanced maintenance of stem-like $CD8^+$ effector T-cells and antigen presentation to $CD8^+$ T-cells [76]. Additionally, it has been shown that TIM3 and galactin-9 are co-expressed by leukemic stem cells and this autocrine secretion of galactin-9 leads to self-renewal of leukemic stem cells [77,78]. This effect is further amplified by preventing IL-1 mediated pyroptosis of leukemic stem cells [78]. As the resistance to cytotoxic chemotherapy of leukemic stem cells is a main contributor to disease relapse, targeting TIM3 could have anti-leukemic properties.

The anti-TIM3 antibody sabatolimab has shown clinical efficacy in phase Ib clinical trials of patients with newly diagnosed AML and higher-risk (HR)-MDS [79]. Among the 51 patients with HR-MDS treated with sabatolimab + hypomethylating agents (HMA), the ORR was 56.9% (19.6% CR) with a median duration of response of 16.1 months and 25% of responding patients experiencing an immune-related adverse event [79]. The randomized, placebo-controlled, phase II STIMULUS-MDS1 trial (NCT03946670), in which sabatolimab was added to HMA for patients with HR-MDS, did not meet its primary endpoint (CR + progression-free survival [PFS]). However, there was a delayed separation of the PFS curves (11.1 months with sabatolimab + HMA vs 8.5 months with placebo + HMA; $p = 0.102$) and a suggestion of benefit among patients with lower disease burden [80]. The randomized phase III trial (NCT04266301) of AZA +/− sabatolimab with a primary endpoint of OS has now fully accrued more than 500 patients and will provide the definitive answer on use of sabatolimab in HR-MDS [81].

4.2. Anti-CD47

CD47 is expressed on a variety of cells and interacts with signal regulatory protein (SIRP)-α on macrophages and dendritic cells resulting in the inhibition of phagocytosis [82,83]. Upregulation of CD47 by leukemic stem cells has been identified as an adverse prognostic factor in myeloid malignancies and blockade of CD47 by monoclonal antibodies has been shown to induce phagocytosis of tumor cells by macrophages as well as activation of the adaptive immune system via presentation of tumor antigens by dendritic cells [82,83]. Upregulation of CD47 has also been identified in MDS patients, particularly higher risk patients, and could be a harbinger for progression of MDS to AML [84,85].

Based on preclinical studies showing that treatment with anti-CD47 antibodies enabled phagocytosis of AML leukemic stem cells in murine models [83], the anti-CD47 antibody magrolimab has demonstrated synergistic effects in combination with AZA [82,86]. In a single arm phase Ib/II trial of previously untreated patients with HR-MDS treated with magrolimab + AZA the ORR was 74.7% (32.6% CR) with numerically comparable efficacy among *TP53* wild-type (ORR: 78.7%; CR

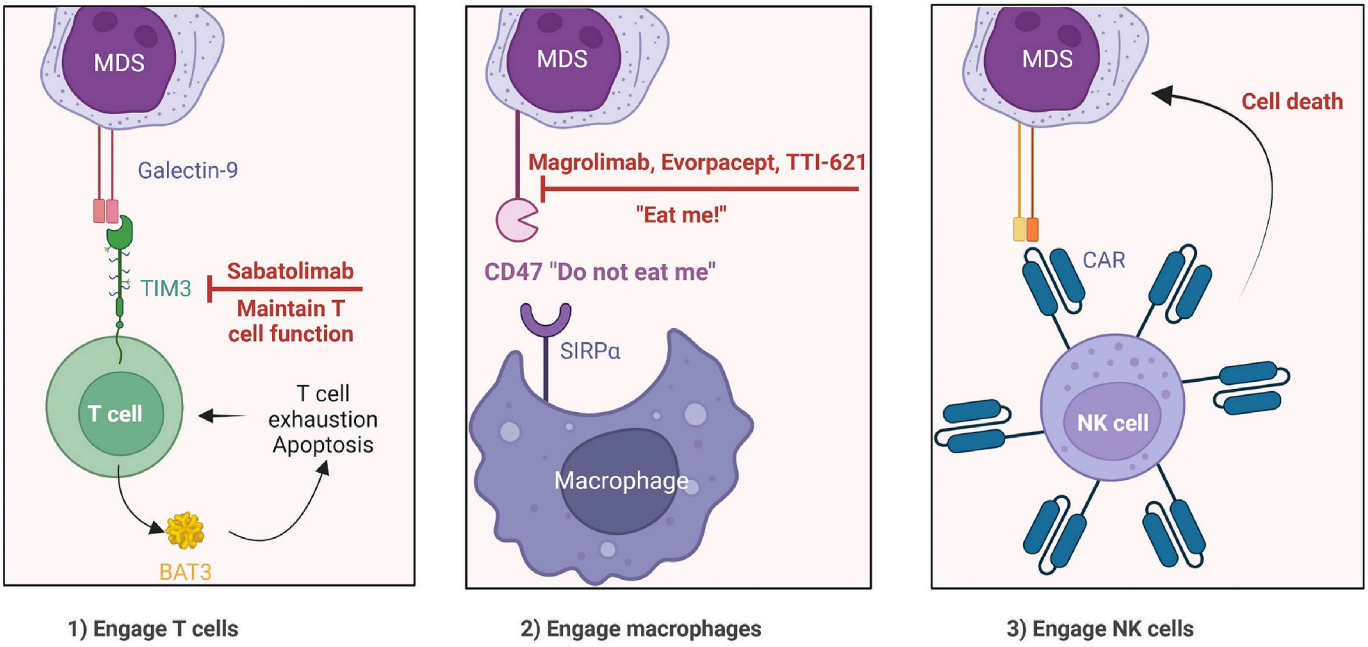

Fig. 2. Novel immunotherapy targets in MDS: 1) T cell immunoglobulin and mucin domain-containing protein 3 (TIM3) is a co-inhibitory receptor that was identified on T-cells and is up-regulated on exhausted T-cells. The anti-TIM3 antibody sabatolimab has shown clinical efficacy in phase Ib clinical trials of patients with newly diagnosed AML and higher-risk (HR)-MDS; 2) CD47 is expressed on a variety of cells and interacts with signal regulatory protein (SIRP)-α on macrophages and dendritic cells resulting in the inhibition of phagocytosis. Anti-CD47 antibodies enabled phagocytosis of AML leukemic stem cells. Several anti-CD47 are being investigated, including magrolimab, evorpacept, and TT1–621. 3) Cellular therapies such as CAR-NK cells are being tested in AML patients.

31.1%) and *TP53*-mutant patients (ORR: 68.0%; CR 40.0%) [86]. While encouraging, these results will need to be confirmed in the ongoing, fully accrued ENHANCE trial, which compares magrolimab + AZA with AZA + placebo (NCT04313881). Whether addition of magrolimab to AZA + venetoclax in older or intensive chemotherapy-ineligible patients with AML also increases therapeutic efficacy is currently being evaluated in a randomized phase III trial (NCT05079230). Notably, early data from a single arm triplet therapy trial have shown the combination to be safe without worsening of cytopenias and to be efficacious in both *TP53* mutant and *TP53* wildtype patients [87].

As treatment with magrolimab can be limited by on-target hemolytic anemia (especially during the first cycle of treatment), novel anti-CD47 antibodies with reduced on-target, off-tumor effects would be welcome additions to improve safety, while maintaining anti-tumor efficacy [86]. Evorpacept (ALX148) is a fusion protein consisting of a modified SIRPα D1 domain fused to an inactive human immunoglobulin Fc region [88]. In a first-in-human, phase I dose-escalation and dose-expansion study that enrolled 110 patients with various advanced solid tumors, evorpacept was given as monotherapy or combined with either pembrolizumab or trastuzumab (in HER2-positive tumors) demonstrating safety and some anti-tumor activity [89]. Evorpacept is also being studied in MDS patients in combination with AZA (NCT04417517) and in combination with AZA + venetoclax in newly-diagnosed AML patients (NCT04755244) [90,91]. Among patients enrolled in the phase I studies, the safety profile was comparable to AZA monotherapy and AZA + venetoclax, respectively, with objective responses documented in patients with both newly diagnosed and relapsed/refractory (R/R) disease [90,91]. There are numerous additional CD47/SIRPα agents in both pre-clinical and clinical development in myeloid malignancies (e.g., TTI-621) [92].

4.3. Other investigational immunologic targets

PD-1H (also known as VISTA) is a homolog to PD-1 or PD-L1 within the CD28 family and is expressed by hematopoietic cells including myeloid cells and human AML blasts [93–95]. PD-1H is also present on tumor infiltrating immune cells including macrophages and has been shown to induce resistance to ipilimumab in prostate cancer models [96,97]. Additionally, PD-1H blockade suppresses tumor growth in various solid tumor and AML models with potential synergy in combination with PD-1 blockade [93,94]. However, whether anti-PD-1H therapy could provide clinically meaningful efficacy in AML and MDS patients remains to be elucidated.

Activation of pattern recognition receptors is essential to the function of the innate immune system. The overlap of lower-risk MDS with autoimmunity and aberrant inflammatory responses has been appreciated for several decades with immunosuppressive therapy constituting an option for a subset of lower-risk MDS patients [3,98,99]. This overlap is also exemplified by molecularly and clinically defined disease entities such as VEXAS syndrome [100,101]. Case series as well as a recent multicenter phase II trial also support the use of HMA as a therapeutic strategy especially among patients with an associated MDS [101,102]. Additionally, an enhanced inflammatory response and the emergence of an aberrant inflammatory monocytic cell population have been associated with disease progression in a murine model of *Tet2*-mutant CH [103]. A detailed review of the contribution of the innate immune system to MDS pathophysiology is beyond the scope of this review but is available elsewhere [3,4,14]. IRAK1/4 are downstream mediators of toll-like and IL-1 receptor signaling leading to the activation of several other pathways including NFκB and MAPK as well as cross-talk with other pro-inflammatory signals such as the NLRP3 inflammasome [14,104–106]. In patients with splicing factor-mutant MDS, abnormal oncogenic isoforms of IRAK4 have been shown to be upregulated [107,108]. This aberrant inflammatory signaling leads to defects in the differentiation of hematopoietic stem and progenitor cells resulting in myeloid skewing, dysplasia, cytopenias, and clonal expansion [14,109]. IRAK4 inhibition with emavusertib is currently being explored in a phase I/II clinical trial (NCT0427868) in patients with HR-MDS or R/R AML with predefined subsets of patients with spliceosome or *FLT3* mutations as well as in patients with LR-MDS (NCT05178342) [110]. Among 15 patients with spliceosome and *FLT3* mutations, 7 patients had an objective response (47%; CR + CRh for AML and CR + mCR for MDS patients) with rhabdomyolysis being a dose-limiting toxicity requiring a temporary hold of enrollment in the phase I part of the study [110]. Additional safety and efficacy data are needed especially for potential combination therapies and in patients without splicing factor mutations. Other parts of the inflammasome and Myddosome complex (e.g., HT-6184) are also being explored in preclinical studies but no safety and efficacy data in MDS patients are available to date [111].

Cellular therapies such as chimeric antigen receptor (CAR) T-cells, bispecific T-cell engagers, and CAR-NK cells have primarily been tested in AML patients and data on the safety and efficacy of these products in MDS are limited [72,112–114]. Recent data have also suggested synergy between BH3 mimetics such as venetoclax and NK cells in vitro and in vivo [115]. However, dedicated research is needed to define the optimal position of cellular therapies in the MDS treatment landscape in order to gather robust safety and efficacy data on potential combination therapies.

5. Targeting the pro-survival machinery in MDS

Targeting pro-survival BCL-2 has re-shaped the treatment landscape of older AML patients and those considered unfit for intensive chemotherapy based on pivotal phase III studies combining venetoclax with low-intensity therapies [116,117]. Enhanced expression of BCL-2 has been observed in more advanced stages of MDS, supporting the clinical rationale to investigate the role of venetoclax in combination with AZA in patients with higher-risk MDS [118]. A phase Ib study (NCT02942290) evaluating venetoclax in combination with AZA in 78 patients with treatment-naïve IPSS intermediate-2 or high-risk MDS demonstrated an intention-to-treat ORR of 80%, including CRs in 40% of patients [119]. In contrast to historical experience with AZA monotherapy, clinical responses appeared to be achieved more rapidly, with a median time to CR of 2.6 months (range 1.2–19.6). Median OS for all AZA + venetoclax treated patients was 28.2 months (95% CI: 17.7–not reached), with post-study allo-HCT performed in 23% of the study population [119]. In contrast to AML, venetoclax in MDS was only tolerable for 14 days per 28-day cycle [120]. Genomic subgroup analyses revealed high response rates across mutation subgroups, including an ORR of 83% in *TP53* mutated MDS. Similar results have been reported from a single center, phase I/II study (NCT04160052) of 23 HR-MDS patients treated with AZA + venetoclax at MD Anderson Cancer Center. The ORR in this study was 87% (95% CI: 66–97%) with AZA 75 mg/m [2] for 5 days + venetoclax 400 mg for 14 days established as the recommended phase II dose [121]. Accrual to the pivotal phase III study (VERONA; NCT04401748) comparing AZA + venetoclax with AZA + placebo in newly diagnosed patients with HR-MDS has been completed and results are awaited. Although longer-term tolerability of AZA + venetoclax in MDS is limited, the rapid and high response rate associated with this regimen may be beneficial for patients planned to undergo allo-HCT. Similarly encouraging results have been reported from a phase Ib study of AZA + venetoclax in patients with R/R MDS. Among 44 patients enrolled, the ORR was 39% with most responses being mCRs ($n = 14$ patients; 32%). Notably, 36% of patients achieved transfusion independence and the median OS among the cohort was 12.6 months [122].

6. Epigenetic aspects of MDS pathogenesis

Epigenetics refers to the reversible, spatiotemporal regulation of gene expression by means of DNA methylation and histone modification without changing the underlying DNA sequence (Fig. 3) [123,124]. While mutations in epigenetic regulators such as *DNMT3A, ASXL1,* and

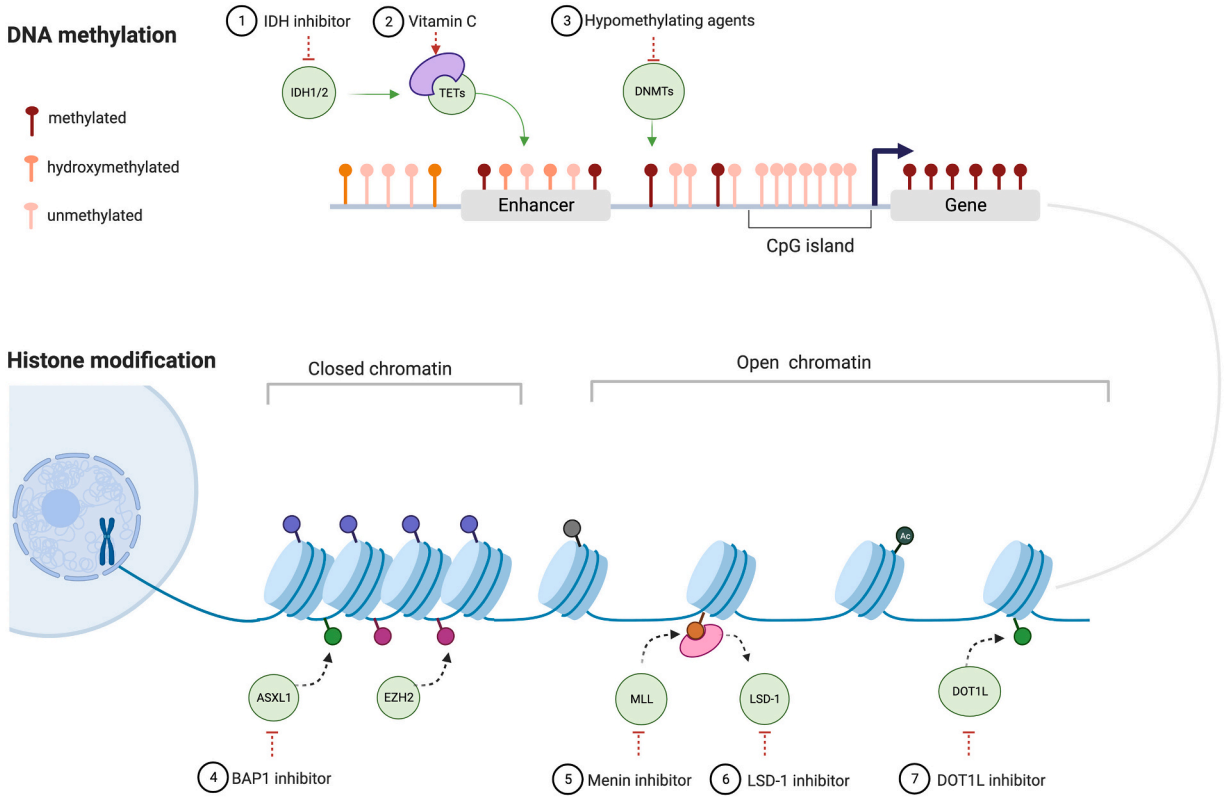

Fig. 3. Epigenetic therapeutics in MDS: Modifying aberrant epigenetic processes is specific to the underlying mutation. 1) IDH1 inhibitor ivosidenib and IDH2 inhibitor enasidenib are currently being investigated in MDS; 2) vitamin C is a cofactor for TET2, treatment with vitamin C has been shown to promote myeloid differentiation and reverse DNA hypermethylation; 3) hypomethylating agents are approved for the treatment of MDS. 4) A first in class BAP1 inhibitor abrogated truncated ASXL1 gene expression and tumor growth in vivo, which could provide a rationale for future clinical trials in MDS; 5) Various approaches to targeting *MLL* rearrangements are currently in various stages of clinical development and include DOT1L inhibitors as well as menin inhibitors. 6) Lysine-specific demethylase (LSD)-1 is a histone H3 lysine demethylase that regulates hematopoiesis and terminal differentiation of hematopoietic progenitor cells. Several LSD-1 inhibitors have been developed.

TET2 are among the most frequently encountered mutations in MDS, and aberrant DNA methylation patterns have been shown in MDS and AML patients [5,6,125], neither the presence of these mutations nor promoter methylation status predicts clinical response to HMA therapy [126,127].

A major challenge with studying epigenetics in MDS is that methylation changes affect not only the promoter region of a given gene but also other regulatory elements both proximally and distally to the gene of interest requiring extensive, genome-wide studies to fully capture the extent of epigenetic dysregulation [128,129]. Thus, assessing gene expression and DNA methylation patterns simultaneously could be an important novel technology to predict response to epigenetic therapies. Baseline differences in DNA methylation in MDS cases sensitive vs resistant to AZA have been demonstrated as well as in chronic myelomonocytic leukemia (CMML) cases sensitive and resistant to decitabine [130,131]. In both instances differentially methylated regions are enriched at distal intergenic regions and enhancers [131].

Modifying aberrant epigenetic processes is specific to the underlying mutation. While *DNMT3A* mutations have been implicated in the self-renewal process of hematopoietic stem cells via the regulation of transcription factor expression (e.g., PU.1, RUNX1, FLI1), a second-hit mutation appears to be necessary for leukemogenesis and no specific therapeutics targeting aberrant DNMT3A function have been developed to date [132–135]. Similar to *DNMT3A, TET2* loss-of-function mutations result in the increased self-renewal and impaired differentiation of hematopoietic stem and progenitor cells [136,137]. As vitamin C is a required cofactor for TET2 function, treatment with vitamin C has been shown to promote myeloid differentiation and reverse DNA hypermethylation in preclinical AML models leading to an ongoing clinical trial of patients with intermediate or high risk MDS with a *TET2* mutation (NCT03433781) [63,138]. Mutations in *IDH1* and *IDH2* are infrequent in MDS but cause epigenetic changes via the generation of the oncometabolite 2-hydroxyglutarate [139–141]. While the IDH1 inhibitor ivosidenib and IDH2 inhibitor enasidenib have been approved for the treatment of AML for several years, data in MDS are limited but early results appear promising with an ORR of 81% (95% CI: 54–96%; 44% CR) among *IDH1*-mutant R/R MDS patients treated with ivosidenib [142–145]. Similar results from a recent trial of *IDH2*-mutant HR-MDS patients treated in the frontline setting with enasidenib + AZA (ORR: 74%; CR 26%) or with enasidenib monotherapy after HMA failure (ORR: 35%; CR 22%) have recently been published [146]. Olutasidenib is another IDH1 inhibitor that has recently been approved for the treatment of R/R *IDH1*-mutant AML and has also demonstrated efficacy in MDS patients [147].

In addition to the reversal of aberrant DNA methylation, targeting posttranslational histone modifications has been another area of active research [123]. *KMT2A* gene rearrangements promote leukemogenesis by the overexpression of *HOXA* genes but are rarely present in MDS [148]. For example, DOT1L has been identified as a histone modifier and implicated in the inappropriate expression of *HOXA* genes in patients with *NPM1*-mutant AML and AML with *KMT2A* gene fusions [149,150]. Inhibition of DOT1L has been shown to have anti-leukemic effects in several genetically defined AML models (e.g., cohesion and *DNMT3A* mutations) [150,151]. However, clinical efficacy of the DOT1L inhibitor pinometostat in patients with AML and *KMT2A* gene rearrangements was limited with only 2 CRs reported among 51 patients enrolled in a

phase I study [152]. Various additional approaches such as menin inhibitors targeting *KMT2A* gene rearrangements are currently in various stages of clinical [153,154]. Although current trials with menin inhibitors (e.g., NCT04065399, NCT04067336) are focusing on patients with *KMT2A* rearrangements as well as mutations in *NPM1* in AML, a number of early phase studies will include R/R MDS and patients with *MLL-PTD* alterations. Additionally, MDS patients with >10% blasts are also eligible for enrollment in some AML trials (e.g., BEAT-AML; NCT03013998). Of note, *KMT2A* rearrangements are considered an AML-defining genetic abnormality in both the ICC and WHO classifications, and a diagnosis of AML rather than MDS can be made in patients with <20% blasts in such cases [1,2].

Lysine-specific demethylase (LSD)-1 is a histone H3 lysine demethylase and scaffolding protein that in conjunction with the corepressor CoREST regulates hematopoiesis and terminal differentiation of hematopoietic progenitor cells via the transcription factors GFI1 and GFI1b [155,156]. Thus, LSD1 inhibition leads to the differentiation of AML blasts by reactivation of PU.1 and CEBP alpha-dependent enhancers [157–159]. Several LSD-1 inhibitors have been developed and are being tested either as monotherapy or in combination with AZA and venetoclax (NCT04748848) or all-trans retinoic acid (NCT02273102) in clinical trials enrolling patients with myeloid malignancies. However, data available to date are limited [160,161].

Finally, mutations in chromatin modifier genes such as *ASXL1* and *EZH2* constitute another class of epigenetic targets. Mutations in *ASXL1* have been identified as an early founder event in the preleukemic hematopoietic stem cell clone and are also among the most common mutations encountered in CH [44]. Additionally, *ASXL1* mutations confer an adverse prognosis in both AML and MDS with no mutation-specific therapy available [162,163]. Although there remains uncertainty regarding the specific mechanisms by which *ASXL1* mutations drive leukemogenesis, the most common *ASXL1* mutations lead to a truncated protein that enhances activity of the histone H2A lysine 119 deubiquitinase BAP1 [164–166]. A first in class BAP1 inhibitor abrogated truncated *ASXL1* gene expression and tumor growth in vivo, which could provide a rationale for future clinical trials [166]. Additional preclinical data also suggest that *ASXL1*-mutant cells are vulnerable to inhibition of Polycomb Repressive Complex (PRC1), which protects *ASXL1*-mutant cells from apoptosis and cellular senescence [167,168].

7. Telomere biology as a therapeutic target in MDS

Telomerase activity and expression of human telomerase reverse transcription (hTERT) have been shown to be increased in MDS cells [169,170]. The telomerase inhibitor imetelstat has been shown to induce apoptosis in malignant cells and to have a disease-modifying potential in MDS and myeloproliferative neoplasms [171,172]. Among 57 patients with transfusion-dependent, ESA-refractory LR-MDS without del(5q) treated with imetelstat in the phase II IMerge trial 37% of patients achieved RBC transfusion independence for at least 8 weeks (23% ≥24 weeks; median duration of transfusion independence of 65 weeks) [169]. Independent of molecular subtypes, imetelstat also led to CR and mCRs in 10% and 13% of patients, respectively, with resolution of dysplasia and reduction in VAF of driver mutations suggesting a potential disease-modifying treatment effect on the malignant clone [169]. Additionally, inhibition of telomerase activity and expression of hTERT among responders was demonstrated supporting the on-target effect of imetelstat [169]. Hematologic AEs seen were common with imetelstat (grade ≥ 3 thrombocytopenia 54%, neutropenia 60%, anemia 19%) with AST elevations and bronchitis being the most common grade ≥ 3 non-hematologic AEs (18% of patients) [169]. The placebo-controlled IMerge phase III trial of imetelstat vs placebo (NCT02598661) in transfusion-dependent LR-MDS patients has completed accrual, and a press release from the sponsor indicated that the trial has met its primary endpoint and results are expected to be shared in near future [173].

8. Targeting aberrant splicing in MDS

Mutations in RNA splicing factor genes such as *SF3B1, SRSF2, U2AF1,* and *ZRSR2* are among the most frequently encountered mutations in MDS [174]. While *SF3B1* mutations define a distinct molecular subtype of MDS in both the WHO and ICC classifications and have been associated with a favorable prognosis, *U2AF1* and *SRSF2* mutations are enriched in HR-MDS and AML and confer an adverse prognosis [1,8,153,175–177]. Pre-mRNA splicing is essential for the regulation of gene expression and proteomic diversity via alternative splicing with mutations affecting diverse pathways including DNA damage response, immune signaling, and epigenetic regulations [174]. While splicing factor mutations confer a high risk of progression to an overt myeloid malignancy among patients with CH, these mutations are not clearly leukemogenic in isolation based on murine models, although further work is needed to elucidate how mutations in splicing factor genes lead to the development of myeloid malignancies [54,174]. Splicing mutations are also detected in other malignancies (e.g., chronic lymphocytic leukemia), and it is important to verify the identification of its clonal presence with the associated diagnosis within each patient [178].

It has been proposed that cells with splicing factor mutations are dependent on the residual function of the remaining wild-type gene, which might render them susceptible to synthetic lethality induced by treatment with a splicing modulator [179]. This concept has been tested in a recent phase I trial of H3B-8800, an oral SF3b complex modulator [180]. Among 84 patients with AML, MDS, or CMML who were enrolled irrespective of the presence of splicing mutations there were no CRs, although 15% of patients with lower-risk MDS achieved red blood cell transfusion independence [181]. Whether restricting enrollment to patients with splicing factor mutations would have yielded different results is unclear and further investigation of H3B-8800 in patients with *SF3B1* mutant MDS with transfusion dependence is ongoing [181]. Interestingly, elevated pre-treatment expression of aberrant transcripts of Transmembrane Protein 14C (TMEM14C), an SF3B1 splicing target encoding a mitochondrial porphyrin transporter, could serve as a predictive biomarker but warrants additional validation [181].

RBM39 is an RNA splicing factor that is required for survival of AML cells with splicing factor mutations and can be targeted for proteasomal degradation by molecular glues such as E7820 or indisulam [153,174,182]. In vitro studies showing the anti-leukemic effects of RBM39 degradation provided the preclinical rationale for clinical trials of both indisulam and E7820 in AML and MDS [183,184]. The addition of indisulam to idarubicin and cytarabine in patients with R/R-AML (independent of the presence of splicing factor mutations) had only limited activity (ORR 35% for the triplet), while E7820 monotherapy in splicing factor-mutant R/R AML and MDS did not yield any responses [183,184]. Correlative studies from the E7820 trial showed evidence of successful RBM39 degradation and splicing inhibition in bone marrow samples of clinical trial patients, illustrating a disconnect between effective splicing inhibition and clinical efficacy, which may suggest that targeting splicing mutations alone might be insufficient for clinical efficacy in R/R higher-risk disease [184]. A recent study suggested that splicing modulators can lead to the generation of neoantigens, which could provide the rationale for combining splicing modulators with immune checkpoint inhibitors [185].

Another strategy is the targeting of dysregulated cellular processes and pathways that are downstream of mutated splicing factors. Splicing factor mutations have been shown to induce accumulation of R-loops and associated DNA damage. This leads to ataxia telangiectasia and Rad3-related protein (ATR) pathway activation and preferential sensitivity to ATR inhibitors, providing a rationale for targeting ATR signaling in patients with splicing factor mutant myeloid malignancies [186–189]. A phase 1b clinical trial assessing AZD6738 (ceralasertib), an ATR inhibitor, in MDS or CMML patients is being conducted (NCT03770429).

9. Future considerations and clinical implications

While preclinical advances in the pathophysiology of MDS have yet to be translated into clinical practice, several recent developments are encouraging. Better animal models and an improved ability to model clonal evolution over time provide important tools for both preclinical screening of novel therapeutics. Collection of serial samples from patients on both standard and investigational therapies will allow us to better understand the impact of these therapies on clonal architecture [54]. Molecular testing is also likely going to become more broadly available and used more widely for both risk stratification and definition of MDS subtypes as well as differentiating MDS from other disorders such as aplastic anemia and MDS/MPN overlap syndromes [1,5,190,191]. A detailed discussion of the implications of molecular testing results for risk stratification, clinical trial design, and clinical management decisions is beyond the scope of this review. We have discussed our top 10 research agenda items in a separate commentary [192]. Importantly, the recently published IPSS-M has been retrospectively validated and is being increasingly incorporated into clinical practice, but additional prospective validation is needed [5,9,28]. The icMDS endorses the recently published International Working Group (IWG) 2023 response criteria for HR-MDS which proposed the use of IPSS-R of >3.5 and IPSS-M risk categories of moderate-high, high, and very high to define HR-MDS for clinical trials [193].

Additionally, the recent publication of the 5th edition of the WHO classification of myeloid neoplasms and the ICC has introduced several areas of inconsistency with regards to the diagnostic criteria and definition of MDS subtypes [1,2]. A notable difference between the two classifications is the introduction of an "AML/MDS overlap" category in the ICC for patients with 10–19% bone marrow blasts based on data showing that the prognosis of MDS patients with increased blasts is comparable to patients with oligoblastic AML [194,195]. As the WHO classification retains a $\geq$ 20% bone marrow blast threshold, this poses challenges for clinical trial design, access to and reimbursement for medications, and psychological consequences for patients who receive different opinions and diagnoses. It will remain to be seen how this dichotomy affects clinical practice and how the differences can be reconciled. The icMDS has published a commentary stressing the urgent need for one harmonized classification system to be used in clinical practice and clinical trials and presented the first data from an ongoing comparative analysis that uses large, international, molecularly annotated databases to highlight areas of potential improvements for both classifications in an evidence-based fashion [23,192,196]. These efforts have led to the proposal of a data-driven roadmap to harmonize the two classifications that will be discussed in more depth in subsequent publications [23,192,196].

Longitudinal assessment of measurable residual disease (MRD) assessment in MDS is not part of routine clinical practice yet and will require a better understanding of the association between genetic and immunophenotypic changes during therapy and meaningful clinical benefit. Any utility of MRD testing in MDS is likely to be context dependent but may have important implications for treatment decision-making (e.g., maintenance therapy after allo-HCT) [197–200].

Elucidating the various contributing factors to MDS pathophysiology including the role of inflammation has enabled the development of several novel investigational therapies such as IRAK1/4 inhibitors and targeting of the NLRP3 inflammasome [105,109,110]. Additionally, it is conceivable that in the foreseeable future several molecularly targeted therapies might become available (e.g., for patients with splicing factor or *TP53* mutations) and that the one-size-fits-all approach of HMA monotherapy will be a relic of the past [86,181]. However, the final results of the ongoing randomized phase III trials of AZA + magrolimab and AZA + venetoclax and longer duration of follow up are needed before a new standard of care can be defined. Table 3 provides an overview of selected ongoing randomized phase III trials in MDS. Additionally, this increasingly complex therapeutic landscape raises questions regarding the optimal sequencing of therapies especially after progression on novel combination treatments. While encouraging, there are currently no on-label targeted therapies for any molecular subtype other than luspatercept for patients with *SF3B1* mutations and lenalidomide in patients with del(5q) [25]. Despite the high expectations for molecular testing and its implications for clinical practice in MDS, it is also important to keep in mind that NGS results are either not readily or not at all available (in resource-limited settings), might not be reimbursed or have a turnaround time for results of several weeks, which could lead to disparities in patient care globally. Standardization and consensus guidelines are thus important and have recently been developed [201].

Another potentially practice-changing trend is the attempt to intervene early at the stage of CH and ideally prevent progression to an overt myeloid neoplasm. However, at this point it remains to be seen whether treatments (e.g., IDH inhibitors) for patients with high-risk CCUS can change the natural history of the disease or if disease progression is inevitable. It is also important to assess whether early intervention

Table 3
Selected ongoing or recently completed, randomized trials in HR-MDS.

Agent	Phase	Intervention	Patient population	Preliminary results	NCT registration
APR-246	Phase III	APR-246 + AZA vs AZA + placebo	*TP53*-mutant HR-MDS; no prior HMA	Not available; phase I/II studies with ORR of 62–73% (47–50% CR) [207,208]	NCT03745716
Magrolimab	Phase III	Magrolimab + AZA vs AZA + placebo	HR-MDS; no prior HMA	Not available; ORR 74.7%, 32.6% CR [86]	NCT04313881
Sabatolimab	Phase II	Sabatolimab + HMA vs HMA + placebo	HR-MDS; previously untreated	No difference in CR rate or OS with addition of sabatolimab (CR rate 21.5% with sabatolimab+HMA vs 17.7% with placebo+HMA; p = 0.769: median OS 19.0 vs 18.0 months (hazard ratio 0.905 [95% CI: 0.565, 1.450]) [80].	NCT03946670
	Phase III	Sabatolimab + AZA vs AZA + placebo	HR-MDS; previously untreated	Not available	NCT04266301
Venetoclax	Phase III	Venetoclax + AZA vs AZA + placebo	HR-MDS; no prior HMA	Not available	NCT04401748
Pevonedistat	Phase III	Pevonedistat + AZA vs AZA monotherapy	HR-MDS, HR-CMML or oligoblastic AML; previously untreated	HR-MDS cohort: median EFS: 19.2 vs 15.6 months (HR, 0.887; 95% CI: 0.659–1.193; P = 0.431). Median OS: 21.6 vs 17.5 months (HR, 0.785; P = 0.092) [209]	NCT03268954
Tamibarotene (SY-1425)	Phase III	Tamibarotene + AZA vs AZA + placebo	RARA-positive, newly diagnosed HR-MDS patients	Not available	NCT04797780

Allo-HCT – allogeneic hematopoietic cell transplantation; AML – acute myeloid leukemia; AZA – azacitidine; CI – confidence interval; CMML – chronic myelomonocytic leukemia; CR – complete remission; EFS – event-free survival; HMA – hypomethylating agent; HR – hazard ratio; HR-MDS – higher-risk myelodysplastic syndrome; ORR – overall response rate; OS – overall survival.

strategies might worsen outcomes due to increased clonal complexity and poorer response to therapy. It remains unclear what the optimal guidance for patients with high-risk CCUS undergoing treatment with cytotoxic chemotherapy/radiation therapy for a solid tumor should be and how cardiovascular complications of CH can be best mitigated [202].

In summary, there have been significant advances in many fields of MDS research that helped to better delineate the genetic and immunologic pathophysiology of MDS, which have enabled several clinical trials of novel agents. However, none of these novel agents have been approved for the treatment of MDS patients to date and additional preclinical and clinical work is needed to enable a better, individualized approach to the care of MDS patients.

10. Research agenda

- Advancing the development and increasing the dissemination of reliable, preclinical models of human MDS
- Establish tools to predict, and ultimately reduce, risk of progression of clonal hematopoiesis to myeloid neoplasms including MDS
- Formulate and validate unified MDS diagnostic and response criteria
- Develop novel treatment strategies directed at the underlying pathophysiology of MDS
- Understanding molecular and biological consequences of different mutations in MDS to allow development of rationally designed therapies

11. Practice points

- The WHO and ICC 2022 classifications have introduced several changes to the MDS subclassification focusing on molecular markers (e.g., *TP53*) to define MDS subgroups
- Molecular testing has enabled a more nuanced and individualized approach to risk stratification of MDS patients
- Novel therapies for MDS using targeting splicing, immunologic and epigenetic disease aspects are in development
- Clonal hematopoiesis can be a precursor to myeloid neoplasms and has been included in the WHO and ICC classifications for the first time

Author contributions

JPB, ZX, MS, and AMZ wrote the initial draft of the manuscript. ZX created figures. All authors participated in the discussions during the workshop in Miami, on which this work is based, reviewed and provided edits to subsequent versions of the manuscript.

Declaration of Competing Interest

Maximilian Stahl consulted for Curis Oncology and Boston Consulting; served on the advisory board for Novartis and Kymera; and participated in GME activity for Novartis, Curis Oncology, Haymarket Media and Clinical care options (CCO). Elizabeth A. Griffiths has received honoraria for advisory board membership from AbbVie, Alexion Pharmaceuticals, Apellis, Celgene/BMS, CTI Biopharma, Genentech, Novartis, Picnic Health, Takeda Oncology, Taiho Oncology. EAG has received research funding from Astex Pharmaceuticals, AstraZeneca Rare Disease, Alexion Pharmaceuticals, Apellis Pharmaceuticals, Blueprint Medicines, Genentech Inc., and honoraria for CME activities from Physicians Educational Resource, MediComWorldwide, American Society of Hematology, AAMDS International Foundation. Ravindra Majeti is on the Advisory Boards of Kodikaz Therapeutic Solutions, Syros Pharmaceuticals, TenSixteen Bio, Roche, and Cullgen Inc. and is an inventor on a number of patents related to CD47 cancer immunotherapy licensed to Gilead Sciences. R.M. receives research support from Gilead Sciences. Ravindra Majeti. is a co-founder and equity holder of Pheast Therapeutics, MyeloGene, and Orbital Therapeutics. Stephanie Halene consulted for Forma Therapeutics. Daniel T. Starczynowski is a consultant and received research funding from Kymera Therapeutics, Kurome Therapeutics, Captor Therapeutics, and Tolero Therapeutics. Daniel T. Starczynowski has equity in Kurome Therapeutics. David A. Sallman served on the advisory board of Aprea, AvenCell, BlueBird Bio, BMS, Intellia, Kite, Novartis, Shattuck Labs, Servier, Syndax. David A. Sallman served as a consultant for AbbVie, Magenta, Molecular Partners AG, Takeda and on the speakers' bureau for BMS, Incyte, Servier; David A. Sallman received research funding from Aprea, Jazz. Mrinal Patnaik received research funding from Kura Oncology and StemLine Pharmaceuticals. Andrew Brunner received consulting or advisory board honoraria from Novartis, Acceleron, Agios, Abbvie, Takeda, Celgene/BMS, Keros Therapeutics, Taiho, Gilead. Andrew Brunner has research support from the NIH SPORE in Myeloid Malignancies, and from the Edward P. Evans Foundation. Tae Kon Kim received research funding from Nextcure and is a consultant for Agenus. Alan List is employed by and has equity in Precision BioSciences, and has served as a consultant for Halia Therapeutics, CTI Biopharma, Aileron. Naval Daver has received research funding from Daiichi-Sankyo, Bristol-Myers Squibb, Pfizer, Gilead, Sevier, Genentech, Astellas, Daiichi-Sankyo, Abbvie, Hanmi, Trovagene, FATE therapeutics, Amgen, Novimmune, Glycomimetics, Trillium, and ImmunoGen and has served in a consulting or advisory role for Daiichi-Sankyo, Bristol-Myers Squibb, Arog, Pfizer, Novartis, Jazz, Celgene, AbbVie, Astellas, Genentech, Immunogen, Servier, Syndax, Trillium, Gilead, Amgen, Shattuck labs, and Agios. Guillermo Sanz received honoraria, advisory board membership or consultation fees from AbbVie, BMS, ExCellThera, Novartis, Roche, and Takeda and participated in sponsored speaker's bureau for BMS, Novartis, and Takeda. Mikkael A. Sekeres has served on advisory boards for BMS, Novartis, Kurome, and Gilead. Pierre Fenaux received research funding from BMS, Abbvie, Jazz Pharmaceuticals, Novartis, and Janssen. Pierre Fenaux had a consultancy with and received honoraria from BMS, Abbvie, Jazz Pharmaceuticals, and Novartis. Omar Abdel-Wahab has served as a consultant for H3B Biomedicine, Foundation Medicine Inc., Merck, Prelude Therapeutics, and Janssen, and is on the Scientific Advisory Board of Envisagenics Inc., AIChemy, Harmonic Discovery Inc., and Pfizer Boulder; Omar Abdel-Wahab has received prior research funding from H3B Biomedicine and LOXO Oncology unrelated to the current manuscript. Andrew H.Wei has served on advisory boards for Novartis, Astra Zeneca, Astellas, Janssen, Jazz, Amgen, Roche, Pfizer, Abbvie, Servier, Gilead, BMS, Shoreline, Macrogenics, Novartis and Agios; receives research funding to the Institution from Novartis, Abbvie, Servier, Janssen, BMS, Syndax, Astex, Astra Zeneca, Amgen; serves on speaker's bureaus for Abbvie, Novartis, BMS, Servier, Astellas; Andrew H.Wei is an employee of the Walter and Eliza Hall Institute (WEHI). WEHI receives milestone and royalty payments related to the development of Venetoclax. Current and past employees of Walter and Eliza Hall Institute may be eligible for financial benefits related to these payments. Andrew H.Wei receives such a financial benefit. Valeria Santini served in advisory boards from Abbvie, BMS, Geron, Gilead, Menarini, Novartis, Servier, Syros, ad received research support from BMS. Amer M. Zeidan received research funding (institutional) from Celgene/BMS, Abbvie, Astex, Pfizer, Medimmune/AstraZeneca, Boehringer-Ingelheim, Cardiff oncology, Incyte, Takeda, Novartis, Aprea, and ADC Therapeutics. AMZ participated in advisory boards, and/or had a consultancy with and received honoraria from AbbVie, Otsuka, Pfizer, Celgene/BMS, Jazz, Incyte, Agios, Boehringer-Ingelheim, Novartis, Acceleron, Astellas, Daiichi Sankyo, Cardinal Health, Taiho, Seattle Genetics, BeyondSpring, Cardiff Oncology, Takeda, Ionis, Amgen, Janssen, Epizyme, Syndax, Gilead, Kura, Chiesi, ALX Oncology, BioCryst, Notable, Orum, and Tyme. AMZ served on clinical trial committees for Novartis, Abbvie, Gilead, BioCryst, Abbvie, ALX Oncology, Geron and Celgene/BMS.

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References

[1] Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization classification of Haematolymphoid tumours: myeloid and histiocytic/dendritic neoplasms. Leukemia. 2022;36:1703–19.
[2] Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM, et al. International consensus classification of myeloid neoplasms and acute Leukemia: integrating morphological, clinical, and genomic data. Blood 2022;140(11):1200–28.
[3] Winter S, Shoaie S, Kordasti S, Platzbecker U. Integrating the "Immunome" in the stratification of myelodysplastic syndromes and future clinical trial design. J Clin Oncol 2020:JCO1901823.
[4] Sallman DA, List A. The central role of inflammatory signaling in the pathogenesis of myelodysplastic syndromes. Blood. 2019;133:1039–48.
[5] Bernard E, Tuechler H, Greenberg PL, Hasserjian RP, Ossa JEA, Nannya Y, et al. Molecular international prognostic scoring system for myelodysplastic syndromes. NEJM Evid 2022;1. EVIDoa2200008.
[6] Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014;28:241–7.
[7] Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, Van Loo P, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013;122:3616–27. quiz 99.
[8] Malcovati L, Stevenson K, Papaemmanuil E, Neuberg D, Bejar R, Boultwood J, et al. SF3B1-mutant MDS as a distinct disease subtype: a proposal from the international working Group for the Prognosis of MDS. Blood. 2020;136:157–70.
[9] Nazha A, Komrokji R, Meggendorfer M, Jia X, Radakovich N, Shreve J, et al. Personalized prediction model to risk stratify patients with myelodysplastic syndromes. J Clin Oncol 2021;39:3737–46.
[10] Bernard E, Nannya Y, Hasserjian RP, Devlin SM, Tuechler H, Medina-Martinez JS, et al. Implications of TP53 allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes. Nat Med 2020;26:1549–56.
[11] Greenberg PL, Stone RM, Al-Kali A, Bennett JM, Borate U, Brunner AM, et al. NCCN guidelines® insights: myelodysplastic syndromes, version 3.2022. J Natl Compr Cancer Netw 2022;20:106–17.
[12] Cazzola M. Myelodysplastic syndromes. N Engl J Med 2020;383:1358–74.
[13] Liu W, Teodorescu P, Halene S, Ghiaur G. The coming of age of preclinical models of MDS. Front Oncol 2022;12:815037.
[14] Stubbins RJ, Platzbecker U, Karsan A. Inflammation and myeloid malignancy: quenching the flame. Blood. 2022;140:1067–74.
[15] Song Y, Rongvaux A, Taylor A, Jiang T, Tebaldi T, Balasubramanian K, et al. A highly efficient and faithful MDS patient-derived xenotransplantation model for pre-clinical studies. Nat Commun 2019;10:366.
[16] Reinisch A, Thomas D, Corces MR, Zhang X, Gratzinger D, Hong WJ, et al. A humanized bone marrow ossicle xenotransplantation model enables improved engraftment of healthy and leukemic human hematopoietic cells. Nat Med 2016;22:812–21.
[17] Medyouf H, Mossner M, Jann JC, Nolte F, Raffel S, Herrmann C, et al. Myelodysplastic cells in patients reprogram mesenchymal stromal cells to establish a transplantable stem cell niche disease unit. Cell Stem Cell 2014;14:824–37.
[18] Muguruma Y, Matsushita H, Yahata T, Yumino S, Tanaka Y, Miyachi H, et al. Establishment of a xenograft model of human myelodysplastic syndromes. Haematologica. 2011;96:543–51.
[19] Song Y, Shan L, Gbyli R, Liu W, Strowig T, Patel A, et al. Combined liver-cytokine humanization comes to the rescue of circulating human red blood cells. Science. 2021;371:1019–25.
[20] Papaemmanuil E, Cazzola M, Boultwood J, Malcovati L, Vyas P, Bowen D, et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. N Engl J Med 2011;365:1384–95.
[21] Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med 2011;364:2496–506.
[22] Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. Nature. 2011;478:64.
[23] Zeidan AM, Bewersdorf JP, Buckstein R, Sekeres MA, Steensma DP, Platzbecker U, et al. Finding consistency in classifications of myeloid neoplasms: a perspective on behalf of the international workshop for myelodysplastic syndromes. Leukemia 2022. Dec;36(12):2939–46.
[24] Malcovati L, Karimi M, Papaemmanuil E, Ambaglio I, Jadersten M, Jansson M, et al. SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. Blood. 2015;126:233–41.
[25] Fenaux P, Platzbecker U, Mufti GJ, Garcia-Manero G, Buckstein R, Santini V, et al. Luspatercept in patients with lower-risk myelodysplastic syndromes. N Engl J Med 2020;382:140–51.
[26] Weinberg OK, Siddon AJ, Madanat Y, Gagan J, Arber DA, Dal Cin P, et al. TP53 mutation defines a unique subgroup within complex karyotype de novo and therapy-related MDS/AML. Blood Adv 2022 May 10;6(9):2847–53.
[27] Grob T, Al Hinai ASA, Sanders MA, Kavelaars FG, Rijken M, Gradowska PL, et al. Molecular characterization of mutant TP53 acute myeloid leukemia and high-risk myelodysplastic syndrome. Blood. 2022;139:2347–54.
[28] Bersanelli M, Travaglino E, Meggendorfer M, Matteuzzi T, Sala C, Mosca E, et al. Classification and personalized prognostic assessment on the basis of clinical and genomic features in myelodysplastic syndromes. J Clin Oncol 2021;39:1223–33.
[29] Zeidan AM, Bewersdorf JP, Hasle V, Shallis RM, Thompson E, de Menezes DL, et al. Prognostic implications of mono-hit and multi-hit TP53 alterations in patients with acute myeloid leukemia and higher risk myelodysplastic syndromes treated with azacitidine-based therapy. Leukemia. 2023. Jan;37(1):240–3.
[30] Bahaj W, Kewan T, Gurnari C, Durmaz A, Ponvilawan B, Pandit I, et al. A clinically practicable approach to predict TP53 allelic configurations in myeloid neoplasia. Blood. 2022;140:2075–7.
[31] Haase D, Stevenson KE, Neuberg D, Maciejewski JP, Nazha A, Sekeres MA, et al. TP53 mutation status divides myelodysplastic syndromes with complex karyotypes into distinct prognostic subgroups. Leukemia. 2019;33:1747–58.
[32] Menssen AJ, Walter MJ. Genetics of progression from MDS to secondary leukemia. Blood. 2020;136:50–60.
[33] Lindsley RC, Mar BG, Mazzola E, Grauman PV, Shareef S, Allen SL, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. Blood. 2015;125:1367–76.
[34] Zeidan AM, Shallis RM, Wang R, Davidoff A, Ma X. Epidemiology of myelodysplastic syndromes: why characterizing the beast is a prerequisite to taming it. Blood Rev 2019;34:1–15.
[35] Drazer MW, Kadri S, Sukhanova M, Patil SA, West AH, Feurstein S, et al. Prognostic tumor sequencing panels frequently identify germ line variants associated with hereditary hematopoietic malignancies. Blood Adv 2018;2:146–50.
[36] Klco JM, Mullighan CG. Advances in germline predisposition to acute leukaemias and myeloid neoplasms. Nat Rev Cancer 2021;21:122–37.
[37] Feurstein S, Churpek JE, Walsh T, Keel S, Hakkarainen M, Schroeder T, et al. Germline variants drive myelodysplastic syndrome in young adults. Leukemia. 2021;35:2439–44.
[38] Polprasert C, Schulze I, Sekeres MA, Makishima H, Przychodzen B, Hosono N, et al. Inherited and somatic defects in DDX41 in myeloid neoplasms. Cancer Cell 2015;27:658–70.
[39] Tawana K, Wang J, Renneville A, Bödör C, Hills R, Loveday C, et al. Disease evolution and outcomes in familial AML with germline CEBPA mutations. Blood. 2015;126:1214–23.
[40] Feurstein SK, Trottier AM, Estrada-Merly N, Pozsgai MJ, McNeely KE, Drazer MW, et al. Germline predisposition variants occur in myelodysplastic syndrome patients of all ages. Blood. 2022. Dec 15;140(24):2533–48.
[41] Konstantinopoulos PA, Norquist B, Lacchetti C, Armstrong D, Grisham RN, Goodfellow PJ, et al. Germline and somatic tumor testing in epithelial ovarian Cancer: ASCO guideline. J Clin Oncol 2020;38:1222–45.
[42] Rio-Machin A, Vulliamy T, Hug N, Walne A, Tawana K, Cardoso S, et al. The complex genetic landscape of familial MDS and AML reveals pathogenic germline variants. Nat Commun 2020;11:1044.
[43] Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126:9–16.
[44] Xie M, Lu C, Wang J, McLellan MD, Johnson KJ, Wendl MC, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med 2014;20:1472–8.
[45] Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med 2014;371:2488–98.
[46] Genovese G, Kahler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med 2014;371:2477–87.
[47] Miller PG, Qiao D, Rojas-Quintero J, Honigberg MC, Sperling AS, Gibson CJ, et al. Association of clonal hematopoiesis with chronic obstructive pulmonary disease. Blood. 2022;139:357–68.
[48] Jaiswal S, Natarajan P, Silver AJ, Gibson CJ, Bick AG, Shvartz E, et al. Clonal Hematopoiesis and risk of atherosclerotic cardiovascular disease. N Engl J Med 2017;377:111–21.
[49] Steensma DP. Cytopenias + mutations - dysplasia = what? Blood. 2015;126:2349–51.
[50] Bewersdorf JP, Ardasheva A, Podoltsev NA, Singh A, Biancon G, Halene S, et al. From clonal hematopoiesis to myeloid leukemia and what happens in between: will improved understanding lead to new therapeutic and preventive opportunities? Blood Rev 2019;37:100587.
[51] Valent P. ICUS, IDUS, CHIP and CCUS: diagnostic criteria, Separation from MDS and clinical implications. Pathobiology. 2018;1-9.

[52] Malcovati L, Galli A, Travaglino E, Ambaglio I, Rizzo E, Molteni E, et al. Clinical significance of somatic mutation in unexplained blood cytopenia. Blood. 2017; 129:3371–8.
[53] Steensma DP. Clinical consequences of clonal hematopoiesis of indeterminate potential. Blood Adv 2018;2:3404–10.
[54] Fabre MA, de Almeida JG, Fiorillo E, Mitchell E, Damaskou A, Rak J, et al. The longitudinal dynamics and natural history of clonal haematopoiesis. Nature. 2022;606:335–42.
[55] van Zeventer IA, de Graaf AO, Wouters HJCM, van der Reijden BA, van der Klauw MM, de Witte T, et al. Mutational spectrum and dynamics of clonal hematopoiesis in anemia of older individuals. Blood. 2020;135:1161–70.
[56] Desai P, Mencia-Trinchant N, Savenkov O, Simon MS, Cheang G, Lee S, et al. Somatic mutations precede acute myeloid leukemia years before diagnosis. Nat Med 2018;24:1015–23.
[57] Gibson CJ, Kennedy JA, Nikiforow S, Kuo FC, Alyea EP, Ho V, et al. Donor-engrafted CHIP is common among stem cell transplant recipients with unexplained cytopenias. Blood. 2017;130:91–4.
[58] Bolton KL, Ptashkin RN, Gao T, Braunstein L, Devlin SM, Kelly D, et al. Cancer therapy shapes the fitness landscape of clonal hematopoiesis. Nat Genet 2020;52: 1219–26.
[59] Bolton K, Ptashkin R, Braunstein L, Kelly D, Devlin SM, Coombs CC, et al. Oncologic therapy for solid Tumors alters the risk of clonal Hematopoiesis. Blood. 2018;132:747.
[60] Gibson CJ, Lindsley RC, Tchekmedyian V, Mar BG, Shi J, Jaiswal S, et al. Clonal Hematopoiesis associated with adverse outcomes after autologous stem-cell transplantation for lymphoma. J Clin Oncol 2017;35:1598–605.
[61] Gillis NK, Ball M, Zhang Q, Ma Z, Zhao Y, Yoder SJ, et al. Clonal haemopoiesis and therapy-related myeloid malignancies in elderly patients: a proof-of-concept, case-control study. Lancet Oncol 2017;18:112–21.
[62] Kwan TT, Oza AM, Tinker AV, Ray-Coquard I, Oaknin A, Aghajanian C, et al. Preexisting TP53-variant clonal Hematopoiesis and risk of secondary myeloid neoplasms in patients with high-grade ovarian cancer treated with Rucaparib. JAMA Oncol 2021;7:1772–81.
[63] Xie Z, McCullough K, Lasho T, Fernandez JA, Finke C, Amundson MR, et al. Phase II study assessing safety and preliminary efficacy of high dose intravenous ascorbic acid in patients with TET2 mutant clonal Cytopenias of undetermined significance. Blood. 2022;140:2893–4.
[64] Sallman DA, McLemore AF, Aldrich AL, Komrokji RS, McGraw KL, Dhawan A, et al. TP53 mutations in myelodysplastic syndromes and secondary AML confer an immunosuppressive phenotype. Blood 2020. Dec 10;136(24):2812–23.
[65] Kubasch AS, Schulze F, Giagounidis A, Götze KS, Krönke J, Sockel K, et al. Single agent talacotuzumab demonstrates limited efficacy but considerable toxicity in elderly high-risk MDS or AML patients failing hypomethylating agents. Leukemia. 2020;34:1182–6.
[66] Zeidan AM, Knaus HA, Robinson TM, Towlerton AMH, Warren EH, Zeidner JF, et al. A multi-center phase I trial of Ipilimumab in patients with myelodysplastic syndromes following Hypomethylating agent failure. Clin Cancer Res 2018;24: 3519–27.
[67] Zeidan AM, Boss I, Beach CL, Copeland WB, Thompson E, Fox BA, et al. A randomized phase 2 trial of azacitidine with or without durvalumab as first-line therapy for higher-risk myelodysplastic syndromes. Blood Adv 2022;6:2207–18.
[68] Chien KS, Kim K, Nogueras-Gonzalez GM, Borthakur G, Naqvi K, Daver NG, et al. Phase II study of azacitidine with pembrolizumab in patients with intermediate-1 or higher-risk myelodysplastic syndrome. Br J Haematol. 2021. Nov;195(3): 378–87.
[69] Kim TK, Vandsemb EN, Herbst RS, Chen L. Adaptive immune resistance at the tumour site: mechanisms and therapeutic opportunities. Nat Rev Drug Discov 2022;21:529–40.
[70] Kohlhapp FJ, Haribhai D, Mathew R, Duggan R, Ellis PA, Wang R, et al. Venetoclax increases Intratumoral effector T cells and antitumor efficacy in combination with immune checkpoint blockade. Cancer Discov 2021;11:68–79.
[71] Bewersdorf JP, Shallis RM, Zeidan AM. Immune checkpoint inhibition in myeloid malignancies: moving beyond the PD-1/PD-L1 and CTLA-4 pathways. Blood Rev 2020;100709.
[72] Daver N, Alotaibi AS, Bücklein V, Subklewe M. T-cell-based immunotherapy of acute myeloid leukemia: current concepts and future developments. Leukemia. 2021;35:1843–63.
[73] Wolf Y, Anderson AC, Kuchroo VK. TIM3 comes of age as an inhibitory receptor. Nat Rev Immunol 2020;20:173–85.
[74] Rangachari M, Zhu C, Sakuishi K, Xiao S, Karman J, Chen A, et al. Bat3 promotes T cell responses and autoimmunity by repressing Tim-3–mediated cell death and exhaustion. Nat Med 2012;18:1394–400.
[75] Nakayama M, Akiba H, Takeda K, Kojima Y, Hashiguchi M, Azuma M, et al. Tim-3 mediates phagocytosis of apoptotic cells and cross-presentation. Blood. 2009;113: 3821–30.
[76] Chiba S, Baghdadi M, Akiba H, Yoshiyama H, Kinoshita I, Dosaka-Akita H, et al. Tumor-infiltrating DCs suppress nucleic acid-mediated innate immune responses through interactions between the receptor TIM-3 and the alarmin HMGB1. Nat Immunol 2012;13:832–42.
[77] Kikushige Y, Shima T, Takayanagi S-i, Urata S, Miyamoto T, Iwasaki H, et al. TIM-3 is a promising target to selectively kill acute myeloid Leukemia stem cells. Cell Stem Cell 2010;7:708–17.
[78] Kikushige Y, Miyamoto T, Yuda J, Jabbarzadeh-Tabrizi S, Shima T, Takayanagi S-i, et al. A TIM-3/Gal-9 autocrine stimulatory loop drives self-renewal of human myeloid Leukemia stem cells and leukemic progression. Cell Stem Cell 2015;17: 341–52.
[79] Brunner AM, Esteve J, Porkka K, Knapper S, Traer E, Scholl S, et al. Efficacy and Safety of Sabatolimab (MBG453) in Combination with Hypomethylating Agents (HMAs) in Patients (Pts) with Very High/High-Risk Myelodysplastic Syndrome (vHR/HR-MDS) and Acute Myeloid Leukemia (AML): Final Analysis from a Phase Ib Study. Blood 2021;138:244.
[80] Zeidan AM, Ando K, Rauzy O, Turgut M, Wang M-C, Cairoli R, et al. Primary results of Stimulus-MDS1: A randomized, double-blind, placebo-controlled phase II study of TIM-3 inhibition with Sabatolimab added to Hypomethylating agents (HMAs) in adult patients with higher-risk myelodysplastic syndromes (MDS). Blood. 2022;140:2063–5.
[81] Zeidan AM, Esteve J, Giagounidis A, Kim H-J, Miyazaki Y, Platzbecker U, et al. The STIMULUS program: clinical trials evaluating Sabatolimab (MBG453) combination therapy in patients (pts) with higher-risk myelodysplastic syndromes (HR-MDS) or acute myeloid Leukemia (AML). Blood. 2020;136:45–6.
[82] Chao MP, Takimoto CH, Feng DD, McKenna K, Gip P, Liu J, et al. Therapeutic targeting of the macrophage immune checkpoint CD47 in myeloid malignancies. Front Oncol 2020;9:1380.
[83] Majeti R, Chao MP, Alizadeh AA, Pang WW, Jaiswal S, Gibbs Jr KD, et al. CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. Cell. 2009;138:286–99.
[84] Jiang H, Fu R, Wang H, Li L, Liu H, Shao Z. CD47 is expressed abnormally on hematopoietic cells in myelodysplastic syndrome. Leuk Res 2013;37:907–10.
[85] Pang WW, Pluvinage JV, Price EA, Sridhar K, Arber DA, Greenberg PL, et al. Hematopoietic stem cell and progenitor cell mechanisms in myelodysplastic syndromes. Proc Natl Acad Sci U S A 2013;110:3011–6.
[86] Sallman DA, Al Malki MM, Asch AS, Wang ES, Jurcic JG, Bradley TJ, et al. S166: Magrolimab in combination with azacitidine for patients with untreated higher-risk myelodysplastic syndromes (HR MDS): 5F9005 phase 1B study results. HemaSphere. 2022;6:67–8.
[87] Daver N, Senapati J, Maiti A, Loghavi S, Kadia TM, DiNardo CD, et al. Phase I/II study of Azacitidine (AZA) with Venetoclax (VEN) and Magrolimab (Magro) in patients (pts) with newly diagnosed (ND) older/unfit or high-risk acute myeloid Leukemia (AML) and relapsed/refractory (R/R) AML. Blood. 2022;140:141–4.
[88] Kauder SE, Kuo TC, Harrabi O, Chen A, Sangalang E, Doyle L, et al. ALX148 blocks CD47 and enhances innate and adaptive antitumor immunity with a favorable safety profile. PLoS One 2018;13:e0201832.
[89] Lakhani NJ, Chow LQM, Gainor JF, LoRusso P, Lee KW, Chung HC, et al. Evorpacept alone and in combination with pembrolizumab or trastuzumab in patients with advanced solid tumours (ASPEN-01): a first-in-human, open-label, multicentre, phase 1 dose-escalation and dose-expansion study. Lancet Oncol 2021;22:1740–51.
[90] Garcia-Manero G, Erba HP, Sanikommu SR, Altman JK, Sayar H, Scott BL, et al. Evorpacept (ALX148), a CD47-Blocking Myeloid Checkpoint Inhibitor, in Combination with Azacitidine: A Phase 1 / 2 Study in Patients with Myelodysplastic Syndrome (ASPEN-02). Blood 2021;138:2601.
[91] Garcia-Manero G, Przespolewski A, Abaza Y, Byrne M, Fong AP, Jin F, et al. Evorpacept (ALX148), a CD47-blocking myeloid checkpoint inhibitor, in combination with Azacitidine and Venetoclax in patients with acute myeloid Leukemia (ASPEN-05): results from phase 1a dose escalation part. Blood. 2022; 140:9046–7.
[92] Ansell SM, Maris MB, Lesokhin AM, Chen RW, Flinn IW, Sawas A, et al. Phase I study of the CD47 blocker TTI-621 in patients with relapsed or refractory hematologic malignancies. Clin Cancer Res 2021;27:2190–9.
[93] Wang L, Jia B, Claxton DF, Ehmann WC, Rybka WB, Mineishi S, et al. VISTA is highly expressed on MDSCs and mediates an inhibition of T cell response in patients with AML. Oncoimmunology. 2018;7:e1469594.
[94] Mostböck S, Wu HH, Fenn T, Riegler B, Strahlhofer S, Huang Y, et al. Distinct immune stimulatory effects of anti-human VISTA antibodies are determined by fc-receptor interaction. Front Immunol 2022;13:862757.
[95] Kim TK, Han X, Hu Q, Hedgepath C, Hong J, Park J, et al. Programmed Death-1 homolog (PD-1H/VISTA) blockade confers a synergistic anti-Leukemia effect with PD-1 blockade. Blood. 2022;140:87–8.
[96] Villarroel-Espindola F, Yu X, Datar I, Mani N, Sanmamed M, Velcheti V, et al. Spatially resolved and quantitative analysis of VISTA/PD-1H as a novel immunotherapy target in human non-small cell lung cancer. Clin Cancer Res 2018;24:1562–73.
[97] Gao J, Ward JF, Pettaway CA, Shi LZ, Subudhi SK, Vence LM, et al. VISTA is an inhibitory immune checkpoint that is increased after ipilimumab therapy in patients with prostate cancer. Nat Med 2017;23:551–5.
[98] Stahl M, Bewersdorf JP, Giri S, Wang R, Zeidan AM. Use of immunosuppressive therapy for management of myelodysplastic syndromes: a systematic review and meta-analysis. Haematologica. 2020;105:102–11.
[99] Passweg JR, Giagounidis AA, Simcock M, Aul C, Dobbelstein C, Stadler M, et al. Immunosuppressive therapy for patients with myelodysplastic syndrome: a prospective randomized multicenter phase III trial comparing antithymocyte globulin plus cyclosporine with best supportive care–SAKK 33/99. J Clin Oncol 2011;29:303–9.
[100] Grayson PC, Patel BA, Young NS. VEXAS syndrome. Blood. 2021;137:3591–4.
[101] Comont T, Heiblig M, Rivière E, Terriou L, Rossignol J, Bouscary D, et al. Azacitidine for patients with vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic syndrome (VEXAS) and myelodysplastic syndrome: data from the French VEXAS registry. Br J Haematol 2022;196:969–74.
[102] Mekinian A, Zhao LP, Chevret S, Desseaux K, Pascal L, Comont T, et al. A phase II prospective trial of azacitidine in steroid-dependent or refractory systemic autoimmune/inflammatory disorders and VEXAS syndrome associated with MDS and CMML. Leukemia. 2022;36:2739–42.

[103] Yeaton A, Cayanan G, Loghavi S, Dolgalev I, Leddin EM, Loo CE, et al. The impact of inflammation-induced tumor plasticity during myeloid transformation. Cancer Discov 2022;12:2392–413.
[104] Mangan MSJ, Olhava EJ, Roush WR, Seidel HM, Glick GD, Latz E. Targeting the NLRP3 inflammasome in inflammatory diseases. Nat Rev Drug Discov 2018;17:588–606.
[105] Bennett J, Starczynowski DT. IRAK1 and IRAK4 as emerging therapeutic targets in hematologic malignancies. Curr Opin Hematol 2022;29:8–19.
[106] Trowbridge JJ, Starczynowski DT. Innate immune pathways and inflammation in hematopoietic aging, clonal hematopoiesis, and MDS. J Exp Med 2021;218.
[107] Smith MA, Choudhary GS, Pellagatti A, Choi K, Bolanos LC, Bhagat TD, et al. U2AF1 mutations induce oncogenic IRAK4 isoforms and activate innate immune pathways in myeloid malignancies. Nat Cell Biol 2019;21:640–50.
[108] Choudhary GS, Pellagatti A, Agianian B, Smith MA, Bhagat TD, Gordon-Mitchell S, et al. Activation of targetable inflammatory immune signaling is seen in myelodysplastic syndromes with SF3B1 mutations. Elife. 2022:11.
[109] McLemore AF, Hou H-A, Meyer BS, Lam NB, Ward GA, Aldrich AL, et al. Somatic gene mutations expose cytoplasmic DNA to co-opt the cGAS/STING/NLRP3 axis in myelodysplastic syndromes. JCI. Insight. 2022:7.
[110] Garcia-Manero G, Winer ES, DeAngelo DJ, Tarantolo SR, Sallman DA, Dugan J, et al. Phase 1/2a study of the IRAK4 inhibitor CA-4948 as monotherapy or in combination with azacitidine or venetoclax in patients with relapsed/refractory (R/R) acute myeloid leukemia or lyelodysplastic syndrome. J Clin Oncol 2022;40:7016.
[111] Chen C, Man N, Liu F, Martin GM, Itonaga H, Sun J, et al. Epigenetic and transcriptional regulation of innate immunity in cancer. Cancer Res 2022;82:2047–56.
[112] Sugita M, Galetto R, Zong H, Ewing-Crystal N, Trujillo-Alonso V, Mencia-Trinchant N, et al. Allogeneic TCRαβ deficient CAR T-cells targeting CD123 in acute myeloid leukemia. Nat Commun 2022;13:2227.
[113] Ravandi F, Walter RB, Subklewe M, Buecklein V, Jongen-Lavrencic M, Paschka P, et al. Updated results from phase I dose-escalation study of AMG 330, a bispecific T-cell engager molecule, in patients with relapsed/refractory acute myeloid leukemia (R/R AML). J Clin Oncol 2020;38:7508.
[114] Subklewe M, Stein A, Walter RB, Bhatia R, Wei AH, Ritchie D, et al. Preliminary Results from a Phase 1 First-in-Human Study of AMG 673, a Novel Half-Life Extended (HLE) Anti-CD33/CD3 BiTE® (Bispecific T-Cell Engager) in Patients with Relapsed/Refractory (R/R) Acute Myeloid Leukemia (AML). Blood 2019;134:833.
[115] Pan R, Ryan J, Pan D, Wucherpfennig KW, Letai A. Augmenting NK cell-based immunotherapy by targeting mitochondrial apoptosis. Cell. 2022;185(1521–38):e18.
[116] DiNardo CD, Jonas BA, Pullarkat V, Thirman MJ, Garcia JS, Wei AH, et al. Azacitidine and venetoclax in previously untreated acute myeloid leukemia. N Engl J Med 2020;383:617–29.
[117] Wei AH, Panayiotidis P, Montesinos P, Laribi K, Ivanov V, Kim I, et al. Long-term follow-up of VIALE-C in patients with untreated AML ineligible for intensive chemotherapy. Blood. 2022. Dec 22;140(25):2754–6.
[118] Slape CI, Saw J, Jowett JB, Aplan PD, Strasser A, Jane SM, et al. Inhibition of apoptosis by BCL2 prevents leukemic transformation of a murine myelodysplastic syndrome. Blood. 2012;120:2475–83.
[119] Garcia JS, Wei AH, Jacoby MA, Fong CY, Borate U, Baer MR, et al. Molecular responses are observed across mutational Spectrum in treatment-Naïve higher-risk myelodysplastic syndrome patients treated with Venetoclax plus Azacitidine. Blood. 2021;138:241.
[120] Garcia JS, Wei AH, Borate U, Fong CY, Baer MR, Nolte F, et al. Safety, efficacy, and patient-reported outcomes of Venetoclax in combination with Azacitidine for the treatment of patients with higher-risk myelodysplastic syndrome: a phase 1b study. Blood. 2020;136:55–7.
[121] Bazinet A, Darbaniyan F, Jabbour E, Montalban-Bravo G, Ohanian M, Chien K, et al. Azacitidine plus venetoclax in patients with high-risk myelodysplastic syndromes or chronic myelomonocytic leukaemia: phase 1 results of a single-Centre, dose-escalation, dose-expansion, phase 1-2 study. Lancet Haematol 2022;9. e756-e65.
[122] Zeidan AM, Borate U, Pollyea DA, Brunner AM, Roncolato F, Garcia JS, et al. A phase 1b study of venetoclax and azacitidine combination in patients with relapsed or refractory myelodysplastic syndromes. Am J Hematol. 2023. Feb;98(2):272–81.
[123] Huang H-T, Figueroa ME. Epigenetic deregulation in myeloid malignancies. Blood. 2021;138:613–24.
[124] Bewersdorf JP, Shallis R, Stahl M, Zeidan AM. Epigenetic therapy combinations in acute myeloid leukemia: what are the options? Ther Adv Hematol 2019;10.
[125] Figueroa ME, Skrabanek L, Li Y, Jiemjit A, Fandy TE, Paietta E, et al. MDS and secondary AML display unique patterns and abundance of aberrant DNA methylation. Blood. 2009;114:3448–58.
[126] DiNardo CD, Patel KP, Garcia-Manero G, Luthra R, Pierce S, Borthakur G, et al. Lack of association of IDH1, IDH2 and DNMT3A mutations with outcome in older patients with acute myeloid leukemia treated with hypomethylating agents. Leuk Lymphoma 2014;55:1925–9.
[127] Prebet T, Sun Z, Figueroa ME, Ketterling R, Melnick A, Greenberg PL, et al. Prolonged administration of azacitidine with or without entinostat for myelodysplastic syndrome and acute myeloid leukemia with myelodysplasia-related changes: results of the US Leukemia intergroup trial E1905. J Clin Oncol 2014;32:1242–8.
[128] Glass JL, Hassane D, Wouters BJ, Kunimoto H, Avellino R, Garrett-Bakelman FE, et al. Epigenetic identity in AML depends on disruption of nonpromoter regulatory elements and is affected by antagonistic effects of mutations in epigenetic modifiers. Cancer Discov 2017;7:868–83.
[129] Telonis AG, Yang Q, Huang HT, Figueroa ME. MIR retrotransposons link the epigenome and the transcriptome of coding genes in acute myeloid leukemia. Nat Commun 2022;13:6524.
[130] Yang Q, Brogi A, Griffiths EA, Follo MY, Finelli C, Radich JP, et al. DNA methylation profiles capture clinical and molecular heterogeneity of MDS and can be harnessed for the development of robust biomarkers predictive of response to Azacitidine. Blood. 2022;140:9769–70.
[131] Meldi K, Qin T, Buchi F, Droin N, Sotzen J, Micol JB, et al. Specific molecular signatures predict decitabine response in chronic myelomonocytic leukemia. J Clin Invest 2015;125:1857–72.
[132] Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification and prognosis in acute myeloid Leukemia. N Engl J Med 2016;374:2209–21.
[133] Challen GA, Sun D, Jeong M, Luo M, Jelinek J, Berg JS, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. Nat Genet 2011;44:23–31.
[134] Jeong M, Park HJ, Celik H, Ostrander EL, Reyes JM, Guzman A, et al. Loss of Dnmt3a immortalizes hematopoietic stem cells in vivo. Cell Rep 2018;23:1–10.
[135] Yang L, Rodriguez B, Mayle A, Park HJ, Lin X, Luo M, et al. DNMT3A loss drives enhancer Hypomethylation in FLT3-ITD-associated Leukemias. Cancer Cell 2016;29:922–34.
[136] Ko M, Huang Y, Jankowska AM, Pape UJ, Tahiliani M, Bandukwala HS, et al. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. Nature. 2010;468:839.
[137] Moran-Crusio K, Reavie L, Shih A, Abdel-Wahab O, Ndiaye-Lobry D, Lobry C, et al. Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. Cancer Cell 2011;20:11–24.
[138] Cimmino L, Dolgalev I, Wang Y, Yoshimi A, Martin GH, Wang J, et al. Restoration of TET2 function blocks aberrant self-renewal and Leukemia progression. Cell. 2017;170(1079–95):e20.
[139] Thol F, Weissinger EM, Krauter J, Wagner K, Damm F, Wichmann M, et al. IDH1 mutations in patients with myelodysplastic syndromes are associated with an unfavorable prognosis. Haematologica. 2010;95:1668–74.
[140] Gross S, Cairns RA, Minden MD, Driggers EM, Bittinger MA, Jang HG, et al. Cancer-associated metabolite 2-hydroxyglutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations. J Exp Med 2010;207:339–44.
[141] Lu C, Ward PS, Kapoor GS, Rohle D, Turcan S, Abdel-Wahab O, et al. IDH mutation impairs histone demethylation and results in a block to cell differentiation. Nature. 2012;483:474–8.
[142] Stein EM, DiNardo CD, Pollyea DA, Fathi AT, Roboz GJ, Altman JK, et al. Enasidenib in mutant IDH2 relapsed or refractory acute myeloid leukemia. Blood. 2017;130:722–31.
[143] Montesinos P, Recher C, Vives S, Zarzycka E, Wang J, Bertani G, et al. Ivosidenib and Azacitidine in IDH1-mutated acute myeloid Leukemia. N Engl J Med 2022;386:1519–31.
[144] DiNardo CD, Stein EM, de Botton S, Roboz GJ, Altman JK, Mims AS, et al. Durable remissions with Ivosidenib in IDH1-mutated relapsed or refractory AML. N Engl J Med 2018;378:2386–98.
[145] Sallman DA, Foran JM, Watts JM, Stein E, Botton SD, Fathi AT, et al. Ivosidenib in patients with IDH1-mutant relapsed/refractory myelodysplastic syndrome (R/R MDS): updated enrollment and results of a phase 1 dose-escalation and expansion substudy. J Clin Oncol 2022;40:7053.
[146] DiNardo CD, Venugopal S, Lachowiez CA, Takahashi K, Loghavi S, Montalban-Bravo G, et al. Targeted therapy with the mutant IDH2 inhibitor enasidenib for high-risk IDH2-mutant myelodysplastic syndrome. Blood Adv. 2022 Aug 16.
[147] Watts JM, Baer MR, Yang J, Prebet T, Lee S, Schiller GJ, et al. Olutasidenib alone or with azacitidine in IDH1-mutated acute myeloid leukaemia and myelodysplastic syndrome: phase 1 results of a phase 1/2 trial. Lancet Haematol.
[148] Sun Y, Zhou B, Mao F, Xu J, Miao H, Zou Z, et al. HOXA9 reprograms the enhancer landscape to promote Leukemogenesis. Cancer Cell 2018;34(643–58):e5.
[149] Bernt KM, Zhu N, Sinha AU, Vempati S, Faber J, Krivtsov AV, et al. MLL-rearranged leukemia is dependent on aberrant H3K79 methylation by DOT1L. Cancer Cell 2011;20:66–78.
[150] Heimbruch KE, Fisher JB, Stelloh CT, Phillips E, Reimer Jr MH, Wargolet AJ, et al. DOT1L inhibitors block abnormal self-renewal induced by cohesin loss. Sci Rep 2021;11:7288.
[151] Rau RE, Rodriguez BA, Luo M, Jeong M, Rosen A, Rogers JH, et al. DOT1L as a therapeutic target for the treatment of DNMT3A-mutant acute myeloid leukemia. Blood. 2016;128:971–81.
[152] Stein EM, Garcia-Manero G, Rizzieri DA, Tibes R, Berdeja JG, Savona MR, et al. The DOT1L inhibitor pinometostat reduces H3K79 methylation and has modest clinical activity in adult acute leukemia. Blood. 2018;131:2661–9.
[153] Bewersdorf JP, Abdel-Wahab O. Translating recent advances in the pathogenesis of acute myeloid leukemia to the clinic. Genes Dev 2022;36:259–77.
[154] Krivtsov AV, Evans K, Gadrey JY, Eschle BK, Hatton C, Uckelmann HJ, et al. A Menin-MLL inhibitor induces specific chromatin changes and eradicates disease in models of MLL-rearranged Leukemia. Cancer Cell 2019;36(660–73):e11.
[155] Zhang S, Liu M, Yao Y, Yu B, Liu H. Targeting LSD1 for acute myeloid leukemia (AML) treatment. Pharmacol Res 2021;164:105335.
[156] Saleque S, Kim J, Rooke HM, Orkin SH. Epigenetic regulation of hematopoietic differentiation by Gfi-1 and Gfi-1b is mediated by the cofactors CoREST and LSD1. Mol Cell 2007;27:562–72.

[157] Cusan M, Cai SF, Mohammad HP, Krivtsov A, Chramiec A, Loizou E, et al. LSD1 inhibition exerts its antileukemic effect by recommissioning PU.1- and C/EBPalpha-dependent enhancers in AML. Blood. 2018;131:1730–42.
[158] Barth J, Abou-El-Ardat K, Dalic D, Kurrle N, Maier A-M, Mohr S, et al. LSD1 inhibition by tranylcypromine derivatives interferes with GFI1-mediated repression of PU.1 target genes and induces differentiation in AML. Leukemia. 2019;33:1411–26.
[159] Kohrogi K, Hino S, Sakamoto A, Anan K, Takase R, Araki H, et al. LSD1 defines erythroleukemia metabolism by controlling the lineage-specific transcription factors GATA1 and C/EBPα. Blood Adv 2021;5:2305–18.
[160] Watts JM, Bradley TJ, Thomassen A, Dinh YT, Tejera D, Englund K, et al. The Lysine-Specific Demethylase 1 (LSD1) Inhibitor Tranylcypromine (TCP) in Combination with ATRA Is Tolerable and Has Anti-Leukemic Activity in Adult Patients with Relapsed/Refractory AML and MDS. Blood 2018;132:2721.
[161] DiNardo CD, Borthakur G, Ball B, Erba HP, Mawad R, Kremyanskaya M, et al. Phase 1/2 Study Investigating CC-90011, a Potent, Selective, and Reversible Oral Inhibitor of Lysine-Specific Demethylase 1 (LSD1), Plus Concurrent Venetoclax (VEN) and Azacitidine (AZA) in Patients with Acute Myeloid Leukemia (AML). Blood 2021;138:4430.
[162] Devillier R, Mansat-De Mas V, Gelsi-Boyer V, Demur C, Murati A, Corre J, et al. Role of ASXL1 and TP53 mutations in the molecular classification and prognosis of acute myeloid leukemias with myelodysplasia-related changes. Oncotarget. 2015;6:8388–96.
[163] Döhner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H, et al. Diagnosis and management of AML in adults: 2022 ELN recommendations from an international expert panel. Blood. 2022. Sep 22;140(12):1345–77.
[164] Balasubramani A, Larjo A, Bassein JA, Chang X, Hastie RB, Togher SM, et al. Cancer-associated ASXL1 mutations may act as gain-of-function mutations of the ASXL1-BAP1 complex. Nat Commun 2015;6:7307.
[165] Asada S, Goyama S, Inoue D, Shikata S, Takeda R, Fukushima T, et al. Mutant ASXL1 cooperates with BAP1 to promote myeloid leukaemogenesis. Nat Commun 2018;9:2733.
[166] Wang L, Birch NW, Zhao Z, Nestler CM, Kazmer A, Shilati A, et al. Epigenetic targeted therapy of stabilized BAP1 in ASXL1 gain-of-function mutated leukemia. Nat Can 2021;2:515–26.
[167] Campagne A, Lee MK, Zielinski D, Michaud A, Le Corre S, Dingli F, et al. BAP1 complex promotes transcription by opposing PRC1-mediated H2A ubiquitylation. Nat Commun 2019;10:348.
[168] Uni M, Masamoto Y, Sato T, Kamikubo Y, Arai S, Hara E, et al. Modeling ASXL1 mutation revealed impaired hematopoiesis caused by derepression of p16Ink4a through aberrant PRC1-mediated histone modification. Leukemia. 2019;33:191–204.
[169] Steensma DP, Fenaux P, Van Eygen K, Raza A, Santini V, Germing U, et al. Imetelstat achieves meaningful and durable transfusion Independence in high transfusion-burden patients with lower-risk myelodysplastic syndromes in a phase II study. J Clin Oncol 2021;39:48–56.
[170] Dong W, Qian Y, Yang L. Telomerase, hTERT and splice variants in patients with myelodysplastic syndromes. Leuk Res 2014;38:830–5.
[171] Wang X, Hu CS, Petersen B, Qiu J, Ye F, Houldsworth J, et al. Imetelstat, a telomerase inhibitor, is capable of depleting myelofibrosis stem and progenitor cells. Blood Adv 2018;2:2378–88.
[172] Baerlocher GM, Oppliger Leibundgut E, Ottmann OG, Spitzer G, Odenike O, McDevitt MA, et al. Telomerase inhibitor Imetelstat in patients with essential Thrombocythemia. N Engl J Med 2015;373:920–8.
[173] Corporation G. Geron announces positive top-line results from IMerge phase 3 trial of Imetelstat in lower risk MDS. 2023.
[174] Chen S, Benbarche S, Abdel-Wahab O. Splicing factor mutations in hematologic malignancies. Blood. 2021;138:599–612.
[175] Bejar R, Papaemmanuil E, Haferlach T, Garcia-Manero G, Maciejewski JP, Sekeres MA, et al. Somatic mutations in MDS patients are associated with clinical features and predict prognosis independent of the IPSS-R: analysis of combined datasets from the international working Group for Prognosis in MDS-Molecular Committee. Blood. 2015;126:907.
[176] Ohgami RS, Ma L, Merker JD, Gotlib JR, Schrijver I, Zehnder JL, et al. Next-generation sequencing of acute myeloid leukemia identifies the significance of TP53, U2AF1, ASXL1, and TET2 mutations. Mod Pathol 2015;28:706–14.
[177] Thol F, Kade S, Schlarmann C, Löffeld P, Morgan M, Krauter J, et al. Frequency and prognostic impact of mutations in SRSF2, U2AF1, and ZRSR2 in patients with myelodysplastic syndromes. Blood. 2012;119:3578–84.
[178] Nadeu F, Delgado J, Royo C, Baumann T, Stankovic T, Pinyol M, et al. Clinical impact of clonal and subclonal TP53, SF3B1, BIRC3, NOTCH1, and ATM mutations in chronic lymphocytic leukemia. Blood. 2016;127:2122–30.
[179] El Marabti E, Abdel-Wahab O. Therapeutic modulation of RNA splicing in malignant and non-malignant disease. Trends Mol Med 2021;27:643–59.
[180] Seiler M, Yoshimi A, Darman R, Chan B, Keaney G, Thomas M, et al. H3B-8800, an orally available small-molecule splicing modulator, induces lethality in spliceosome-mutant cancers. Nat Med 2018;24:497–504.
[181] Steensma DP, Wermke M, Klimek VM, Greenberg PL, Font P, Komrokji RS, et al. Phase I first-in-human dose escalation study of the oral SF3B1 modulator H3B-8800 in myeloid neoplasms. Leukemia. 2021;35:3542–50.
[182] Han T, Goralski M, Gaskill N, Capota E, Kim J, Ting TC, et al. Anticancer sulfonamides target splicing by inducing RBM39 degradation via recruitment to DCAF15. Science. 2017;356:eaal3755.
[183] Assi R, Kantarjian HM, Kadia TM, Pemmaraju N, Jabbour E, Jain N, et al. Final results of a phase 2, open-label study of indisulam, idarubicin, and cytarabine in patients with relapsed or refractory acute myeloid leukemia and high-risk myelodysplastic syndrome. Cancer. 2018;124:2758–65.
[184] Bewersdorf JP, Stahl MF, Taylor J, Chandhok NS, Watts J, Derkach A, et al. A phase II clinical trial of E7820 for patients with relapsed/refractory myeloid malignancies with mutations in splicing factor genes. Blood. 2022;140:9065–7.
[185] Lu SX, De Neef E, Thomas JD, Sabio E, Rousseau B, Gigoux M, et al. Pharmacologic modulation of RNA splicing enhances anti-tumor immunity. Cell. 2021;184(4032–47):e31.
[186] Flach J, Jann JC, Knaflic A, Riabov V, Streuer A, Altrock E, et al. Replication stress signaling is a therapeutic target in myelodysplastic syndromes with splicing factor mutations. Haematologica. 2021;106:2906–17.
[187] Singh S, Ahmed D, Dolatshad H, Tatwavedi D, Schulze U, Sanchi A, et al. SF3B1 mutations induce R-loop accumulation and DNA damage in MDS and leukemia cells with therapeutic implications. Leukemia. 2020;34:2525–30.
[188] Nguyen HD, Leong WY, Li W, Reddy PNG, Sullivan JD, Walter MJ, et al. Spliceosome mutations induce R loop-associated sensitivity to ATR inhibition in myelodysplastic syndromes. Cancer Res 2018;78:5363–74.
[189] Chen L, Chen JY, Huang YJ, Gu Y, Qiu J, Qian H, et al. The augmented R-loop is a unifying mechanism for myelodysplastic syndromes induced by high-risk splicing factor mutations. Mol Cell 2018;69(412–25):e6.
[190] Nagata Y, Makishima H, Kerr CM, Przychodzen BP, Aly M, Goyal A, et al. Invariant patterns of clonal succession determine specific clinical features of myelodysplastic syndromes. Nat Commun 2019;10:5386.
[191] Zhang X, List AF, Lancet JE, Jinming S, Moscinski LC, Sokol L, et al. Clinicopathological features and mutational analysis of patients with aplastic Anemia and Hypoplastic myelodysplastic syndrome. Blood. 2016;128:1993.
[192] Stahl M, Abdel-Wahab O, Wei AH, Savona MR, Xu ML, Xie Z, et al. An agenda to advance research in MDS: a TOP 10 priority List from the first international workshop in MDS (iwMDS). Blood Adv. 2022 Oct 19.
[193] Zeidan AM, Platzbecker U, Bewersdorf JP, Stahl M, Adès L, Borate U, et al. Consensus proposal for revised international working group response criteria for higher risk myelodysplastic syndromes. Blood. 2023. Feb 1.
[194] Chen X, Othus M, Wood BL, Walter RB, Becker PS, Percival ME, et al. Comparison of myeloid blast counts and variant allele frequencies of gene mutations in myelodysplastic syndrome with excess blasts and secondary acute myeloid leukemia. Leuk Lymphoma 2021;62:1226–33.
[195] Estey E, Hasserjian RP, Döhner H. Distinguishing AML from MDS: a fixed blast percentage may no longer be optimal. Blood. 2022;139:323–32.
[196] Ball S, Singh AM, Ali NA, Aguirre LE, Jain AG, Schwabkey ZI, et al. A product of "clash of titans" or true reflection of disease biology? Validation of 2022 WHO and ICC classifications in a large dataset of patients with myelodysplastic syndrome. Blood. 2022;140:1118–20.
[197] Rodriguez-Meira A, Norfo R, Wen WX, Chédeville AL, Rahman H, O'Sullivan J, et al. Deciphering TP53 mutant cancer evolution with Single-Cell Multi-Omics. bioRxiv 2022. 2022.03.28.485984.
[198] Platzbecker U, Middeke JM, Sockel K, Herbst R, Wolf D, Baldus CD, et al. Measurable residual disease-guided treatment with azacitidine to prevent haematological relapse in patients with myelodysplastic syndrome and acute myeloid leukaemia (RELAZA2): an open-label, multicentre, phase 2 trial. Lancet Oncol 2018;19:1668–79.
[199] Miles LA, Bowman RL, Merlinsky TR, Csete IS, Ooi AT, Durruthy-Durruthy R, et al. Single-cell mutation analysis of clonal evolution in myeloid malignancies. Nature. 2020;587:477–82.
[200] Dillon LW, Gui G, Logan BR, Fei M, Ghannam J, Li Y, et al. Impact of conditioning intensity and genomics on relapse after allogeneic transplantation for patients with myelodysplastic syndrome. JCO precis. Oncol. 2021:265–74.
[201] Duncavage EJ, Bagg A, Hasserjian RP, DiNardo CD, Godley LA, Iacobucci I, et al. Genomic profiling for clinical decision making in myeloid neoplasms and acute Leukemia. Blood. 2022. Nov 24;140(21):2228–47.
[202] Bolton KL, Gillis NK, Coombs CC, Takahashi K, Zehir A, Bejar R, et al. Managing clonal hematopoiesis in patients with solid Tumors. J Clin Oncol 2018:JCO1800331.
[203] Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391–405.
[204] Stengel A, Meggendorfer M, Walter W, Baer C, Nadarajah N, Hutter S, et al. Interplay of TP53 allelic state, blast count and karyotype on survival of patients with AML and MDS. Blood. 2022;140:2073–4.
[205] Gómez MJM, Palomo L, Vidal P, Fuster-Tormo F, Such E, Ortega M, et al. TP53 allelic state in myelodysplastic syndromes with 5q deletion. Blood. 2022;140:6949–51.
[206] Zeidan AM, Boss I, Beach CL, Copeland WB, Thompson E, Fox BA, et al. A randomized phase 2 trial of azacitidine with or without durvalumab as first-line therapy for older patients with AML. Blood Adv 2022;6:2219–29.
[207] Sallman DA, DeZern AE, Garcia-Manero G, Steensma DP, Roboz GJ, Sekeres MA, et al. Eprenetapopt (APR-246) and Azacitidine in TP53-mutant myelodysplastic syndromes. J Clin Oncol 2021;39:1584–94.
[208] Cluzeau T, Sebert M, Rahmé R, Cuzzubbo S, Lehmann-Che J, Madelaine I, et al. Eprenetapopt plus Azacitidine in TP53-mutated myelodysplastic syndromes and acute myeloid Leukemia: a phase II study by the Groupe francophone des Myélodysplasies (GFM). J Clin Oncol 2021;39:1575–83.
[209] Adès L, Girshova L, Doronin VA, Díez-Campelo M, Valcárcel D, Kambhampati S, et al. Pevonedistat plus azacitidine vs azacitidine alone in higher-risk MDS/chronic myelomonocytic leukemia or low-blast-percentage AML. Blood Adv 2022;6:5132–45.