Molecular insights and treatment innovations: Advancing outcomes in acute myeloid leukemia with myelodysplasia-related changes (Review)

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Abstract. Acute myeloid leukemia, myelodysplasia-related (AML-MR), a challenging and aggressive subtype of AML, is characterized by unique genetic abnormalities and molecular features, which contribute to its poor prognosis compared with other AML subtypes. The present review summarizes the current understanding of AML-MR pathogenesis, highlighting notable advancements in genetic and cytogenetic insights. Critical mutations, such as those in the tumor antigen p53 and additional sex combs like 1 genes, and their role in disease progression and resistance to treatment, are explored. The review further investigates how clonal evolution and cellular microenvironment alterations drive AML-MR transformation and impact patient outcomes. Despite the poor outlook typically associated with AML-MR, developments in treatment approaches offer hope. The present review considers the efficacy of novel therapeutic agents, including CPX-351, hypomethylating agents and targeted molecular therapies. Additionally, innovations in immunotherapy and allogeneic hematopoietic stem cell transplantation are discussed as promising avenues to improve patient survival rates. The challenges of treating AML-MR, particularly in elderly and pretreated patients, underline the necessity for individualized treatment strategies that consider both the biological complexity of the disease and the overall health profile of the patient. The present review focuses on the mechanisms of AML-MR transformation, highlighting factors that may offer a crucial theoretical foundation and pave the way for future applications in precision medicine. Future research directions include exploring novel targeted therapies and combination regimens to mitigate the transformation risks and enhance the quality of life of patients with AML-MR.

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Contents

1. Introduction

Acute myeloid leukemia (AML) is a malignant tumor of myeloid hematopoietic stem cells (HSCs) that is characterized by abnormal proliferation of primitive and immature myeloid cells in the bone marrow and peripheral blood (1). The clinical manifestations include pancytopenia, anemia, fever, infection, hemorrhage and extramedullary infiltration. Patients with this disease are often critically ill and have a poor prognosis (2-4). Prognosis is related to age, and patients have a median age of 68 years at diagnosis (5). AML is divided into different subtypes based on genetic characteristics, including AML with recurrent genetic abnormalities, AML without recurrent genetic abnormalities (classified according to the differentiation level of the leukemia cells) and special types of AML (such as treatment-related AML, germline susceptibility-related myeloid malignancies and myeloid malignancies associated with Down syndrome) (6). AML, myelodysplasia-related (AML-MR) is one of the subtypes of AML with recurrent genetic abnormalities. AML-MR accounts for ~22.2% of AML cases and the median age at diagnosis is 61 years (7). Patients with AML-MR who have previously developed myelodysplastic syndromes (MDS) have shorter survival times and more severe side effects from treatment than those with newly developed AML-MR due to advanced age, previous hematological disorders and poor karyotypes (8,9).

The definition and diagnostic criteria of AML-MR have undergone continuous updates and are becoming

more precise. In 1982, the concept of AML associated with myelodysplasia-related features was first proposed by the French-American-British classification (10). In 2001, a new subtype of AML was introduced as 'AML with multilineage dysplasia', a category in which patients with AML were characterized by MDS-like manifestations (11). In 2008, AML with multilineage dysplasia was changed to 'AML with myelodysplastic syndrome-related changes', with the following diagnostic criteria: i) A history of MDS or MDS/myeloproliferative neoplasm (MPN) that has evolved to AML; ii) cytogenetic abnormalities associated with myelodysplasia; and iii) ≥50% of the cells in ≥2 bone marrow lineages are dysplastic (12). In 2016, patients were excluded according to the following criteria: i) Multilineage hyperplasia due to nucleophosmin 1 (NPM1) mutation or CCAAT enhancer binding protein α diallelic mutation; and ii) cytogenetically-related abnormalities unrelated to deletion (del) (9q) (13). In the fifth edition of the World Health Organization (WHO) guidelines in 2022, the most significant change was the separation of AML with defining genetic abnormalities from AML defined by differentiation (6). AML-MR is classified as a type of AML characterized by defining genetic abnormalities. As one of the subtypes of AML, AML-MR is a neoplasm with ≥20% blasts that expresses a myeloid immunophenotype, and harbors cytogenetic changes and molecular biological gene mutations associated with MDS (6). In 2022, the fifth edition of the WHO diagnostic criteria renamed AML with associated myelodysplasia-related changes as AML-MR. The update involved: i) Removal of morphology alone as a diagnostic premise for AML-MR; ii) an amendment of the defining cytogenetic criteria; and iii) the introduction of a mutation-based definition based on a set of eight genes (*ASXL1*, *BCOR*, *EZH2*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1* and *ZRSR2*) (6).

With the rapid development of molecular biology, the diagnosis and treatment of diseases have made great progress. The development of second-generation sequencing technology, which can detect common gene mutations and help determine the target of gene mutations for the selection of corresponding targeted drugs for treatment, has progressed the research on AML-MR gene mutations (14). The gene mutation detection rate in patients with AML is >20%, as detected by next-generation sequencing (15). Advances in genomics, proteomics and artificial intelligence have enhanced early genetic testing, aiding risk stratification and personalized treatment. Whole-genome sequencing and minimal residual disease detection are increasingly guiding individualized treatment decisions (16,17). Compared with patients with newly diagnosed AML, patients with AML-MR exhibit a poorer overall response to induction chemotherapy and low-intensity therapy, a higher relapse rate and a poorer overall survival (OS) (18,19). A study has shown that there is no significant difference in age or sex between patients with AML-MR with a history of MDS (MRC-MDS) and those with MDS-related cytogenetic abnormalities (MRC-Cyto) (20). The median OS time for patients with AML-MR with morphologic dysplasia is 20.4 months, compared with 5.3 months for patients with MRC-MDS and 6.3 months for patients with MRC-Cyto (20). Among patients with MRC-Cyto, those with a history of MDS have a shorter median OS time of 3.5 months, compared with 6.3 months in those without a history (20). However, there is no significant difference in OS between patients with and without a history of MDS in the normal karyotype group (20).

The present review discusses AML-MR and its mechanism in detail, with a focus on the notable role of cytogenetics and molecular biology in the diagnosis, treatment and prognostication of this disease. In addition, the present review summarizes the current treatment methods for AML-MR and suggests potential treatment directions, providing a valuable reference for the further research and clinical treatment of AML-MR.

2. Factors promoting MDS transformation

MDS is a malignant disease characterized by the clonal proliferation of HSCs, myelodysplasia, ineffective hematopoiesis, peripheral blood cytopenia and a high risk of developing into AML (21). The transformation into AML is mainly related to genetics, molecular biology, the bone marrow microenvironment and clonal evolution (22-25). The mechanisms driving the progression of AML-MR are illustrated in Fig. 1.

Genetic changes

Chromosomal changes. The diagnostic criteria of the WHO fifth edition guidelines suggest that certain chromosome karyotype abnormalities are related to myelodysplastic disorders. Cytogenetic abnormalities associated with AML-MR are listed in Table I (6). The most common abnormalities are trisomy 8 [-7/del (7q) or -5/del (5q)] and complex karyotypes (≥3 chromosomal abnormalities) (26,27). These abnormalities may lead to deletion or inactivation of important tumor suppressor genes. The deletion of 5q activates p53 by increasing ribosomal protein levels, which initially suppresses the progression of MDS to AML. However, tumor antigen p53 (TP53) mutations disrupt the role of p53 in maintaining genomic stability, increasing the risk of transformation. Consequently, the coexistence of TP53 mutations and 5q deletion represents a critical driver and an early warning signal for the progression of MDS to AML (28,29). Similar to 5q, the deletion of 7q is associated with high genomic complexity and a short survival time in patients with AML-MR. Chromosome 7q contains several key tumor suppressor genes related to myeloid malignancies such as sterile α motif domain containing 9 (SAMD9), SAMD9-like, enhancer of zeste homolog 2 (EZH2), cut like homeobox 1 and mixed-lineage leukemia protein 3. The deletion or mutation of these genes confers survival and growth advantages to hematopoietic stem and progenitor cells, thereby accelerating their progression to AML-MR (29-33). Complex karyotypes are associated with poor survival and a high risk of transformation to AML in patients with MDS. This risk is independent of blast percentage, cytopenias and transfusion dependence (34-36).

Gene mutations. A next-generation sequencing study has revealed that MDS and AML-MR share mutations in genes involved in cellular processes such as RNA splicing, epigenetic regulation, transcriptional regulation and DNA damage repair (14). The identification of these mutations across various cellular processes highlights the progression of clonal myeloid neoplasms from the early disease stages, such as clonal hematopoiesis of indeterminate potential, to MDS and AML-MR (3).

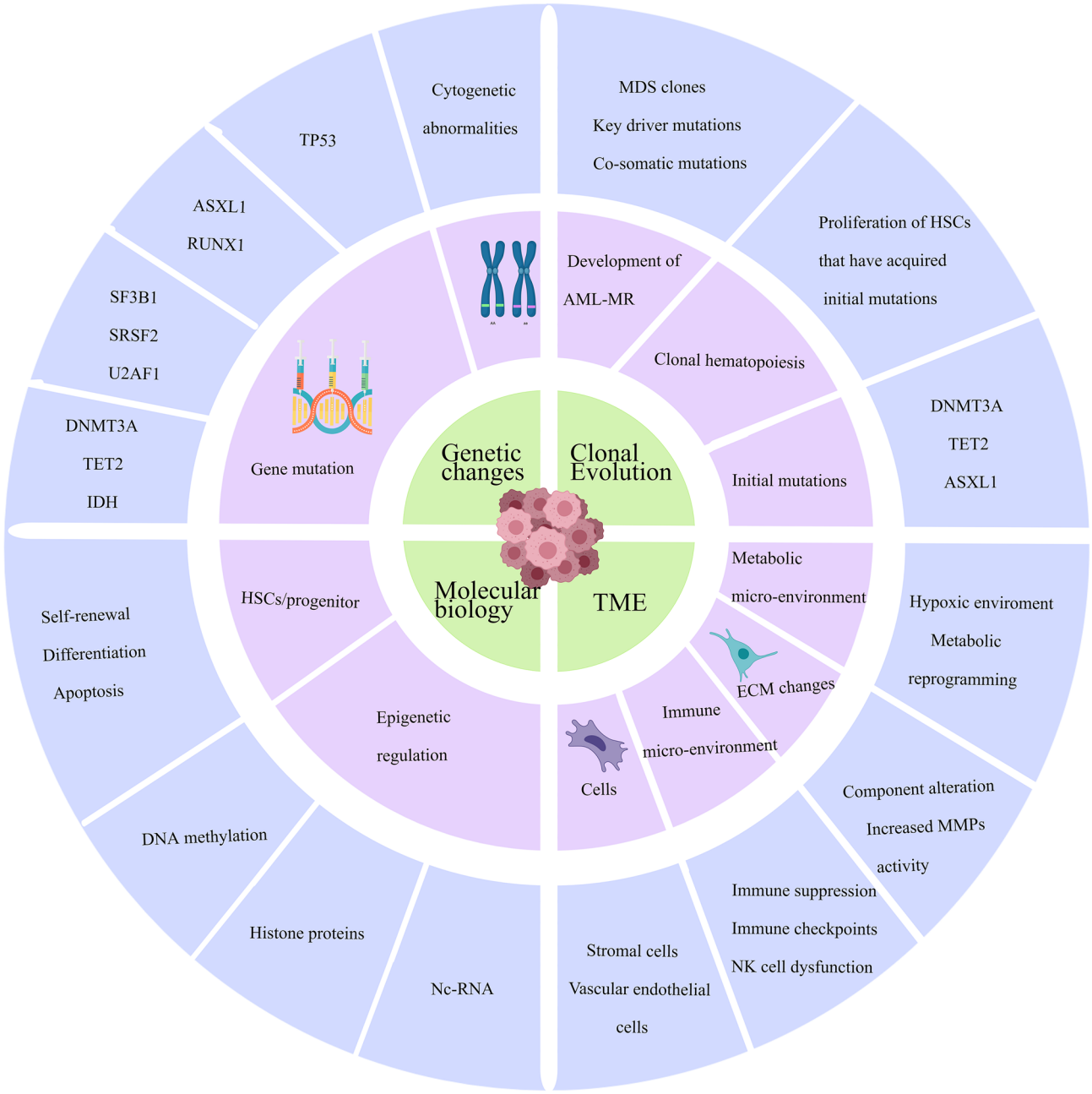

Figure 1. Factors that promote the transformation of AML-MR. AML-MR-related cytogenetic abnormalities are shown in Table I. ASXL1 and RUNX1, epigenetic regulation genes; SF3B1, SRSF2 and U2AF1, splicing-related genes; DNMT3A, TET2 and IDH, DNA methylation-related genes. The abnormalities in hematopoietic stem/progenitor cells are primarily manifested as enhanced self-renewal ability, blocked differentiation and resistance to apoptosis. Epigenetic regulation abnormalities are primarily manifested as altered DNA methylation, changes in histone protein post-translational modifications and dysregulated expression of nc-RNAs. The genetic mutations associated with histone post-translational modifications serve a critical role in promoting the progression from MDS to AML. Abnormalities in the bone marrow micro-environment include defects in mesenchymal stem cells and vascular endothelial cells. HSCs in patients with MDS carry epigenetic mutations, leading to the formation and expansion of specific clones. As mutations accumulate, these clonal cells gradually acquire driver mutations and evolve into leukemic cells, ultimately leading to the development of AML. AML-MR, acute myeloid leukemia, myelodysplasia-related; ECM, extracellular matrix; HSC, hematopoietic stem cell; MDS, myelodysplastic syndromes; MMPs, matrix metalloproteinases; nc-RNA, non-coding RNA; NK, natural killer; TME, tumor microenvironment.

TP53 mutations. The tumor suppressor TP53 is the most common mutated gene in human cancer (37). TP53 encodes the p53 protein, which serves a key regulatory role in cells and mediates key antitumor activity by inducing apoptosis during DNA damage (38). The TP53 mutation disables the p53 protein (38). Cancer types with TP53 mutations are typically characterized by the accumulation of DNA damage and a poor response to cytotoxic therapy (37). An analysis of 3,324 patients with MDS revealed that multi-hit TP53 alterations were associated with high-risk features of a complex karyotype, an increased rate of progression to AML-MR and poor OS. By contrast, monoallelic TP53 alterations did not exhibit these associations (39). Patients with TP53 gene mutations are primarily classified into two groups: One with monoallelic mutations, and one with biallelic or multitarget mutations (40). However, a study of AML has found that the

Table I. Defining cytogenetic abnormalities.

Abnormality	Mutation status
Complex karyotype	≥3 abnormalities
Chromosome 5	5q deletion or loss of 5q due to unbalanced translocation
Chromosome 7	Monosomy 7, 7q deletion or loss of 7q due to unbalanced translocation
Chromosome 11	11q deletion
Chromosome 12	12p deletion or loss of 12p due to unbalanced translocation
Chromosome 13	Monosomy 13 or 13q deletion
Chromosome 17	17p deletion or loss of 17p due to unbalanced translocation, isochromosome 17q
Chromosome X	Idic (X)(q13)

Idic (X), isodicentric X chromosome.

frequency of TP53 mutations was higher in AML-MR than in non-MRC AML (41). Prognostic assessment of AML-MR showed that the TP53 mutation was an independent predictor of poor OS and progression-free survival (PFS), and that the presence of TP53 mutation affects the prognosis of AML-MR (42).

Mutations in epigenetic regulatory genes. Additional sex combs like 1 (ASXL1) encodes a nuclear protein of 1,541 amino acids (43). This chromatin-modifying protein is involved in the activation and inhibition of transcription (44). Thol *et al* (44) reported that ASXL1 mutations occur mostly in MDS/MPN, occurring in 20.7% of patients with MDS, and those with ASXL1 frame-shift mutations have a shorter progression time to AML. There was no significant difference in the clinical parameters(including age, sex, bone marrow blasts, hemoglobin, transfusion dependence and ferritin levels) between patients with high and low ASXL1 expression, but patients with low ASXL1 expression harbored more isocitrate dehydrogenase NADP (+) 1 (IDH1) mutations (P=0.012) (44). Additionally, patients with MDS harboring ASXL1 and SET binding protein 1 mutations were more likely to progress to AML than patients with ASXL1 mutations alone (45). The ASXL1 mutation rate in patients with AML-MR (31%) is higher than that in patients with AML not otherwise specified (AML-NOS; 4.3%), suggesting that ASXL1 may be a surrogate marker in AML-MR (46). Furthermore, ASXL1 and EZH2 mutations often occur at the same time, indicating a poor prognosis (47).

The runt-related transcription factor 1 (RUNX1) gene encodes a protein with a heterodimer of α and β subunits and a core binding factor, which binds to DNA (48). RUNX1 mutations are found in 10-15% of patients with MDS and are associated with thrombocytopenia, poor OS, a high risk of progression to AML-MR, co-occurrence with RAS mutations and the loss of chromosome 7/7q (49-51). Additionally, germline point mutations in RUNX1 have been identified in an autosomal dominant platelet disorder, which carries an increased risk of transformation to AML (52). Patients with MDS harboring RUNX1 mutations have a significantly shorter time to AML (12.9±3.1 vs. 21.7±4.7 months; P=0.008) (45). RUNX1 mutations occur in 5.6-17.9% of newly diagnosed AML cases, whereas they occur in 27.7% of newly diagnosed AML-MR cases (53-55).

Mutations in RNA splicing-related genes. Spliceosome mutations can further alter the ability of cells to differentiate into fully mature blood cells by altering the intron-exon boundaries of selected transcribed genes, resulting in dysplastic phenotypes (47). Splicing factor 3b subunit 1 (SF3B1) encodes subunit 1 of the splicing factor 3b protein complex (56). In MDS, SF3B1 mutations are predominantly missense substitutions that disrupt spliceosome function and alter the proteome (57). These mutations are commonly associated with MDS with ring sideroblasts and carry a relatively low risk of progression to AML (58). However, specific mutations, such as the K666N hotspot, increase the risk of progression to AML-MR (57).

Serine/arginine rich splicing factor 2 (SRSF2) encodes a serine/arginine rich pre-RNA splicing factor that serves an important role in splice site selection and splice assembly (59). This mutation is more common in chronic myelomonocytic leukemia (59-61). SRSF2 mutations are also associated with neutropenia and overt thrombocytopenia (62). In low-risk MDS, patients with SRSF2 mutations have a shorter OS time and a higher risk of the MDS transforming to AML within 5 years, which may be an independent risk factor for poor prognosis (63).

The U2 small nuclear RNA auxiliary factor 1 (U2AF1) gene encodes a 35 kD protein. U2AF1 mutations can down-regulate splicing and RNA recognition motif genes, increase the transcripts of unspliced intron sequences or affect splicing and exon skipping (59,64,65). U2AF mutations are present in 5-12% of MDS cases and are strongly associated with a high risk of progression to AML-MR (66). Studies have shown that these mutations cause aberrant splicing, leading to suppressed autophagy-related 7 expression and reduced autophagy, which results in increased oxidative stress and chromosomal instability (67-69). Furthermore, research has revealed that U2AF1 mutations upregulate the expression of the long isoform of interleukin-1 receptor-associated kinase 4, thereby activating the nuclear factor κB signaling pathway, a mechanism closely linked to leukemic cell proliferation (66-68).

Mutations in DNA methylation-related genes. DNA (cytosine-5-) methyltransferase 3α (DNMT3A), catalyzes the methyl transfer to cytosine in CpG dinucleotides within DNA. DNMT3A is an important component in effectively maintaining the methylation of active chromosomal domains (70). DNMT3A mutations are present in 22% of AML cases, and

most are missense mutations (71). A study has demonstrated that the median OS time of patients with AML harboring DNMT3A mutations was significantly shorter than that of patients without DNMT3A mutations (12.3 vs. 41.1 months; P<0.001). The study showed that the mutation rate of DNMT3A in MDS was 2.6%, most of the mutations were missense mutations, the AML transformation rate was 29.4% and that there was no significant difference in the OS and AML transformation time between patients with low and high DNMT3A expression (72).

Tet methylcytosine deoxygenase 2 (TET2) catalyzes the hydroxylation of 5-methylcytosine to hydroxymethylcytosine on DNA, resulting in DNA methylation deletion (73). Due to mutations in HSCs and their progenitor cells, decreased TET2 activity contributes to the self-renewal and clonal dominance of HSCs, and enhances MDS transformation to AML (74). Seethy *et al* (75) showed that TET2 expression was reduced in AML-MR and International Prognostic Scoring System (IPSS-R) (76) high-risk MDS cases. A study on IDH has shown that TET2 is also involved in the production of α-ketoglutarate (αKG) by IDH (77). IDH catalyzes the conversion of isocitrate to αKG in a NADP-dependent manner (77). IDH mutations result in altered substrate specificity, in which the IDH1/2 enzymes cannot convert isocitrate to αKG and instead use isocitrate as a substrate to produce β-hydroxyglutarate (2-HG) (77). IDH1/2 mutations lead to accumulation of 2-HG in cells, resulting in TET2 inhibition and a corresponding increase in DNA methylation in genomic promoter regions (77). This leads to the impaired differentiation of hematopoietic cells *in vitro* and promotes MDS transformation to AML (77). IDH1/2 mutations are found in 4-12% of MDS cases and 10-15% of AML cases, and result in a poorer prognosis compared with that of patients without IDH1/2 mutations. Specifically, patients with IDH mutations have a lower OS than patients without these mutations (78). IDH mutations are frequently associated with NPM1 mutations (47.2%), and patients with AML harboring IDH and NPM1 mutations have an improved prognosis compared with patients harboring IDH mutations alone (OS rate, 66.5 vs. 35.2%; P<0.001) (79).

Molecular biology abnormalities. AML-MR involves complex molecular biological mechanisms. These changes are closely related to genetic alterations, but also exhibit distinct characteristics. Hematopoietic stem cell abnormalities, often linked to gene mutations and genetic alterations, result in the accumulation of abnormal hematopoietic cells (24,80). Epigenetic modifications, such as DNA methylation, drive the transformation from MDS to AML by altering gene expression (23). Alterations in the bone marrow microenvironment, including the loss of stromal cell function and immune surveillance, facilitate the growth of malignant clones (22,25). Clonal evolution manifests as the progressive accumulation of genetic mutations within the hematopoietic clone, ultimately promoting disease progression (22,80). These mechanisms serve a crucial role in the transformation of MDS to AML (22-25,80). The specific mechanisms of this transformation are subsequently described in detail (22-25).

Hematopoietic stem/progenitor cell abnormalities. HSCs are cells with the potential for self-renewal (to produce progeny HSCs by cell division) and pluripotent differentiation (to produce any mature adult hematopoietic cell type) (81). Myeloid malignancies enhance the self-renewal of HSCs through genetic and epigenetic alterations that stimulate HSC proliferation and lead to differentiation defects (82). IDH gene mutation leads to abnormal DNA hypermethylation and differentiation arrest in myeloid precursor cells, which is one of the important causes of AML (77). Survival-promoting signals strongly inhibit programmed cell death pathways so that activated HSCs are not eliminated through apoptosis or necrosis during cell division (81).

Epigenetic regulation abnormalities. Epigenetic changes are clonally heritable changes in gene expression without nucleotide sequence changes and include DNA methylation, histone modifications and non-coding RNA regulation. DNA methylation is one of the key epigenetic signals that regulate gene expression in eukaryotic cells. Changes in DNA methylation patterns affect CpG islands in intergenic and intragenic regions, especially enhancers regions. Inactivation ('silencing') of tumor suppressor genes and growth suppressor genes is typically mediated by DNA methylation in gene promoters, and demethylation of CpG islands in gene promoters is a major epigenetic transcriptional control mechanism for hematological tumorigenesis (83,84). Epigenetic changes can lead to the hypermethylation of genes that control proliferation (85,86). In MDS, adhesion and disease-specific changes in HSCs, as well as the abnormal silencing of tumor suppressor and DNA repair genes through promoter-associated CpG island hypermethylation, increase transformation to AML (85,86).

After protein modification, abnormal protein post-translational modification can take the following forms: Acetylation, methylation, phosphorylation, ubiquitination, and ADP ribosylation of histones and non-histones (lysine residue or protein N terminal residue). Modified histones can link with other proteins to form nucleosomes with different structures, which can promote transcription or expression of repressor transcription factors, resulting in gene silencing (87). The mutated genes associated with histone modification mainly include the EZH2, ASXL1 and UTX genes (88). The regulatory effect of histone modification on AML transformation is twofold: i) Lysine acetylation limits IDH2 activity and reduces AML transformation (89); and ii) the phosphorylation of actin-stabilizing proteins in patients with MDS increases the risk of AML transformation (90).

The dysregulated expression of non-coding RNA may participate in the regulation of tumor cell proliferation and apoptosis, thus serving an important role in the occurrence and development of hematological malignancies (91). O'Connell *et al* (92) demonstrated that microRNA (miRNA)-125 could enhance the self-renewal ability of HSCs or precursor cells and further induce AML in mice. Another study has also shown that the upregulation of miRNA-196b-5p may promote the high-risk transformation of MDS into leukemia in patients (93).

Changes in the tumor microenvironment. The bone marrow microenvironment is the internal environment for hematopoietic cells to survive; it supports and regulates the adhesion, homing, self-renewal, proliferation, differentiation and development of hematopoietic cells in a number of ways, and

also serves an important role in AML resistance. The surface molecule integrin β3 on LSCs can adhere to the bone marrow microenvironment, promoting LSC homing and enabling them to evade the cytotoxic effects of chemotherapy drugs (94). Research has shown that CXCR4 regulates the interaction between leukemia cells and bone marrow mesenchymal stem cells (MSCs) in the bone marrow microenvironment, protecting leukemia cells from chemotherapy-induced apoptosis (95). LSCs promote the survival of leukemia cells by inducing reversible changes in the function and composition of bone marrow stromal cells, thereby reconstructing a bone marrow microenvironment conducive to LSC survival (96).

Changes in interstitial cells. Bone marrow MSCs are essential for regulating the number of HSCs and for maintaining normal hematopoietic function (97). MSCs regulate the fate of HSCs by interacting with other cells in the microenvironment and secreting cytokines (97). When abnormal, MSCs secrete abnormal levels of cytokines and growth factors (such as IL-6, VEGF and stromal cell-derived factor 1), which support the self-renewal and survival of LCSs (97). In AML, the morphology and function of MSCs are influenced by BMMs; MSC dysfunction is associated with the impairment of BMMs that promotes leukemia development (98). The downregulation of C-X-C motif chemokine ligand 12 (CXCL12) expression affects the homing of normal HSCs. Specifically, compared with normal subjects, the hematopoietic capacity of patients with MDS is associated with the decrease in CXCL12 expression, and MDS-MSCs support the reduction in hematopoietic function and cell apoptosis, which makes patients with high-risk MDS prone to the immune escape of leukemia clones and further transformation into leukemia (99). Bhagat *et al* (100) reported that the abnormal hypermethylation and low expression of frizzled-related protein, an antagonist of the WNT signaling pathway, in MDS-MSCs led to the activation of WNT/β-catenin signaling. In addition, activation of the WNT/β-catenin signaling pathway in MDS-MSCs may lead to the transformation of AML (101). The downregulation of serine peptidase inhibitor Kunitz type 2 (also known as HAI-2) gene expression in MSCs is beneficial for the survival and the adhesion of tumor stem cells (101), which serves an auxiliary role in MDS transformation.

Changes in vascular endothelial cells. Increased secretion of angiogenic factors (such as VEGF) leads to the formation of abnormal blood vessels (102,103). LSCs secrete VEGF-A, which can activate endothelial cells, increase vascular niche protection for leukemia cells, promote leukemia cell proliferation, support tumor angiogenesis and immune escape, and reduce the apoptosis of AML cells (102,103).

Endothelial cells enhance the adhesion and invasion of leukemia cells (104). In AML, LSCs block the adhesion mechanism of healthy HSCs by upregulating the expression levels of C-X-C motif chemokine receptor 4, very late activation antigen 4 and CD44 (104). In addition, endothelial cells can promote the survival of leukemia cells and enhance their drug resistance. Inflammatory mediators secreted by LSCs serve a key role in regulating the expression of endothelial adhesion molecules (such as E-selectin) and initiating leukemia-endothelial cell interaction, which is upregulated 5-10-fold in AML and mediates drug resistance in vascular niches (105).

Changes in the immune microenvironment. i) Immune suppression. Regulatory T (Treg) cells are a unique class of negatively regulated immune cells that inhibit the proliferation and differentiation of other immune cells, including effector T lymphocytes, B lymphocytes, macrophages and dendritic cells (106). Treg cells mainly inhibit the proliferation and differentiation of immune cells, so that abnormal clonal cells undergo immune escape, continue to expand and replace normal hematopoietic cells to serve a role in promoting transformation (107). Kotsianidis *et al* (106) detected the levels of Treg cells in the peripheral blood and bone marrow of patients with newly diagnosed MDS and healthy individuals. The Treg cell levels in patients with early MDS were lower than those in patients with advanced MDS and AML as well as in relapsed patients after chemotherapy; however, the Treg cell levels in patients with MDS that did not transform into AML did not change significantly within 9 months.

Myeloid-derived suppressor cells (MDSCs) are important innate immune cells that contribute to trilineage cytopenia in patients with low-risk MDS (108). MDSCs are a group of immature myeloid cells, and bone marrow MDSC levels are significantly higher in patients with MDS than in healthy individuals ($P<0.05$) (108). In addition to MDSCs, other innate immune cells in patients with MDS also have abnormal regulation, including macrophages and natural killer (NK) cells (109).

ii) Immune checkpoint expression. Immune checkpoint expression changes serve a regulatory role in the immune system. Immune checkpoint receptors are mainly expressed in immune cells, while the ligands are mainly expressed in tumor cells (110). The ligands interact with the receptors and, on the one hand, participate in maintaining the immune tolerance of the body and preventing autoimmune reactions. On the other hand, they suppress the functions of immune cells and promote the immune evasion of tumor cells (110). The increased expression of programmed cell death-ligand 1 (PD-L1)/programmed death protein 1 (PD-1) suppresses the function of T cells (111), which in turn leads to immune escape, and thus, tumor development (112). PD-L1 is an important ligand of PD-1, which is widely expressed on the surface of antigen-presenting cells (110). PD-1 inhibits cell activation when bound to its ligands, PD-L1 and programmed death ligand 2 (113).

iii) NK cells dysfunction. NK cell dysfunction leads to a decline in their killing activity and an inability to effectively eliminate abnormal cells (114). NK cells are a member of the innate lymphoid cell family and are key effector lymphocytes mediating tumor immune surveillance and clearance (114). NK cells are also key components of the antitumor defense system (114). AML cells achieve immune escape by inhibiting the development and function of NK cells, leading to a reduction in their cytotoxic activity and an inability to effectively eliminate abnormal cells (115).

Changes in the extracellular matrix (ECM). Changes in the ECM components result in the abnormal expression of fibronectin and laminin, increased activity of matrix metalloproteinases (MMPs), upregulation of tumor necrosis factor-α and the secretion of MMP-2 and MMP-9 in normal $CD34^+$ cells (116). High expression of MMPs is considered to contribute to cancer progression and leukemia spread, and to promote the invasion and metastasis of leukemia cells (116).

Changes in the metabolic microenvironment. The hypoxic bone marrow microenvironment supports leukemia transformation and progression by regulating LSC silencing, self-renewal capacity and cell homing (117). Hypoxia-inducible factor-1 (HIF-1) serves an important role in tumor progression and tumor matrix formation (118). Abnormal HIF-1α expression of also exists in LSCs. The leukemia microenvironment can induce increased expression of HIF-1α under hypoxia, which can regulate the VEGF expression, increase angiogenesis in the leukemia microenvironment, and provide nutrition for the survival and proliferation of LSCs (118).

The metabolism of glucose and amino acids leads to metabolic reprogramming, which affects the metabolic microenvironment (119-123). Branched chain amino acids (BCAAs) are essential for tumor growth, and HSCs are highly sensitive to valine. Fluctuation of the valine level induces BCAA imbalance and reduces the proliferation and survival of HSCs (124). Raffel *et al* (125) demonstrated that the BCAA pathway was enriched in human AML stem cells (compared with non-stem cell populations) and AML cells exhibited upregulated expression levels of BCAA aminotransferase 1 (BCAT1), suggesting that the BCAA-BCAT1 pathway may represent a therapeutic target and that drugs inhibiting this pathway may effectively inhibit the clonal proliferation of human AML blasts. Normal cells mainly rely on mitochondrial oxidative phosphorylation to produce ATP, but cancer cells increasingly prefer aerobic glycolysis, that is, energy production by glycolysis and lactic acid fermentation, even in the presence of oxygen (119). This phenomenon is referred to as the 'Warburg effect'. Glycolysis metabolism is also enhanced in AML cells and studies have found that hexokinase, phosphofructokinase-1 and pyruvate kinase, which are the rate-limiting enzymes in glycolysis, may be potential therapeutic targets (120,121).

Clonal evolution of AML-MR. Normal HSCs typically acquire initial mutations in epigenetic regulatory genes (such as DNMT3A, TET2 and ASXL1) or RNA splicing genes (such as SF3B1 and SRSF2) (122). Preleukemic mutations confer a competitive advantage on HSCs without causing the transformation of downstream progenitor cells (123). In patients with MDS, cells carrying DNMT3A mutations have a clonal advantage (126). These mutations may not result in immediate phenotypic changes but may increase the risk of cells acquiring further mutations (126). The transplantation of HSCs with SF3B1 mutations from patients with MDS-ring sideroblasts into mice has been shown to induce the generation of human circular sideroblasts, demonstrating that SF3B1 mutations originate in HSCs and persist in differentiated cells (127).

The cells carrying the initial mutation gain a proliferation advantage and form preleukemia clones (122). This stage may not have notable clinical manifestations and is termed 'clonal hematopoiesis' (122). Over time, preleukemia clones accumulate more genetic alterations (122,123). Common additional alterations include mutations in TP53 and deletions in chromosome 5q or 7q (123). These alterations lead to hematopoietic dysfunction, clinically manifested as MDS (122,123). Makishima *et al* (128) found that there was not only a linear evolution reported in patients with MDS, but there were also clone cleanups where existing or newly emerging subclones 'cleared' one or more previous subclones and eventually became dominant clones. This form of clonal evolution emphasizes the critical role of driver gene mutations in MDS.

MDS clones acquire key driver mutations (such as fms related receptor tyrosine kinase 3-internal tandem duplication mutations, and NPM1 and RUNX1 mutations). Some evidence suggests that RUNX1 and RUNX1 partner transcriptional co-repressor 1 mutations are acquired in preleukemic HSCs, and that leukemia transformation requires additional co-somatic mutations, such as in ASXL1 (123). These mutations further promote cell proliferation, inhibit differentiation and ultimately lead to AML (122,123). Leukemic clones expand rapidly in the bone marrow, inhibiting normal hematopoiesis (122). Since the disease originates from MDS, it retains the genetic and morphological features associated with MDS and is therefore diagnosed as AML-MR (122). Clonal evolution is a continuous process and continues even after AML-MR formation (122). Treatment may lead to the selection of subclones and the emergence of resistant clones (122).

3. Current treatment status of AML-MR

Challenges in the treatment of AML-MR

Elderly, frail patients. The challenge of treating AML-MR in older patients primarily arises from the demographic profile of those affected by the disease (129). AML-MR predominantly occurs in older individuals (129). This age group typically presents with a higher incidence of comorbidities and a poorer overall physical performance compared with younger populations (130). Such health complications can limit the ability to endure the aggressive nature of intensive chemotherapy regimens traditionally used to combat AML-MR (131). The physiological resilience required to withstand the side effects of such treatments is often diminished in older patients, making it challenging to administer therapies with curative intent (26,130).

Furthermore, aging is accompanied by a natural decline in organ function, impacting the metabolism and clearance of chemotherapy agents from the body (132). This can result in increased toxicity and adverse effects, further complicating the management of AML-MR in elderly patients (133). For instance, the common use of anthracyclines and cytarabine in intensive regimens can exacerbate underlying cardiovascular issues or affect renal and hepatic functions, which are crucial for drug processing (134,135). These factors create a delicate balance for clinicians who must weigh the potential benefits of intensive treatment against the heightened risk of severe, life-threatening side effects in older patients.

Additionally, older patients with AML-MR often exhibit a poorer performance status compared with younger adults with AML-MR, which is a prognostic factor for short- and long-term mortality (136). The performance status, an evaluation of a patient's general well-being and the ability to perform daily activities, directly influences treatment outcomes, as those with lower performance scores are less likely to benefit from or complete intensive treatment courses (137). Consequently, healthcare providers may need to opt for less aggressive treatment strategies, such as lower-intensity chemotherapy or supportive care, which may not be as effective in inducing remission, but may reduce the risk of severe side effects (138). The limitations imposed by age and associated health

conditions necessitate personalized treatment approaches, tailored not only to the disease prognosis but also to each patient's comprehensive health profile, to optimize outcomes while maintaining the quality of life.

Prior treatment history. The treatment history of patients poses challenges in managing the disease effectively. A number of patients diagnosed with AML-MR have had prior exposure to cytotoxic therapies due to pre-existing conditions such as MDS or MPN (139). These previous treatments often include hypomethylating agents (HMAs) such as azacitidine or decitabine, and cytotoxic agents such as ruxolitinib or lenalidomide (140). As these therapies can alter the bone marrow environment and immune response, they may contribute to treatment resistance in patients who subsequently develop AML-MR (141). This resistance can manifest as lower complete remission (CR) rates when intensive chemotherapy is attempted, complicating the clinical course of AML-MR (141).

Prior cytotoxic exposure not only impacts the efficacy of future treatments but can also influence the ability of patients to tolerate more intensive chemotherapy regimens (26). Treatments such as HMAs are known to upregulate immune checkpoint molecules, including PD-1, PD-L1 and cytotoxic T-lymphocyte associated protein 4, which are associated with immune evasion by malignant cells (142-145). This mechanism may further diminish the responsiveness to subsequent therapeutic interventions (142-145). As a result, clinicians face the difficult task of designing treatment regimens that consider past therapies and their potential impact on current treatment options. The diminished therapeutic response due to prior treatments can also lead to an overall reduced survival rate compared with patients with non-MRC AML, highlighting the need for innovative approaches to improve outcomes (19).

These challenges underscore the critical need for individualized treatment strategies that account for the cumulative effects of previous therapies (146). Leveraging genomic and molecular data to guide therapy selection may offer novel avenues for overcoming resistance and improving the efficacy of treatment for patients with AML-MR (147). Clinical trials exploring novel therapeutic combinations and personalized medicine approaches hold promise for this patient group, who face a historically challenging prognosis due to their complex treatment history.

Current treatments. In the past few decades, the standard induction regimen for patients with AML has been a combination of cytarabine and anthracycline drugs (7+3 regimen) (148). However, with the approval of CPX-351 (Vyxeos) by the US Food and Drug Administration (FDA) in August 2017, a fixed molar ratio liposomal formulation of cytarabine and daunorubicin for the treatment of newly diagnosed AML-MR and treatment-related AML (t-AML), there have been changes in the treatment of AML-MR (149). In the 2024 edition of the National Comprehensive Cancer Network guidelines (150), AML-MR is classified under the adverse-risk category. Preferred treatments include CPX-351 (liposomal cytarabine and daunorubicin) for patients aged ≥60 years and the standard 7+3 regimen (daunorubicin or idarubicin) for those aged <60 years. Other recommended options include CPX-351 for patients <60 years, the standard 7+3 regimen for patients ≥60 years and combination therapies such as decitabine (days 1-5) + venetoclax, azacitidine + venetoclax or low-dose cytarabine + venetoclax. The consolidation treatment options include allogeneic hematopoietic cell transplantation (allo-HSCT; preferred), cytarabine, CPX-351 (dual-drug liposomal cytarabine and daunorubicin; preferred only if administered during induction), fludarabine, cytarabine, granulocyte colony-stimulating factor-containing regimens (preferred only if administered during induction) and continuation of the lower intensity regimen used during induction (for instance, HMAs such as azacitidine or decitabine + venetoclax). In this section, the aforementioned treatment methods and their efficacy in AML-MR are described.

CPX-351. CPX-351 liposomes are formed from a 5:1 combination of cytarabine and daunorubicin and are the only treatment specifically approved for newly diagnosed adult patients with AML-MR and t-AML (151,152). Increasing the drug concentration in the bone marrow promotes the entry of drugs into AML cells, thereby exerting a stronger anti-leukemia effect (151). This regimen reduces the pharmacokinetic differences between the two drugs compared with the previous daunorubicin 3 days + cytarabine 7 days (DA3+7) regimen, and solves the issue of continuous infusion of cytarabine due to transient bioavailability (153). A phase III clinical trial (NCT01696084) enrolled 309 patients with AML-MR and t-AML aged 60-75 years and noted a longer OS time (9.56 vs. 5.95 months; P=0.003) and higher CR rate (37.4 vs. 24.4%; P=0.04) in the CPX-351 group compared with the standard chemotherapy regimen 'DA3+7' group. An analysis of 246 patients with AML-MR enrolled in this trial showed that patients treated with CPX-351 had a higher median survival time (19.2 vs. 11.6 months) and a higher allogeneic transplantation rate (54 vs. 43%) than patients receiving the DA3+7 regimen (151). A study of 103 patients with t-AML and AML-MR treated with CPX-351 in the first line treatment showed an overall response rate (CR/CR with incomplete blood count recovery) of 59% after induction and a median survival time of 16.1 months after a median follow-up of 8.6 months (154). Compared with the DA3+7 regimen, patients treated with CPX-35 exhibit a longer median time to neutrophil and platelet recovery, but no increase in early mortality (5.9 vs. 10.6%) (155).

HMAs (including decitabine and azacitidine), as specific DNA methyltransferase inhibitors, can reverse DNA methylation and induce the apoptosis or differentiation of tumor cells into normal cells, and have become standard chemotherapy regimens for high-risk MDS (156). HMAs remain the fundamental treatment for the majority of elderly patients with AML-MR (157).

In a phase III multicenter randomized trial of 158 patients with AML-MR, the median OS time was 12.7 months for azacitidine compared with 6.3 months for conventional care regimens (CCR) (P=0.036) (158). Another study, which included 262 patients with AML-MR, showed a higher median OS time in patients treated with azacitidine compared with patients treated with CCR (8.9 vs. 4.9 months; hazard ratio, 0.74), and the 1-year survival rates were 44.3 and 27.2%, respectively. The study also found that patients with only morphological polypedigree dysplasia AML-MR (≥50% cytodysplasia in at least two of the three myeloid lineages) had an

improved OS time (azacitidine, 16.3 months; CCR, 7.1 months) compared with other patients with AML-MR, whereas patients with only MDS with cytogenetic abnormalities had a much shorter median survival time (azacitidine, 5.3 months; CCR, 2.9 months) (129).

A study comparing decitabine + low-dose cytarabine, idarubicin, and granulocyte colony-stimulating factor (CAG) with standard CAG in MDS with blastosis and AML-MR found a significantly higher CR rate in the decitabine + reduced-dose CAG group compared with the conventional CAG group (81.0 vs. 52.4%; P=0.05), but no significant difference in the OS rate at 12 months (90.9±8.7 vs. 61.5±13.5%) (159).

Allo-HSCT. The continuous optimization of chemotherapy regimens and the application of novel targeted drugs and cellular immunotherapy have improved the prognosis of patients with AML to a certain extent, but allo-HSCT is still the only potential means to cure AML (4,160). The 2017 European Leukemia Network (ELN) guidelines recommend allo-HSCT as the first choice for patients with intermediate and high-risk AML (4). A study comparing patients with AML-MR (n=4,091) and AML-NOS (n=3,964) undergoing allo-HSCT showed that patients with AML-MR after transplantation had a lower OS rate (35.5 vs. 50.6%) and a higher relapse rate (42.3 vs. 32.1%) than the patients with AML-NOS (161). A study on 95 patients with secondary myeloid leukemia by Yuan *et al* (161) showed that the 3-year OS rates in the MDS-AML and MDS/MPN-AML group and the t-AML group were 69.7 and 75.4%, respectively, and there was no statistically significant difference between the two groups (P=0.233).

Targeted therapy and immunotherapy. The antiapoptotic protein BCL-2 protein family regulates the mitochondrial apoptotic pathways, and inhibiting BCL-2 expression inhibits tumor neovascularization, thereby inhibiting tumor metastasis and reducing resistance to antitumor drugs (162). Venetoclax has been approved in combination with HMA or low-dose cytarabine in patients ≥75 years of age with newly diagnosed AML or who cannot tolerate intensive chemotherapy (163). Azacitidine + venetoclax combination therapy has shown superior efficacy compared with azacitidine alone in patients with *de novo* AML and AML-MR, particularly in terms of the OS time (14.7 vs. 9.6 months) and the CR rate (66.4 vs. 28.3%) (163). However, it remains unclear whether azacitidine + venetoclax combination therapy is superior to intensive chemotherapy for fit patients with high-risk MDS and AML-MR. At present, an ongoing clinical trial is primarily focused on comparing intensive chemotherapy with the azacitidine + venetoclax combination, with particular emphasis on evaluating the treatment outcomes in patients with AML-MR (164). A study of 188 patients with AML-MR showed that after the first cycle of induction therapy, patients receiving venetoclax +HMA had a higher overall response rate than patients receiving 7+3 (75 vs. 46%). The minimal residual disease-negative CR rate in the venetoclax +HMA group was also higher than that in the 7+3 group (46 vs. 19%; P<0.001) (165).

PD-1 is the most common immune checkpoint of T cells identified so far. PD-1 regulates the immune system by down-regulating the immune system response to human cells and inhibiting T cell inflammatory activity (113,145). A study has observed that the expression of PD-1 and PD-L1 on T cells and AML cells increased gradually during the progression of MDS to AML, which suggested that PD-1/PD-L1 may participate in the development of AML-MR (160). Lysine-specific demethylase 1 (LSD1) was the first histone demethylase identified in 2004 (166). LSD1 mediates a number of cell signal transduction pathways and is involved in tumor genesis and development (166). LSD1 inhibits the expression of genes related to myeloid differentiation and ultimately inhibits cell proliferation and tumor growth *in vivo* (167). An *in vivo* experiment showed that the LSD1 inhibitor, NCD38, had good efficacy against AML-MR with a complex karyotype (167).

Lenalidomide (a thalidomide analog) is an immunosuppressant approved by the FDA for the treatment of transfusion-dependent anemia in patients with IPSS low/intermediate-1 risk and del (5q) MDS with or without additional cytogenetic abnormalities, and is more effective in patients with low-risk MDS with del (5q) chromosomal abnormalities (168-170). Currently, to the best of our knowledge, there is no clear research on the therapeutic effects of lenalidomide in patients with AML-MR. Brune *et al* (171) found that the microvessel density decreased more rapidly in patients with AML-MR receiving lenalidomide than in patients receiving standard therapy (daunorubicin + cytarabine). However, due to the small number of cases included in the study, the difference in PFS was not statistically significant, and a large number of studies are still needed to further verify the effectiveness of lenalidomide.

It is noteworthy that the combination of magrolimab, azacitidine and venetoclax has demonstrated a CR rate of >80% in patients with newly diagnosed ELN adverse-risk AML (172). This suggests that this approach may become a novel standard of care for patients with high-risk MDS/AML-MR, especially those who are ineligible for intensive chemotherapy but can tolerate the triple therapy (172). In addition, several phase III clinical trials (such as NCT05079230 and NCT04313881) have compared magrolimab-based therapy with standard treatment options, such as azacitidine and azacitidine + venetoclax combination therapy. Furthermore, clinical trials evaluating the efficacy of IDH1/2 inhibitors in patients with MDS and AML-MR, including NCT03503409, NCT04603001, NCT04493164 and NCT03471260, are also ongoing. These trials hold the potential to improve the survival rates of patients with AML-MR in the future.

4. Conclusion

AML-MR remains a common, highly aggressive subtype of AML and is associated with poorer long-term prognosis compared with that of patients with AML. Specifically, patients with AML-MR generally have a worse prognosis than patients with non-AML-MR, with lower complete response rates and OS. The pathogenesis of AML-MR is driven by a combination of genetic alterations, molecular biology, bone marrow microenvironment abnormalities and clonal evolution. Current research focuses on genetic factors, with chromosomal abnormalities and somatic mutations central to AML-MR development. However, the interactions between the bone marrow microenvironment and clonal evolution remain underexplored. Future research should focus on clarifying these complex interactions, particularly at the single-cell

level, to gain a deeper understanding of how genetic alterations influence clonal evolution and the development of the disease. Uncovering these complex mechanisms will pave the way for novel strategies for early diagnosis and the development of targeted therapies. Treatments such as CPX-351, HSCT, HMAs, and venetoclax have shown promise, but their long-term effectiveness requires further validation. Emerging therapies targeting BCL-2, PD-1 inhibitors, and lenalidomide may serve key roles in improving outcomes by targeting clonal cells. With continued advances in therapies and technologies, future research will enable earlier diagnosis, reveal optimized treatment strategies and enhance the prognosis of patients with AML-MR.

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Authors' contributions

HQ and CZ performed the literature search, wrote major parts of the manuscript, edited the manuscript, and prepared the figures and tables. XM and YL contributed to revising the manuscript critically for important intellectual content. Data authentication is not applicable. All authors have read and approved the final version of the manuscript.

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Competing interests

The authors declare that they have no competing interests.

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