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Prognostic implications of myelodysplasia-related gene mutations in *NPM1*-mutated acute myeloid leukemia: a systematic review and meta-analysis

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Data-sharing statement

This meta-analysis utilized data extracted from publicly available, previously published studies. All relevant data analyzed in this study are included in the main article and its supplementary materials. Additional information can be requested by contacting the corresponding author.

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Author contributions

YS Chang: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization; **YW Lee:** Methodology, Formal analysis, Investigation, Data curation, Validation, Writing – review & editing; **CY Liu:** Investigation, Data curation, Supervision, Validation, Writing – review & editing; **J Othman, J-N Eckardt, Q Zhou:** Data curation; **FM Tien, XC-H Tsai, TW Lyu, CC Lin:** Investigation, Resources, Writing – review & editing; **BS Ko, WC Chou:** Validation, Resources, Writing – review & editing; **H Chang, C Röllig, N Russell, R Dillon:** Data curation, Supervision; **HA Hou:** Conceptualization, Methodology, Supervision, Validation, Writing – review & editing, Project administration, Funding acquisition. All authors have reviewed and approved the final manuscript.

Conflict of Interest

YS. Chang: Honoraria from PharmaEssentia; **J. Othman:** Honoraria from Astellas,

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Abstract

NPM1-mutated (*NPM1*^{mut}) acute myeloid leukemia (AML) is generally classified as favorable-risk under the 2022 European LeukemiaNet (ELN-2022) guidelines, except in the presence of *FLT3*-internal tandem duplication or adverse-risk cytogenetics. However, the prognostic significance of co-occurring myelodysplasia-related gene (MRG) mutations remains unclear, with prior studies yielding inconsistent results. To clarify this issue, we conducted a systematic review and meta-analysis in accordance with PRISMA guidelines, searching PubMed, Embase, and MEDLINE through March 2025. MRG mutations were defined as pathogenic variants in *ASXL1*, *BCOR*, *EZH2*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, *ZRSR2* and *RUNX1*, and the primary analysis incorporated studies regardless of *RUNX1* inclusion. Data from ten cohorts across nine studies (one of which included an independent validation cohort), encompassing a total of 4,363 patients, were analyzed. Of these, 655 patients (15.0%) harbored co-occurring MRG mutations. Among the patients with ELN-2022 intermediate risk (n=1,294), 108 (8.3%) had MRG mutations. The presence of MRG mutations was significantly associated with inferior overall survival (pooled hazard ratio [HR] 1.30; 95% confidence interval [CI], 1.11–1.51; $p<0.001$; $I^2=39.2\%$), shorter event-free survival (HR 1.43; 95% CI, 1.11–1.85; $p=0.006$; $I^2=18.2\%$), and lower complete

remission rates (risk ratio 0.94; 95% CI, 0.90–0.99; p=0.01; I^2=0.0%). Subgroup analyses confirmed the adverse prognostic impact in patients receiving intensive therapy and those classified as ELN-2022 favorable risk. These findings suggest that MRG mutations confer an adverse prognostic effect in *NPM1*^{mut} AML and support the integration of MRG status into future risk stratification frameworks for *NPM1*^{mut} AML.

Introduction

Acute myeloid leukemia (AML) is a heterogenous hematologic malignancy characterized by distinct genetic and molecular alterations that influence disease prognosis and therapeutic response. *Nucleophosmin* (*NPM1*) mutated (*NPM1*^{mut}) AML accounts for approximately 30% of AML cases[1] and is recognized as a distinct disease entity in the contemporary World Health Organization (WHO) Classification of Haematolymphoid Tumours[2] and International Consensus Classification (ICC) of Myeloid Neoplasms and Acute Leukemias.[3] According to the European LeukemiaNet 2022 (ELN-2022) recommendations, *NPM1*^{mut} AML is categorized as favorable risk in intensively treated patients unless accompanied by a *FLT3*-internal tandem duplication (*FLT3*-ITD) or adverse-risk cytogenetics, which modify its prognostic classification.[4]

On the other hand, mutations in *ASXL1*, *BCOR*, *EZH2*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, and *ZRSR2*, are strongly associated with secondary AML arising from myelodysplastic neoplasms/syndromes (MDS).[5] These eight genes, along with *RUNX1*, are categorized as myelodysplasia-related gene (MRG) mutations in the 2022 ICC,[3] and the adverse-risk group in the ELN-2022 risk classification. Their presence has been linked to adverse outcomes in *de novo* AML.[5-7] However, the prognostic

relevance of MRG mutations in the context of *NPM1*^{mut} AML remains uncertain. While some studies suggest that the presence of MRG abrogate the favorable prognosis of *NPM1*^{mut} AML,[8, 9] others indicate that their effect may be dependent on additional co-mutations, treatment regimens, or measurable residual disease (MRD) status.[10, 11] The inconsistency across studies highlights the need for a comprehensive synthesis of available evidence to determine whether the presence of MRG mutations would influence risk stratification and treatment decisions in patients with *NPM1*^{mut} AML. To address these uncertainties, this systematic review and meta-analysis aims to quantify the prognostic impact of MRG mutations in *NPM1*^{mut} AML, aiding clinicians in decision making and refining current AML risk classifications.

Methods

Systemic Literature Review

This systematic review and meta-analysis were conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[12] The study was registered on INPLASY (registration number: INPLASY202530128; https://inplasy.com/inplasy-2025-3-0128/). A comprehensive literature search was performed across PubMed, Embase, and MEDLINE databases to

identify relevant studies published up to March 2025 (Supplementary Table 1). The search strategy combined Medical Subject Headings (MeSH) terms and free-text keywords, specifically targeting "Acute Myeloid Leukemia", "NPM1", and "Myelodysplasia-related", along with their relevant synonyms. Boolean operators were applied to refine the search. Additionally, conference abstracts from the American Society of Clinical Oncology, American Society of Hematology and European Hematology Association were manually reviewed to capture the latest findings in the field.

Eligibility Criteria

Both prospective and retrospective studies were included in the meta-analysis. Studies were eligible if they met the following criteria: (1) enrolled patients diagnosed with *NPM1*^{mut} AML and assessed the prognostic impact of co-occurring MRG mutations; (2) provide detailed survival data, including hazard ratios (HRs), 95% confidence interval (CI), and *p* values. Studies were excluded if they lacked sufficient survival data, reported only median survival without an accompanying survival curve or HR, focused on *NPM1*^{mut} AML with MDS-related cytogenetic abnormalities, or were review articles, animal studies, or case reports.

Study Selection

After removing duplicates, two independent reviewers (YS Chang and YW Lee) screened the titles and abstracts to identify studies meeting the predefined eligibility criteria. Full-text articles of potentially relevant studies were then reviewed in detail to confirm inclusion. Discrepancies between reviewers were resolved through discussion, and if necessary, a third reviewer (HA Hou) was consulted. Finally, nine studies (one study with an independent validation cohort) were included in the meta-analysis, as shown in Figure 1.

Data Extraction, Quality Assessment, Data Synthesis and Statistical Analysis

Full details of data extraction procedures, risk of bias assessment and statistical methodology are provided in the Supplementary Methods.

Ethics Statement

This study is a systematic review and meta-analysis of previously published data and did not involve any direct contact with patients or access to individual-level

data. As such, institutional review board approval was not required. However, the study was conducted in accordance with the ethical standards and regulations of the country in which it was performed.

Results

Literature Search

A systematic search of Embase, MEDLINE, and PubMed identified 955 records (Embase, n=578; MEDLINE, n=300; PubMed, n=77). After removing 142 duplicates, 813 unique records underwent screening, with 777 excluded due to irrelevance (n=664), article type (n=104), or non-English language (n=9). The full texts of 36 articles were reviewed, resulting in the exclusion of 27 studies due to the absence of relevant outcome data (*n*=15), an ineligible patient population (*n*=7), or overlapping cohorts (*n*=5). Ultimately, nine studies met the inclusion criteria, with one study contributing an additional independent validation cohort (Figure 1).[8-11, 13-17]

Characteristics of Studies

The baseline characteristics of the nine primary studies and one validation cohort

are summarized in Supplementary Table 2. All studies were retrospective in design and published between 2022 and 2025. Collectively, these studies included 4,363 patients with *NPM1*^{mut} AML, of whom 655 (15.0%) harbored co-occurring MRG mutations. Three studies defined MRG mutations according to WHO-2022 classification criteria[2] and therefore did not include *RUNX1* mutations. The most frequently co-occurring MRG were *SRSF2* (range: 3.3 – 10.5%), *ASXL1* (range: 0.8% – 16.5%), and *STAG2* (range: 0 – 5%) (Supplementary Table 3). Seven of the nine studies reported median age comparisons between MRG and non-MRG groups, all showing significantly older age in the MRG group (all $p < 0.01$). Among the ten cohorts, three included only patients with ELN-2022 favorable risk, while the remaining seven cohorts (n=3,656) included all three ELN-2022 risk categories. The majority were classified as favorable risk (n=2,203; 60.3%), followed by intermediate (n=1,294; 35.4%) and adverse risk (n=109; 3.0%). The distribution of MRG mutations differed significantly across ELN-2022 risk groups, with 516 (23.4%) in favorable, 108 (8.3%) in intermediate, and 31 (28.4%) in adverse-risk patients ($p < 0.001$). Additionally, among the seven studies that included both *FLT3*-ITD positive and negative patients, MRG mutations were associated with a lower frequency in concomitant *FLT3*-ITD (MRG^{mut} 20% *vs*. MRG^{WT} 38%; $p < 0.001$).

Risk of bias was assessed using the QUIPS tool. All studies were judged to have an overall low risk of bias (Supplementary Figure 1A). Domains related to outcome measurement and prognostic factor ascertainment were consistently rated as low risk. Moderate risk was noted in select domains, including participation, attrition, confounding, and statistical reporting; however, these were limited in number and did not compromise overall study quality (Supplementary Figure 1B).

Pooled Analysis

Meta-analysis of 10 cohorts (nine studies and one with additional validation cohort) demonstrated that MRG mutations were significantly associated with inferior overall survival (OS) in *NPM1*^{mut} AML, with a pooled HR of 1.30 (95% CI: 1.11–1.51; $p < 0.001$; Figure 2A). Between-study heterogeneity was moderate ($I^2 = 39.2\%$, $\tau^2 = 0.0062$; $p = 0.10$). To evaluate the stability of the overall estimate, leave-one-out sensitivity analyses were performed (Figure 2B). Sequential exclusion of individual studies yielded consistent results (HR range: 1.27–1.37), all of which remained statistically significant ($p < 0.01$). Heterogeneity estimates remained within an acceptable range (I^2: 0.0%–45.8%), and no single study exerted disproportionate influence. Potential publication bias was assessed via funnel plot, which demonstrated

visible asymmetry suggestive of small-study effects (Supplementary Figure 2A). Egger's regression test supported the presence of bias (t = 2.34, $p = 0.047$), with a bias estimate of 1.59 (SE = 0.68). To adjust for potential publication bias, the trim-and-fill method was applied. One imputed study was added, resulting in a total of 11 cohorts included in the adjusted analysis. After adjustment, the association between MRG mutations and OS remained statistically significant (pooled HR = 1.28; 95% CI: 1.10–1.48; $p = 0.001$; Supplementary Figure 2B-C).

Subgroup and Meta-Regression Analyses

To evaluate the prognostic relevance of MRG mutations in intensively treated *NPM1*^{mut} AML, six cohorts[9-11, 14, 16] along with the intensively treated subgroup from Zhou et al.[17] were included in a predefined subgroup analysis. The presence of MRG mutations remained significantly associated with inferior OS (pooled HR = 1.20, 95% CI: 1.02–1.41; $p = 0.027$), and low between-study heterogeneity ($I^2 = 14.1\%$, $p = 0.32$; Figure 3A). A further subgroup analysis assessed the prognostic impact of MRG mutations according to the ELN-2022 risk classification. Due to the small number of patients in the ELN-adverse risk category ($n = 109$, 2.5%), the model for this subgroup in some individual studies did not converge,[10, 11] and its prognostic effect

was therefore not evaluated. Three cohorts[9, 15, 16] and the ELN-favorable subgroups from five additional cohorts[10, 11, 14, 17] contributed data for the ELN-favorable category, while three cohorts[10, 14, 17] provided data for the ELN-intermediate category. In the ELN-favorable subgroup, MRG mutations were significantly associated with inferior OS (pooled HR, 1.34; 95% CI, 1.14–1.59; I^2 = 0.0%; Figure 3B). In contrast, no significant association was observed in the ELN-intermediate subgroup (pooled HR, 0.85; 95% CI, 0.57–1.27; I^2 = 0.0%). The difference between subgroups was statistically significant (p = 0.04).

In the age-stratified subgroup analysis, three cohorts[10, 15, 17] provided data for patients aged ≥60 years and <60 years. MRG mutations were not significantly associated with OS in either subgroup (age ≥60: pooled HR, 1.04; 95% CI, 0.72–1.49; I^2 = 54.5%, and age <60: pooled HR, 1.24; 95% CI, 0.84–1.81; I^2 = 46.5%), and the difference between age groups was not significant (p = 0.52; Figure 4A). When the analysis was restricted to patients with ELN-favorable risk, age <60 years was associated with inferior outcomes (pooled HR, 1.50; 95% CI, 1.12–2.02; I^2 = 0.0%), whereas age ≥60 years was not (pooled HR, 0.85; 95% CI, 0.46–1.57; I^2 = 59.2%); the age-group difference remained nonsignificant (p = 0.10; Figure 4B). However, the absence of a statistically significant interaction between age subgroups may be

noteworthy, given the modest number of elderly patients with *NPM1* mutation in the pooled cohort and should therefore be interpreted with caution.

For the hematopoietic stem cell transplantation (HSCT) in first complete remission (CR1) subgroup analysis, four cohorts[10, 14, 15, 17] contributed data for patients who underwent HSCT in CR1 and for those who did not. In the HSCT group, MRG mutations were not significantly associated with OS (pooled HR, 1.46; 95% CI, 0.72–2.95; I^2 = 45.8%), and no significant association was observed in the no-HSCT group (pooled HR, 1.09; 95% CI, 0.89–1.33; I^2 = 0.0%). The difference between HSCT and no-HSCT subgroups was not statistically significant (p = 0.43; Figure 5). These findings may be confounded by the fact that patients with *NPM1*^{mut} AML typically do not undergo transplantation in CR1; those who did receive HSCT in CR1 were often ELN intermediate-risk due to co-occurring *FLT3*-ITD, representing a population with inherently higher baseline risk. Across all subgroup analyses, the absence of statistical significance outside the ELN-favorable subgroup should be interpreted with caution. These analyses were based on a limited number of studies and relatively small sample sizes, which may have reduced statistical power to detect modest effects.

We performed study-level meta-regression to examine whether key cohort

characteristics influenced the prognostic effect of MRG mutations on OS (Table 1). In univariable analyses, median age (coefficient (β), 0.009; 95% CI, –0.033 to 0.051; p = 0.668), proportion of patients receiving intensive treatment (β, –1.165; 95% CI, –2.939 to 0.609; p = 0.198) or HSCT (β, 4.831; 95% CI, –0.232 to –2.604; p = 0.101), and frequency of *FLT3* co-mutation (β, 0.223; 95% CI, –0.661 to 1.108; p = 0.621) were not significantly associated with the HR for OS. In multivariable analysis adjusting for these factors, none reached statistical significance (all $p > 0.05$). These findings indicate that the prognostic effect of MRG mutations was consistent across studies, with no evidence of modification by median age, *FLT3* co-mutation prevalence, or the proportion of patients receiving intensive therapy or HSCT.

RUNX1 Inclusion and the Prognostic Significance of MRG Mutations

To assess whether the inclusion of *RUNX1* in the definition of MRG mutations impacts their prognostic association with OS, we conducted a network meta-analysis (NMA) using non-MRG as the common comparator (Supplementary Figure 3A). In this analysis, MRG defined with *RUNX1* was associated with significantly inferior OS compared to the non-MRG group (pooled HR = 1.48; 95% CI: 1.16–1.89; Supplementary Figure 3B). In contrast, MRG defined with *RUNX1* demonstrated a

trend toward worse outcomes relative to MRG without *RUNX1*, but the association did not reach statistical significance (pooled HR = 1.35; 95% CI: 0.88–2.08). These findings suggest that inclusion of *RUNX1* may enhance the prognostic discrimination of MRG mutations in identifying patients at higher risk for adverse outcomes.

Prognostic impact of Individual MRG Mutations

To assess the prognostic contribution of individual MRG mutations, we conducted subgroup analyses of the most frequent co-occurring mutations among four cohorts[10, 14, 15, 17] with available data: *ASXL1* (n=28, 1.5%), *SRSF2* (n=98, 7.6%), and *STAG2* (n=73, 4.1%). Compared with patients without MRG mutations, only *ASXL1* was associated with inferior survival (pooled HR, 2.27; 95% CI, 1.44–3.59; I^2 = 0.0%; Supplementary Figure 4), whereas *SRSF2* (pooled HR, 1.14; 95% CI, 0.85–1.54; I^2 = 0.0%) and *STAG2* (pooled HR, 0.86; 95% CI, 0.36–2.10; I^2 = 71.5%) were not. The difference between subgroups was statistically significant (p = 0.03). We further performed univariable meta-regression using the frequency of each gene mutation among MRG-positive patients as study-level covariates. A higher frequency of *ASXL1* mutations was significantly associated with increased hazard of death (β = 5.45, p = 0.004; Supplementary Table 4), corresponding to a 1.73-fold increase in HR per 10%

increase in *ASXL1* prevalence. *ASXL1*, *RUNX1*, *SF3B1* and *STAG2* explained over 99% of between-study heterogeneity (R^2 > 99%), while other mutations did not show statistically significant associations.

Event-Free Survival (EFS) and Complete Remission (CR)

Four studies[11, 13, 14, 17] and one additional validation cohort reported EFS data stratified by MRG mutation status in patients with *NPM1*^{mut} AML. Pooled analysis demonstrated a significant association between MRG mutations and inferior EFS (pooled HR = 1.31; 95% CI: 1.04–1.65; p = 0.024), and a moderate between-study heterogeneity (I^2 = 29.7%; p = 0.22; Figure 6A). On the other hand, eight studies[8-10, 13-17] reported CR rates. MRG mutations were associated with a modestly lower likelihood of achieving CR, with a pooled risk ratio of 0.94 (95% CI: 0.90 – 0.99; p = 0.010), with minimal heterogeneity (I^2 = 0.0%; p = 0.73; Figure 6B). Among the four studies[9, 10, 14, 15] reporting time to CR data in patients who achieved CR1, no significant difference was observed between those with and without MRG mutations (Supplementary Table 2).

Discussion

This meta-analysis demonstrates that MRG mutations confer adverse prognostic significance in patients with *NPM1*^{mut} AML. Among 4,363 patients included across nine studies (ten cohorts), the presence of MRG mutations was significantly correlated with inferior OS, EFS, and lower CR rates. These findings challenge the current risk stratification paradigm, which classifies *NPM1*^{mut} AML with MRG mutations as favorable-risk subgroup under the ELN-2022 guidelines,[4] and highlight the importance of incorporating co-occurring high-risk molecular alterations into future prognostic models.

Although prior studies have explored the prognostic impact of MRG mutations in *NPM1*^{mut} AML, their findings have been inconsistent. Some reports suggest that MRG mutations attenuate the favorable prognosis typically associated with *NPM1*^{mut} AML,[8, 9, 15, 18] while others have not demonstrated a significant effect.[10, 11, 13, 14, 16, 17] Notably, despite clinical and biological heterogeneity across the included studies, the adverse impact of MRG mutations remained statistically significant in prespecified subgroup analyses, including intensively treated patients and those classified as ELN-2022 favorable risk. Although potential patient overlap existed between the studies by Lachowiez et al.[15] and Othman et al.,[10] a leave-one-out sensitivity analysis that sequentially excluded each study yielded similar results, and the prognostic

association remained robust after adjustment for potential publication bias and across multiple sensitivity analyses, reinforcing the consistency of the pooled estimates. Furthermore, meta-regression demonstrated that treatment intensity did not account for the observed heterogeneity in outcomes, suggesting that the adverse prognostic effect is likely attributable to the intrinsic biology of MRG mutations rather than therapeutic context alone. While MRG mutations were significantly associated with worse outcomes in *NPM1*^{mut} AML, the magnitude of risk (pooled HR = 1.30 in the overall cohort and HR = 1.34 in the ELN favorable-risk subgroup) seemed less pronounced than the prognostic differential typically observed between ELN intermediate- and favorable-risk categories (HR ~1.5–2.27).[19, 20] This suggests that MRG mutations may function as moderate risk-modifying factors within an otherwise favorable subset of AML.

In most studies (seven of nine), patients with *NPM1*^{mut} AML harboring co-occurring MRG mutations were older at diagnosis. In the study by Chan et al.,[8] the adverse prognostic impact of MRG mutations was confined to patients aged ≥60 years; however, another study demonstrated that this association remained significant after adjusting for age, indicating that the effect of MRG mutations on prognosis is not solely age-dependent.[15] In the age-stratified subgroup analysis (n=1,810), MRG

mutations were not significantly associated with poor OS and no differences among age groups were found. However, when the age-stratified subgroup was confined to ELN-2022 favorable group patients, younger patients with MRG mutations was associated with poorer outcomes, suggesting potential age-dependent effect.

Moreover, Tazi et al.[21] reported that the presence of ≥2 MRG mutations is associated with inferior survival, and a similar trend toward worse outcomes was observed in a study specifically evaluating *NPM1*^{mut} AML.[10] These findings raise the possibility that the total number of MRG mutations may further refine risk stratification beyond their mere presence or absence. However, due to limited reporting in this subset, a pooled analysis of mutation burden could not be performed. Future investigations incorporating individual-level genomic data will be essential to determine whether the number of MRG mutations carries incremental prognostic value within the *NPM1*^{mut} AML population.

This study has several inherent limitations. First, as a study-level meta-analysis, it lacks access to individual participant data, precluding adjustment for patient-level confounders that could refine effect estimates. Specifically, factors such as MRD negativity, microclones with low variant allele frequency, and HSCT could potentially mitigate the adverse outcomes associated with MRG mutations in *NPM1*^{mut} AML but

could not be comprehensively evaluated in this analysis. Second, all included studies were retrospective in design, potentially introducing selection and publication biases; however, this limitation is largely unavoidable in genetic prognostic research, and our findings remained robust across multiple sensitivity analyses. Finally, the interpretation of the adverse prognostic impact of MRG mutations in specific subgroups of *NPM1*^{mut} AML—such as those with co-occurring ELN-2022 adverse risk cytogenetics, *FLT3*-ITD, or those receiving non-intensive therapy—should be approached with caution due to the relatively small sample sizes in the total cohort. Recent advances in menin inhibitors have shown clinical meaningful responses in relapsed or refractory *NPM1*^{mut} AML[22] and are now being evaluated in combination with intensive chemotherapy for newly diagnosed cases.[23] Given the adverse impact of co-occurring MRG mutations, evaluating whether menin inhibitor–based regimens can overcome this high-risk genomic feature and improve outcomes represents an important area for future investigation.

In conclusion, MRG mutations are associated with significantly worse survival and lower remission rates in patients with *NPM1*^{mut} AML, challenging the assumption that this genetic subtype uniformly portends a favorable prognosis. These findings advocate for the incorporation of MRG mutation status into contemporary AML risk

models and underscore the need for prospective studies to elucidate the biological and therapeutic implications of this high-risk molecular subgroup.

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Table 1. Meta-regression analysis using study-level characteristics

	Univariable analysis			Multivariable analysis		
	Coefficient (β)	95% CI	*p* value	Coefficient (β)	95% CI	*p* value
Median age (years)	0.009	-0.033 - 0.051	0.668	-0.052	-0.333 – 0.230	0.720
Intensive treatment (%)	-1.165	-2.939 - 0.609	0.198	-1.963	-13.909 - 9.984	0.748
FLT3 co-mutation (%)	0.223	-0.661 - 1.108	0.621	-1.334	-5.404 – 2.737	0.521
HSCT (%)	4.831	-0.232 - 2.604	0.101	8.049	-6.637 - 22.735	0.283

Abbreviations: CI, confidence interval; HSCT: hematopoietic stem cell transplantation

Figure legends

Figure 1. PRISMA flow diagram of study selection.

Study selection process for the systematic review and meta-analysis, following PRISMA guidelines. A total of 955 records were identified; after removing duplicates and screening, 36 full-text articles were assessed for eligibility. Nine studies met the inclusion criteria and were included in the final analysis.

Figure 2. Association between myelodysplasia-related gene (MRG) mutations and overall survival in *NPM1*-mutated (*NPM1*^{mut}) acute myeloid leukemia (AML).

(A) Forest plot displaying hazard ratios for overall survival in patients with *NPM1*^{mut} AML stratified by presence of MRG mutations.

(B) Leave-one-out sensitivity analysis assessing the influence of individual studies on the overall association between MRG mutations and overall survival.

Figure 3. Subgroup analysis of overall survival (OS) in *NPM1*-mutated (*NPM1*^{mut}) acute myeloid leukemia (AML) with myelodysplasia-related gene (MRG) mutations by treatment and European LeukemiaNet (ELN) risk.

(A) Forest plot of hazard ratios (HRs) for OS in patients who received intensive chemotherapy.

(B) Forest plot of HRs for OS among patients classified as favorable or intermediate

risk according to the 2022 European LeukemiaNet (ELN) criteria.

Figure 4. Subgroup analysis of overall survival (OS) in *NPM1*-mutated (*NPM1*^{mut}) acute myeloid leukemia (AML) with myelodysplasia-related gene (MRG) mutations by age.

(A) Forest plot of hazard ratios for OS among patients aged < 60 years versus those ≥ 60 years.

(B) Forest plot of hazard ratios for OS by age subgroup (<60 vs ≥60 years) restricted to patients in the ELN favorable-risk category.

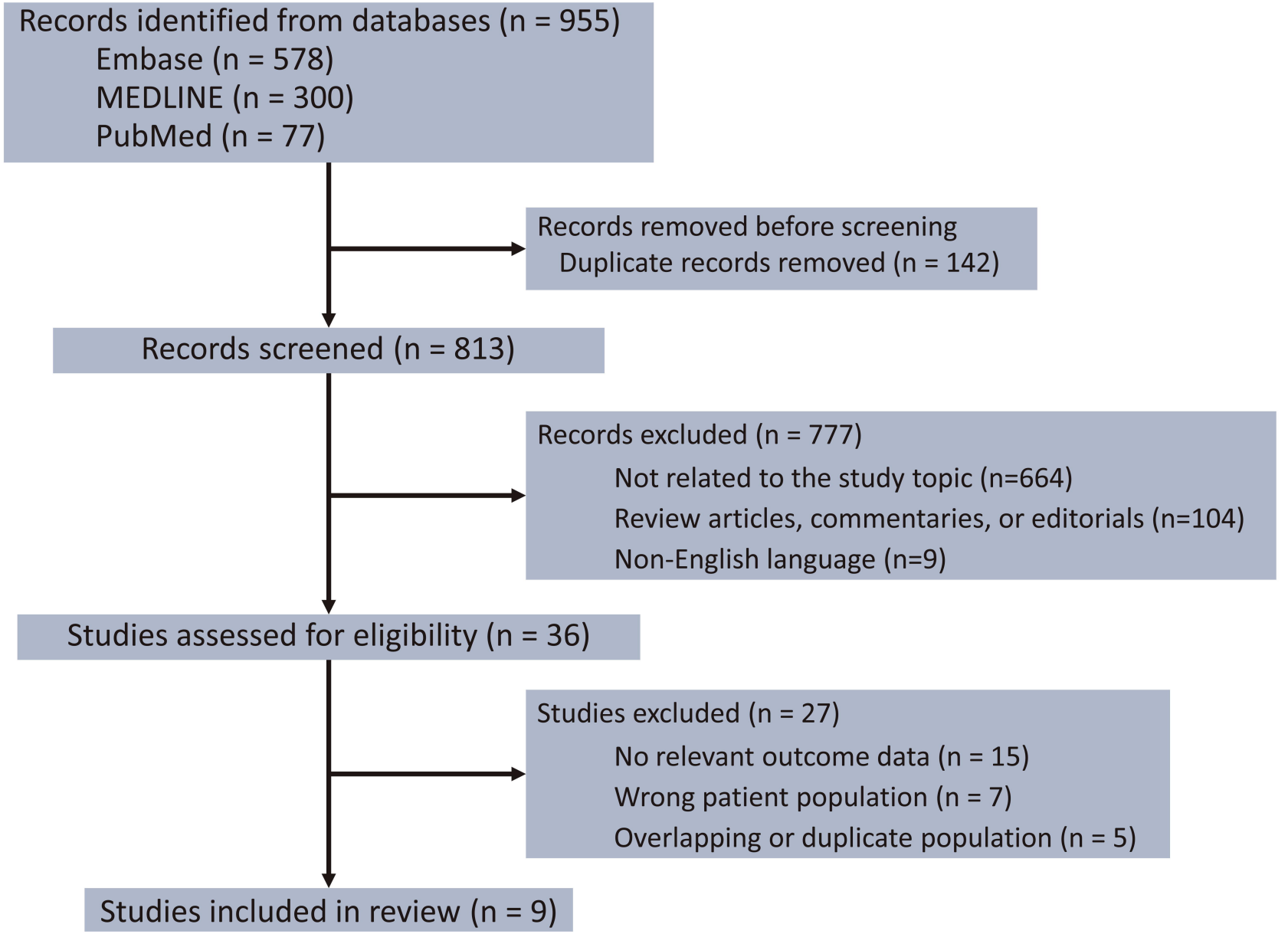
Figure 5. Subgroup analysis of overall survival (OS) in *NPM1*-mutated (*NPM1*^{mut}) acute myeloid leukemia (AML) with myelodysplasia-related gene (MRG) mutations by transplantation status.

Forest plot of hazard ratios for OS among patients who underwent transplantation in first complete remission (CR1) versus those who did not

Figure 6. Event-free survival (EFS) and complete remission (CR) rates in *NPM1*-mutated (*NPM1*^{mut}) acute myeloid leukemia (AML) with or without myelodysplasia-related gene (MRG) mutations.

(A) Forest plot of hazard ratios for EFS comparing patients with versus without MRG mutations.

(B) Forest plot of risk ratios for CR rates comparing patients with versus without MRG mutations.

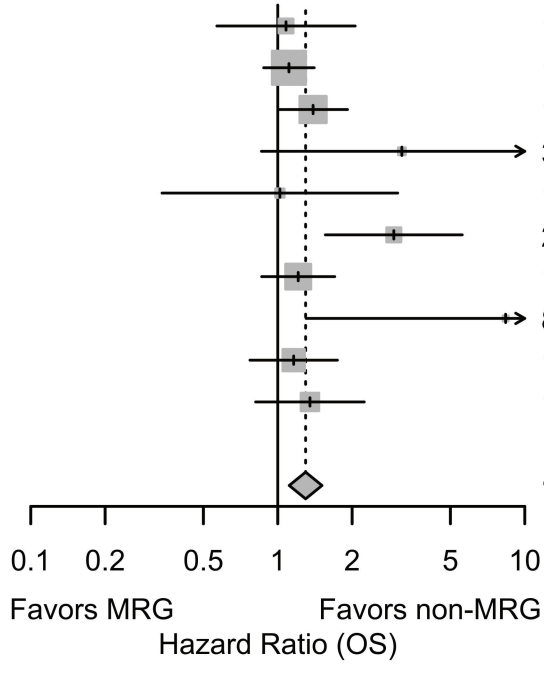

Records identified from databases (n = 955)
Embase (n = 578)
MEDLINE (n = 300)
PubMed (n = 77)
Records removed before screening
Duplicate records removed (n = 142)
Records screened (n = 813)
Records excluded (n = 777)
Not related to the study topic (n=664)
Review articles, commentaries, or editorials (n=104)
Non-English language (n=9)
Studies assessed for eligibility (n = 36)
Studies excluded (n = 27)
No relevant outcome data (n = 15)
Wrong patient population (n = 7)
Overlapping or duplicate population (n = 5)
Studies included in review (n = 9)

(A)

Study	log(HR)	SE	Total(n)	MRG(n)	HR	95%-CI	Weight
Wright 2022	0.0770	0.3290	257	41	1.08	[0.57; 2.06]	5.3%
Eckardt 2023	0.1044	0.1203	936	125	1.11	[0.88; 1.41]	29.5%
Lachowiez 2023	0.3293	0.1637	581	92	1.39	[1.01; 1.92]	18.5%
Wang 2023	1.1569	0.6681	35	10	3.18	[0.86; 11.78]	1.3%
Zhou 2023	0.0198	0.5605	129	25	1.02	[0.34; 3.06]	1.9%
Chan 2024	1.0818	0.3256	233	43	2.95	[1.56; 5.58]	5.4%
Othman 2024	0.1906	0.1738	1098	132	1.21	[0.86; 1.70]	16.7%
Wu 2024	2.1242	0.9491	91	35	8.37	[1.30; 53.75]	0.7%
Cocciardi 2025	0.1484	0.2080	568	103	1.16	[0.77; 1.74]	12.3%
Cocciardi (validation) 2025	0.3001	0.2583	435	49	1.35	[0.81; 2.24]	8.3%
Random effects model					**1.30**	**[1.11; 1.51]**	**100.0%**

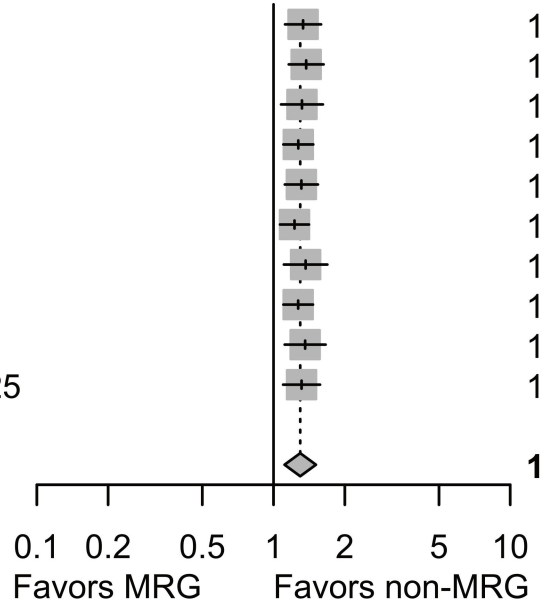
Heterogeneity: I^2 = 39.2%, τ^2 = 0.0062, p = 0.10

Test for overall effect: z = 3.33 (p < 0.001)



(B)

Study	Leave-One-Out Meta-Analysis	HR	95%-CI	P-value	Tau2	I2
Omitting Wright 2022		1.33	[1.12; 1.59]	0.0013	0.015	44.9%
Omitting Eckardt 2023		1.37	[1.16; 1.63]	0.0002	< 0.001	37.2%
Omitting Lachowiez 2023		1.32	[1.08; 1.62]	0.0077	0.023	44.7%
Omitting Wang 2023		1.27	[1.10; 1.47]	0.0012	0.003	38.1%
Omitting Zhou 2023		1.31	[1.12; 1.54]	0.0010	0.009	45.4%
Omitting Chan 2024		1.23	[1.07; 1.41]	0.0046	< 0.001	0%
Omitting Othman 2024		1.37	[1.11; 1.69]	0.0038	0.029	45.5%
Omitting Wu 2024		1.27	[1.10; 1.47]	0.0011	0.002	26.3%
Omitting Cocciardi 2025		1.36	[1.11; 1.66]	0.0024	0.024	45.1%
Omitting Cocciardi (validation) 2025		1.31	[1.10; 1.57]	0.0029	0.015	45.8%
Random effects model		**1.30**	**[1.11; 1.51]**	**0.0009**	**0.006**	**39.2%**

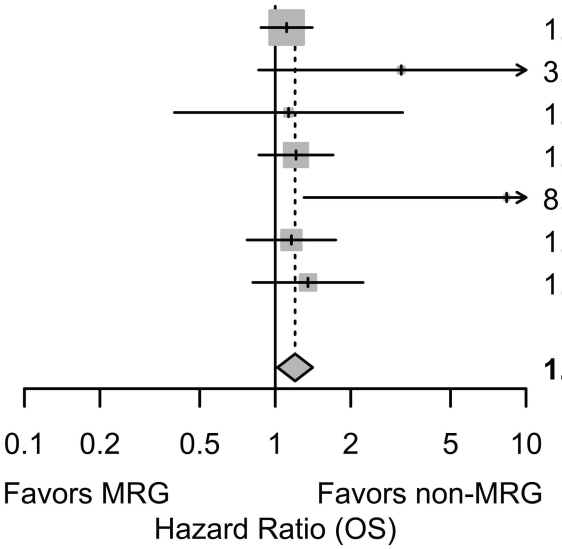


(A)

Study	log(HR)	SE	Total(n)	MRG(n)	HR	95%-CI	Weight
Eckardt 2023	0.1044	0.1203	936	125	1.11	[0.88; 1.41]	47.0%
Wang 2023	1.1569	0.6681	35	10	3.18	[0.86; 11.78]	1.5%
Zhou (intensive) 2023	0.1222	0.5344	104	15	1.13	[0.40; 3.22]	2.4%
Othman 2024	0.1906	0.1738	1098	132	1.21	[0.86; 1.70]	22.5%
Wu 2024	2.1242	0.9491	91	35	8.37	[1.30; 53.75]	0.8%
Cocciardi 2025	0.1484	0.2080	568	103	1.16	[0.77; 1.74]	15.7%
Cocciardi (validation) 2025	0.3001	0.2583	435	49	1.35	[0.81; 2.24]	10.2%
Random effects model					**1.20**	**[1.02; 1.41]**	**100.0%**

Heterogeneity: I^2 = 14.1%, τ^2 < 0.0001, p = 0.32

Test for overall effect: z = 2.21 (p = 0.027)



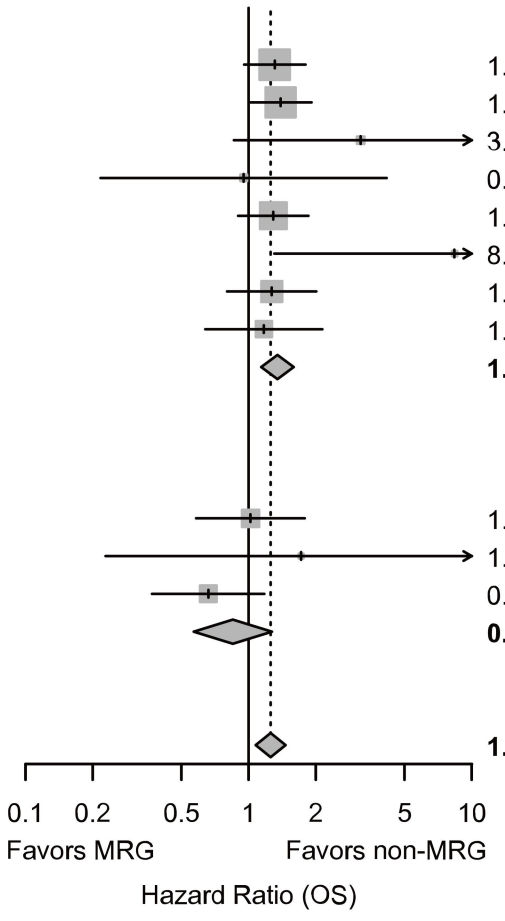
(B)

Study	log(HR)	SE	Total(n)	MRG(n)	HR	95%-CI	Weight
ELN favorable							
Eckardt (ELN favorable) 2023	0.2700	0.1618	496	75	1.31	[0.95; 1.80]	23.4%
Lachowiez 2023	0.3293	0.1637	581	92	1.39	[1.01; 1.92]	22.9%
Wang 2023	1.1569	0.6681	35	10	3.18	[0.86; 11.78]	1.4%
Zhou (ELN favorable) 2023	-0.0513	0.7530	57	7	0.95	[0.22; 4.16]	1.1%
Othman (ELN favorable) 2024	0.2546	0.1852	649	92	1.29	[0.90; 1.85]	17.9%
Wu 2024	2.1242	0.9491	91	35	8.37	[1.30; 53.75]	0.7%
Cocciardi (ELN favorable) 2025	0.2390	0.2350	470	94	1.27	[0.80; 2.01]	11.1%
Cocciardi (validation) (ELN favorable) 2025	0.1570	0.3079	265	36	1.17	[0.64; 2.14]	6.5%
Random effects model					**1.34**	**[1.14; 1.59]**	**84.9%**
Heterogeneity: I^2 = 0%, τ^2 = < 0.0001, p = 0.54							
ELN intermediate							
Eckardt (Dresden)(ELN intermediate) 2023	0.0198	0.2861	181	21	1.02	[0.58; 1.79]	7.5%
Zhou (ELN intermediate) 2023	0.5423	1.0302	45	2	1.72	[0.23; 12.96]	0.6%
Othman (ELN intermediate) 2024	-0.4155	0.2959	434	38	0.66	[0.37; 1.18]	7.0%
Random effects model					**0.85**	**[0.57; 1.27]**	**15.1%**
Heterogeneity: I^2 = 0%, τ^2 = 0.0038, p = 0.45							
Random effects model					**1.25**	**[1.08; 1.46]**	**100.0%**

Heterogeneity: I^2 = 16.3%, τ^2 < 0.0001, p = 0.29

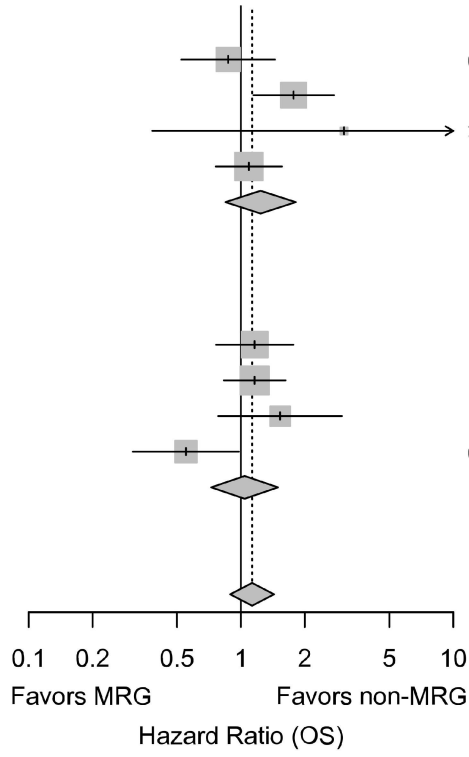
Test for overall effect: z = 2.89 (p = 0.004)

Test for subgroup differences: χ^2_1 = 4.20, df = 1 (p = 0.04)



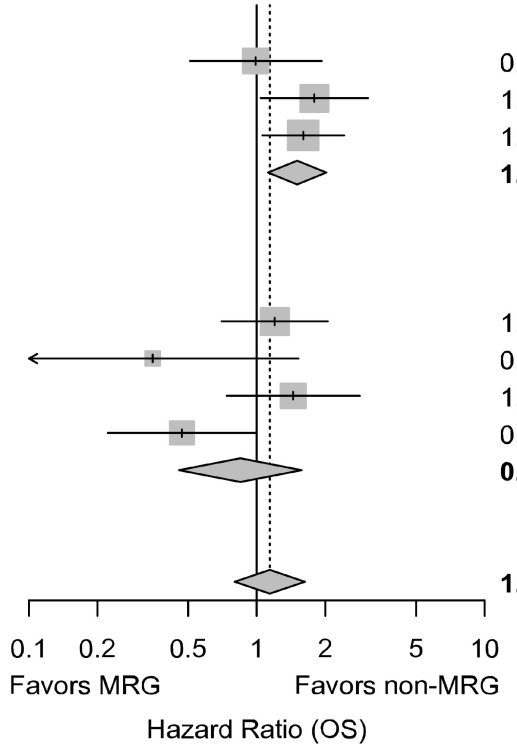
(A)

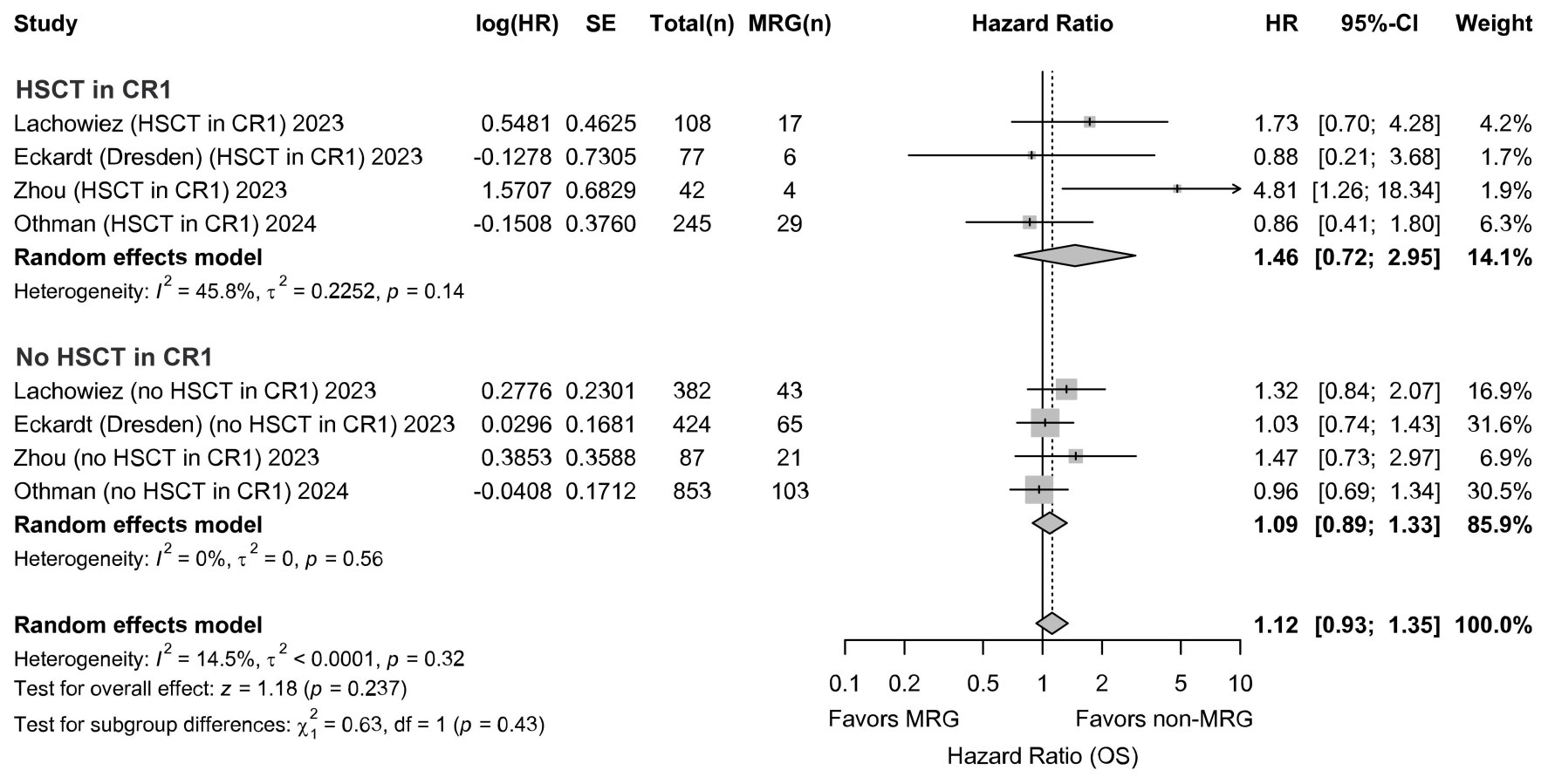
Study	log(HR)	SE	Total(n)	MRG(n)	HR	95%-CI	Weight
Age < 60							
Eckardt (Dresden) (under 60) 2023	-0.1393	0.2598	322	32	0.87	[0.52; 1.45]	12.6%
Lachowiez (under 60) 2023	0.5710	0.2228	383	38	1.77	[1.14; 2.74]	14.8%
Zhou (under 60) 2023	1.1184	1.0613	46	2	3.06	[0.38; 24.49]	1.2%
Othman (under 60) 2024	0.0862	0.1834	890	93	1.09	[0.76; 1.56]	17.7%
Random effects model					**1.24**	**[0.84; 1.81]**	**46.3%**
Heterogeneity: I^2 = 46.5%, τ^2 = 0.0682, p = 0.13							
Age ≥ 60							
Eckardt (Dresden) (over 60) 2023	0.1484	0.2142	179	39	1.16	[0.76; 1.77]	15.4%
Lachowiez (over 60) 2023	0.1484	0.1706	198	54	1.16	[0.83; 1.62]	18.7%
Zhou (over 60) 2023	0.4253	0.3428	85	23	1.53	[0.78; 3.00]	8.8%
Othman (over 60) 2024	-0.5978	0.2936	208	39	0.55	[0.31; 0.98]	10.8%
Random effects model					**1.04**	**[0.72; 1.49]**	**53.7%**
Heterogeneity: I^2 = 54.5%, τ^2 = 0.0750, p = 0.09							
Random effects model					**1.13**	**[0.89; 1.43]**	**100.0%**
Heterogeneity: I^2 = 45.4%, τ^2 = 0.0495, p = 0.08							
Test for overall effect: z = 0.99 (p = 0.324)							
Test for subgroup differences: χ^2_1 = 0.41, df = 1 (p = 0.52)							



(B)

Study	log(HR)	SE	Total(n)	MRG(n)	HR	95%-CI	Weight
Age < 60							
Eckardt (Dresden) 2023	-0.0101	0.3395	184	25	0.99	[0.51; 1.93]	14.2%
Lachowiez (AMLSG) 2023	0.5822	0.2770	225	27	1.79	[1.04; 3.08]	17.0%
Othman 2024	0.4700	0.2116	533	65	1.60	[1.06; 2.42]	20.4%
Random effects model					**1.50**	**[1.12; 2.02]**	**51.6%**
Heterogeneity: I^2 = 0%, τ^2 = < 0.0001, p = 0.37							
Age ≥ 60							
Eckardt (Dresden) 2023	0.1823	0.2741	100	20	1.20	[0.70; 2.05]	17.1%
Lachowiez (AMLSG) 2023	-1.0498	0.7511	39	7	0.35	[0.08; 1.53]	4.8%
Zhou 2023	0.3688	0.3433	84	24	1.45	[0.74; 2.83]	14.0%
Othman 2024	-0.7550	0.3837	131	29	0.47	[0.22; 1.00]	12.5%
Random effects model					**0.85**	**[0.46; 1.57]**	**48.4%**
Heterogeneity: I^2 = 59.2%, τ^2 = 0.2314, p = 0.06							
Random effects model					**1.14**	**[0.80; 1.63]**	**100.0%**
Heterogeneity: I^2 = 54.2%, τ^2 = 0.1163, p = 0.04							
Test for overall effect: z = 0.73 (p = 0.467)							
Test for subgroup differences: χ^2_1 = 2.70, df = 1 (p = 0.10)							

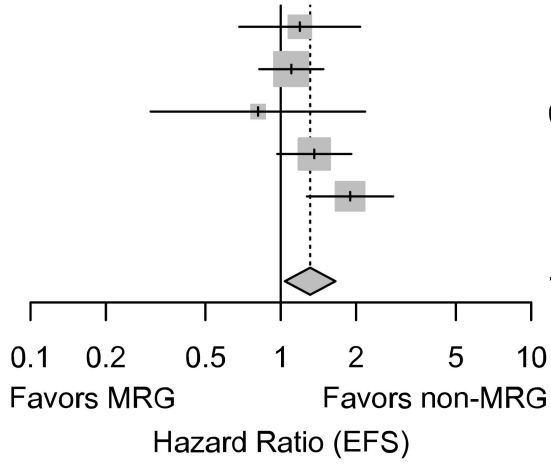



Study
log(HR)
SE
Total(n)
MRG(n)
Hazard Ratio
HR
95%-CI
Weight
HSCT in CR1
Lachowiez (HSCT in CR1) 2023 0.5481 0.4625 108 17 1.73 [0.70; 4.28] 4.2%
Eckardt (Dresden) (HSCT in CR1) 2023 -0.1278 0.7305 77 6 0.88 [0.21; 3.68] 1.7%
Zhou (HSCT in CR1) 2023 1.5707 0.6829 42 4 4.81 [1.26; 18.34] 1.9%
Othman (HSCT in CR1) 2024 -0.1508 0.3760 245 29 0.86 [0.41; 1.80] 6.3%
Random effects model 1.46 [0.72; 2.95] 14.1%
Heterogeneity: I^2 = 45.8%, τ^2 = 0.2252, p = 0.14
No HSCT in CR1
Lachowiez (no HSCT in CR1) 2023 0.2776 0.2301 382 43 1.32 [0.84; 2.07] 16.9%
Eckardt (Dresden) (no HSCT in CR1) 2023 0.0296 0.1681 424 65 1.03 [0.74; 1.43] 31.6%
Zhou (no HSCT in CR1) 2023 0.3853 0.3588 87 21 1.47 [0.73; 2.97] 6.9%
Othman (no HSCT in CR1) 2024 -0.0408 0.1712 853 103 0.96 [0.69; 1.34] 30.5%
Random effects model 1.09 [0.89; 1.33] 85.9%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.56
Random effects model 1.12 [0.93; 1.35] 100.0%
Heterogeneity: I^2 = 14.5%, τ^2 < 0.0001, p = 0.32
Test for overall effect: z = 1.18 (p = 0.237)
Test for subgroup differences: χ^2_1 = 0.63, df = 1 (p = 0.43)
0.1
0.2
0.5
1
2
5
10
Favors MRG
Favors non-MRG
Hazard Ratio (OS)

(A)

Study	log(HR)	SE	Total(n)	MRG(n)	HR	95%-CI	Weight
Wright 2022	0.1740	0.2840	257	41	1.19	[0.68; 2.08]	13.8%
Eckardt 2023	0.0953	0.1506	936	125	1.10	[0.82; 1.48]	31.6%
Zhou 2023	-0.2107	0.5036	129	25	0.81	[0.30; 2.17]	5.1%
Cocciardi 2025	0.3075	0.1742	568	103	1.36	[0.97; 1.91]	27.0%
Cocciardi (validation) 2025	0.6366	0.2026	435	49	1.89	[1.27; 2.81]	22.4%
Random effects model					**1.31**	**[1.04; 1.65]**	**100.0%**

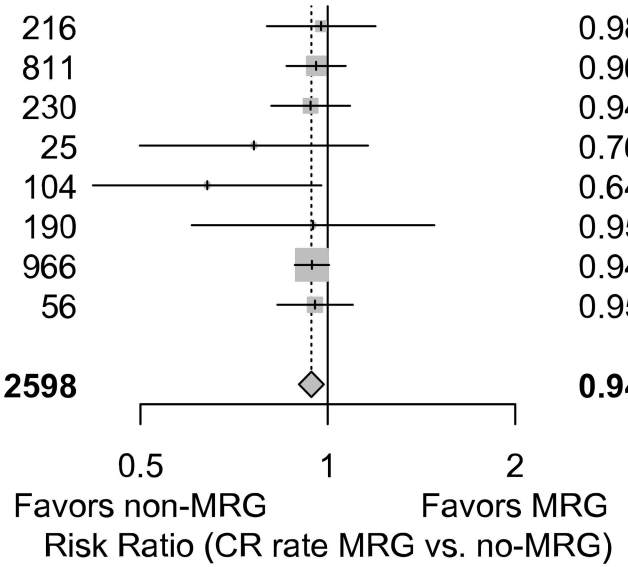
Heterogeneity: I^2 = 29.7%, τ^2 = 0.0221, p = 0.22
Test for overall effect: z = 2.26 (p = 0.024)



(B)

Study	MRG Events	MRG Total	non-MRG Events	non-MRG Total	RR	95%-CI	Weight
Wright 2022	30	41	162	216	0.98	[0.80; 1.19]	5.2%
Eckardt 2023	93	125	630	811	0.96	[0.86; 1.07]	17.7%
Lachowiez (AMLSG) 2023	29	34	209	230	0.94	[0.81; 1.09]	10.0%
Wang 2023	7	10	23	25	0.76	[0.50; 1.16]	1.2%
Zhou 2023	12	25	78	104	0.64	[0.42; 0.98]	1.2%
Chan 2024	15	43	70	190	0.95	[0.60; 1.48]	1.0%
Othman 2024	117	132	908	966	0.94	[0.89; 1.00]	52.8%
Wu 2024	31	35	52	56	0.95	[0.83; 1.10]	10.9%
Random effects model		**445**		**2598**	**0.94**	**[0.90; 0.99]**	**100.0%**

Heterogeneity: I^2 = 0.0%, τ^2 < 0.0001, p = 0.73
Test for overall effect: z = -2.58 (p = 0.010)



Supplementary Material

Prognostic implications of myelodysplasia-related gene mutations in *NPM1*-mutated acute myeloid leukemia: a systematic review and meta-analysis

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Supplementary Methods

Data Extraction and Quality Assessment

Data extraction was performed using a standardized form, capturing key variables including study characteristics (author, year, study design, sample size, study population), treatment intensity, concurrent *FLT3*-ITD, *NPM1* and MRG mutation status, hazard ratios (HRs), 95% CI and *p* values for survival outcomes (overall survival [OS]), event-free survival [EFS]), and response rates (complete remission [CR]). When necessary, the corresponding or first authors were contacted by email to request missing information, including subgroup analysis data. For the study by Lachowiez et al.,[1] which was itself based on the AMLSG[2] and UK-NCRI[3] cohorts, the subgroup data included in our meta-analysis were derived directly from these publicly available datasets. In the study of Eckardt et al.,[4] subgroup analysis was accessible in 501 patients (53.5%), derived from the following cohorts: AML96 (NCT00180115, n=270), AML2003 (NCT00180102, n=91), AML60+ (NCT00180167, n=25), SORAML (NCT00893373, n=74), and the SAL AML registry (n=41).

Risk of bias was evaluated using the Quality in Prognostic Studies (QUIPS) tool,[5] which is designed for prognostic factor studies and visualized by robvis package.[6] Six domains were assessed: study participation, attrition, prognostic factor measurement, outcome measurement, confounding, and statistical analysis and reporting. Each domain was rated as having low, moderate, or high risk of bias according to standardized criteria.

Data Synthesis and Statistical Analysis

The primary outcome was OS, expressed as a pooled HR with corresponding 95% CIs, comparing patients with versus without MRG mutations within *NPM1*^{mut} AML. All HRs were directly extracted from individual studies. Secondary outcomes included EFS, defined as the time from diagnosis to relapse, treatment failure, or death from any cause, and CR rate, assessed according to standard morphological criteria as reported in the original studies. Pooled estimates for primary and secondary outcomes, along with corresponding 95% CIs and *p* values, were calculated using random-effects models. Between-study heterogeneity was quantified using the I^2 statistic, τ^2 (tau-squared), and Cochran's Q test, with I^2 values of 25%, 50%, and 75% interpreted as low, moderate, and high heterogeneity, respectively.

Prespecified subgroup analyses were conducted to evaluate the prognostic impact of MRG mutations in intensively treated cohorts, ELN 2022 risk classification, age (> 60 years), and receipt of hematopoietic stem cell transplantation (HSCT) in first CR (CR1).[7] To explore potential sources of heterogeneity, we conducted univariable and multivariable meta-regression using study-level covariates to assess their contribution to between-study variation. Meta-regression was conducted using the *metareg* function from the meta package in R.[8] Sensitivity analyses were performed using leave-one-out methods to assess the robustness of the pooled estimates.[9] To evaluate the prognostic effect of *RUNX1* inclusion in MRG definitions, a network meta-analysis (NMA) was conducted using non-MRG as a common comparator to indirectly compare MRG (with *RUNX1*) and MRG (without *RUNX1*).[10] A two-sided *p* value < 0.05 was considered statistically significant. All statistical analyses were performed using R software (version 4.4.1; R Foundation for Statistical

Computing, Vienna, Austria) and independently reviewed by a biostatistician (CY Liu).

Supplementary Tables

Supplementary Table 1: Search strategy

Database	Search strategy
PubMed	#1 "Leukemia, Myeloid, Acute"[Mesh]
	#2 "Acute Myeloid Leukemia" OR "AML" OR "Acute Myelogenous Leukemia" OR "Acute Myeloblastic Leukemia"
	#3 #1 OR #2
	#4 "NPM1 gene" OR "NPM1" OR "Nucleophosmin"
	#5 #3 AND #4
	#6 "Secondary-type mutations" OR "secondary AML" OR "Myelodysplasia-related" OR "Myelodysplasia-related mutations" OR "Myelodysplasia-related genes" OR "Myelodysplasia-related gene mutations" OR "MDS-related mutations" OR "co-occurrence mutations"
	#7 #5 AND #6
Embase	#1 'acute myeloid leukemia'/exp OR 'acute myeloid leukemia'
	#2 'Acute Myeloid Leukemia' OR 'AML' OR 'Acute Myelogenous Leukemia' OR 'Acute Myeloblastic Leukemia'
	#3 #1 OR #2
	#4 'npm1 gene' OR 'NPM1' OR 'Nucleophosmin'
	#5 #3 AND #4
	#6 'Secondary-type mutations' OR 'secondary AML' OR 'Myelodysplasia-related' OR 'Myelodysplasia-related mutations' OR 'Myelodysplasia-related genes' OR 'Myelodysplasia-related gene mutations' OR 'MDS-related mutations' OR 'co-occurrence mutations'
	#7 #5 AND #6
Medline	1 MH "Leukemia, Myeloid, Acute+"
	2 ((Acute AND Myeloid AND Leukemia) OR AML OR (Acute AND Myelogenous AND Leukemia) OR (Acute AND Myeloblastic AND Leukemia)).mp.
	3 1 OR 2
	4 ("Nucleophosmin") OR ("NPM1").mp.
	5 3 AND 4
	6 ("Secondary-type mutations" OR "secondary AML" OR "Myelodysplasia-related" OR "Myelodysplasia-related mutations" OR "Myelodysplasia-related genes" OR "Myelodysplasia-related gene mutations" OR "MDS-related mutations" OR "co-occurrence mutations").mp.
	7 5 AND 6

Supplementary Table 2: Baseline characteristics of included studies

Author	Year	Journal	Cohort	Study design	MRG include RUNX1[a]	Total (N)	MRG (n)
Wright et al[11]	2022	Am J Hematol.	VUMC, BWH, UPMC, MGH	Retrospective	Not included	257	41
Eckardt et al[4]	2023	Leukemia	AML96, AML2003, AML60+, SORAML, AMLCG-1999, AMLCG-2008	Retrospective	Not included	936	125
Lachowiez et al[1]	2023	Blood	AMLSG, UKNCRI	Retrospective	Included	581	92
Wang et al[12]	2023	Cancers	MDACC, FSOM	Retrospective	Included	35	10
Zhou et al[13]	2023	Eur J Haematol.	University of Toronto	Retrospective	Not included	129	25
Chan et al[14]	2024	Blood adv.	Moffitt Cancer Center, Weill Cornell, and Memorial Healthcare System	Retrospective	Included	233	43
Othman et al[15]	2024	Blood	UK AML17, AML19	Retrospective	Included	1098	132
Wu et al[16]	2024	Cancer Med.	Nanfang Hospital (Guangzhou, China)	Retrospective	Included	91	35
Cocciardi et al[17]	2025	Hemasphere	AMLSG 09-09	Retrospective	Included	568	103
Cocciardi et al (validation)[17]	2025	Hemasphere	NCT00146120	Retrospective	Included	435	49

Supplementary Table 2: Baseline characteristics of included studies (continued)

Author	Age[b]	Age over 60	Male[c]	Concurrent *FLT3*[c]	ELN 2022 classification
Wright et al[11]	non-MRG: 63.1 (53.9-72.0); MRG: 68.3 (64.0-76.5)[g]	NA	non-MRG: 92 (43%); MRG: 28 (68%)[g]	non-MRG: 98 (45%); MRG: 12 (29%)	Favorable: 147 (57.8%); Intermediate: 110 (42.8%); Adverse: 0 (0.0%)
Eckardt et al[4]	non-MRG: 55 (45-64); MRG: 59 (49-68)[g]	NA	non-MRG: 334 (41.2%); MRG: 62 (49.6%)	non-MRG: 330 (40.7%); MRG: 19 (15.2)[g]	Favorable: 496 (55.7%); Intermediate: 349 (39.2%); Adverse: 45 (5.1%)
Lachowiez et al[1]	non-MRG: 53; MRG: 62[g]	non-MRG: 144 (29.4%); MRG: 54 (58.7%)[g]	NA	Excluded	Favorable: 581 (100.0%); Intermediate: 0 (0.0%); Adverse: 0 (0.0%)
Wang et al[12]	non-MRG: 57 (17–69); MRG: 59 (33–68)	NA	non-MRG: 13 (52%); MRG: 6 (60%)	Excluded	Favorable: 35 (100.0%); Intermediate: 0 (0.0%); Adverse: 0 (0.0%)
Zhou et al[13]	non-MRG: 61 (28-87); MRG: 71 (47-89)[g]	non-MRG: 62 (60%); MRG: 23 (92%)[g]	non-MRG: 44 (42%); MRG: 13 (52%)	non-MRG: 44 (42%); MRG: 8 (32%)	Favorable: 57 (44.2%); Intermediate: 45 (34.9%); Adverse: 27 (20.9%)
Chan et al[14]	non-MRG: 62 (22-80); MRG: 68 (34-86)[g]	non-MRG: 99 (52.1%); MRG: 37 (86.0%)[g]	non-MRG: 84 (44.2%); MRG: 27 (62.8%)[g]	non-MRG: 68 (35.8%); MRG: 11 (25.6%)[g]	Favorable: 119 (51.1%); Intermediate: 88 (37.8%); Adverse: 22 (9.4%)
Othman et al[15]	non-MRG: 52 (44-58); MRG 55 (47-62)[g]	non-MRG: 169 (17%); MRG: 39 (30%)[g]	non-MRG: 392 (41%); MRG: 80 (61%)[g]	non-MRG: 396 (41%); MRG: 38 (29%)[g]	Favorable: 649 (59.1%); Intermediate: 434 (39.5%); Adverse: 15 (1.4%)
Wu et al[16]	non-MRG: 49.0; MRG: 53.0	non-MRG: 9 (16.1%); MRG: 11 (31.4%)	non-MRG: 32 (57.1%); MRG: 17 (48.6%)	Excluded	Favorable: 91 (100.0%); Intermediate: 0 (0.0%); Adverse: 0 (0.0%)
Cocciardi et al[17]	non-MRG: 57 (50-67); MRG: 66 (57-71)[g]	non-MRG: 175 (38.6%); MRG: 65 (63.8%)[g]	non-MRG: 203 (44%); MRG: 61 (59%)[g]	non-MRG: 89 (19%); MRG: 9 (8.7%)[g]	Favorable: 470 (82.7%); Intermediate: 98 (17.3%); Adverse: 0 (0.0%)
Cocciardi et al (validation)[17]	Total: 50 (42-56)	NA	Total: 187 (43%)	non-MRG: 157 (40.7%); MRG: 13 (26.5%)	Favorable: 265 (60.9%); Intermediate: 170 (39.1%); Adverse: 0 (0.0%)

Supplementary Table 2: Baseline characteristics of included studies (continued)

Author	Intensive treatment[c]	HSCT[c]	CR rate	Time to CR[b]	Data
Wright et al[11]	non-MRG: 160 (63%); MRG: 24 (58.5%)	non-MRG:80 (38%); MRG: 10 (26%)	non-MRG: 75%; MRG: 72%	NA	Direct extraction
Eckardt et al[4]	All intensive	non-MRG: 255 (27.2%); MRG: 22 (17.6%)[g]	non-MRG: 77.7%; MRG: 74.4%	non-MRG: 64 (55-81); MRG: 67 (54-85)	Direct extraction, Data request
Lachowiez et al[1]	NA	non-MRG:179 (41.6%); MRG: 26 (43.3%)[h]	non-MRG: 90.7%; MRG: 85.3%[i]	non-MRG: 34 (28-59); MRG: 35 (29-70)[i]	Direct extraction, Data request
Wang et al[12]	All intensive	NA	non-MRG: 92%; MRG: 70%	NA	Direct extraction
Zhou et al[13]	non-MRG: 89 (86%); MRG: 15 (60%)[g]	non-MRG: 38 (37%); MRG: 4 (16%)	non-MRG: 86%; MRG: 80%	NA	Direct extraction, Data request
Chan et al[14]	non-MRG: 151 (79.5%); MRG: 26 (60.5%)[g]	non-MRG: 94 (49.5%); MRG: 15 (34.9%)	non-MRG: 85.3%; MRG: 69.8%	NA	Direct extraction
Othman et al[15]	All intensive	non-MRG: 390 (40.4%); MRG: 44 (33.3%)	non-MRG: 94%; MRG: 89%	non-MRG: 33 (29-39); MRG: 34 (30-39)	Direct extraction, Data request
Wu et al[16]	All intensive	non-MRG: 23 (41.1%); MRG: 12 (34.3%)	non-MRG: 92.9%; MRG: 88.8%	No difference in CR rate among 1-2 cycles of induction or 1st cycle of consolidation	Direct extraction
Cocciardi et al[17]	All intensive	NA	NA	NA	Direct extraction
Cocciardi et al (validation)[17]	All intensive	NA	NA	NA	Direct extraction

[a]Myelodysplasia-related gene (MRG) including ASXL1, BCOR, EZH2, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2 with or without RUNX1.

[b]Time from diagnosis to CR. Presented as median and interquartile range.

[c]Presented as frequency and percentage.

[d]Among 65 patients with *NPM1*-mutated AML (Group A(n=25): no *FLT3*-ITD/MRG, Group B(n=30): with *FLT3*-ITD, Group C(n=10): with MRG).

As hazard ratios were available only for comparisons with Group A, only Groups A and C were included.

[e]Among 1,357 patients with *NPM1*-mutated AML, NGS data were available for 1,098 patients. All variables presented were based on the NGS-available cohort, except for age >60 years, male, and HSCT, which reflect the entire cohort.

[f]Cocciardi et al. 2025 included an independent validation cohort.

[g]Statistically significant differences were observed between the MRG and non-MRG groups ($p<0.05$).

[h]HSCT data available for 490 patients (MRG, n=60; non-MRG, n=430)

[i]Only data of the AMLSG cohort was available

Abbreviations: BWH: Brigham and Women's Hospital, FSOM: Northwestern University Feinberg School of Medicine, MDACC: MD Anderson Cancer Center, MGH: Massachusetts General Hospital, MRG: myelodysplasia-related gene, UPMC: University of Pittsburgh Medical Center, VUMC: Vanderbilt University Medical Center.

Supplementary Table 3: Frequency of myelodysplasia-related gene mutations in *NPM1*-mutated acute myeloid leukemia across included studies

Study	*ASXL1*	*BCOR*	*EZH2*	*RUNX1*	*SF3B1*	*SRSF2*	*STAG2*	*U2AF1*	*ZRSR2*
Wright 2022	7 2.7%	1 0.4%	1 0.4%	NA	4 1.6%	27 10.5%	5 1.9%	0 0.0%	0 0.0%
Eckardt 2023	12 1.3%	16 1.7%	22 2.4%	NA	13 1.4%	48 5.1%	32 3.4%	4 0.4%	5 0.5%
Lachowiez 2023	8 1.4%	8 1.4%	5 0.9%	7 1.2%	5 0.9%	47 8.1%	19 3.3%	1 0.2%	1 0.2%
Wang 2023	2 5.7%	0 0.0%	1 2.9%	2 5.7%	0 0.0%	3 8.6%	0 0.0%	0 0.0%	0 0.0%
Zhou 2023	1 0.8%	1 0.8%	0 0.0%	NA	3 2.3%	13 10.1%	6 4.7%	4 3.1%	0 0.0%
Chan 2024	8 3.4%	6 2.6%	5 2.1%	2 0.9%	4 1.7%	15 6.4%	5 2.1%	3 1.3%	3 1.3%
Othman 2024	13 1.2%	5 0.5%	9 0.8%	11 1.0%	10 0.9%	56 5.1%	40 3.6%	5 0.5%	1 0.1%
Wu 2024	15 16.5%	9 9.9%	10 11.0%	1 1.1%	0 0.0%	3 3.3%	1 1.1%	2 2.2%	2 2.2%
Cocciardi 2025[a]	2%	0%	1%	1%	1%	8%	5%	1%	1%
Cocciardi (validation) 2025[a]	1.4%	1.3%	0.9%	1.3%	0.5%	3.7%	3.2%	0.0%	1.3%

[a]Only the percentage of each mutation was reported; absolute mutation counts were not provided.

Supplementary Table 4: Univariable Meta-Regression of Study Level Individual Myelodysplasia-Related Gene (MRG) Mutation Frequencies on Hazard Ratios for Overall Survival in *NPM1*-Mutated AML

Gene	Coefficient	Fold change in HR	95% CI	*p* value	Accounted heterogeneity	Residual heterogeneity
ASXL1	5.45	1.73	1.72 - 9.19	0.004	100%	0%
BCOR	2.65	1.30	-1.21 - 6.52	0.18	0%	38.07%
EZH2	2.53	1.29	-1.82 - 6.88	0.25	0%	50.66%
RUNX1	2.37	1.27	-0.82 - 5.54	0.15	99.96%	0.00%
SF3B1	-4.39	0.64	-9.86 - 1.08	0.12	99.95%	0.01%
SRSF2	-1.89	0.83	-4.05 - 0.27	0.09	0.00%	40.58%
STAG2	-3.06	0.74	-5.4 - -0.72	0.01	99.96%	0.00%
U2AF1	0.88	1.09	-5.07 - 6.83	0.77	0.00%	18.75%
ZRSR2	1.88	1.21	-3.39 - 7.14	0.48	0.00%	24.09%

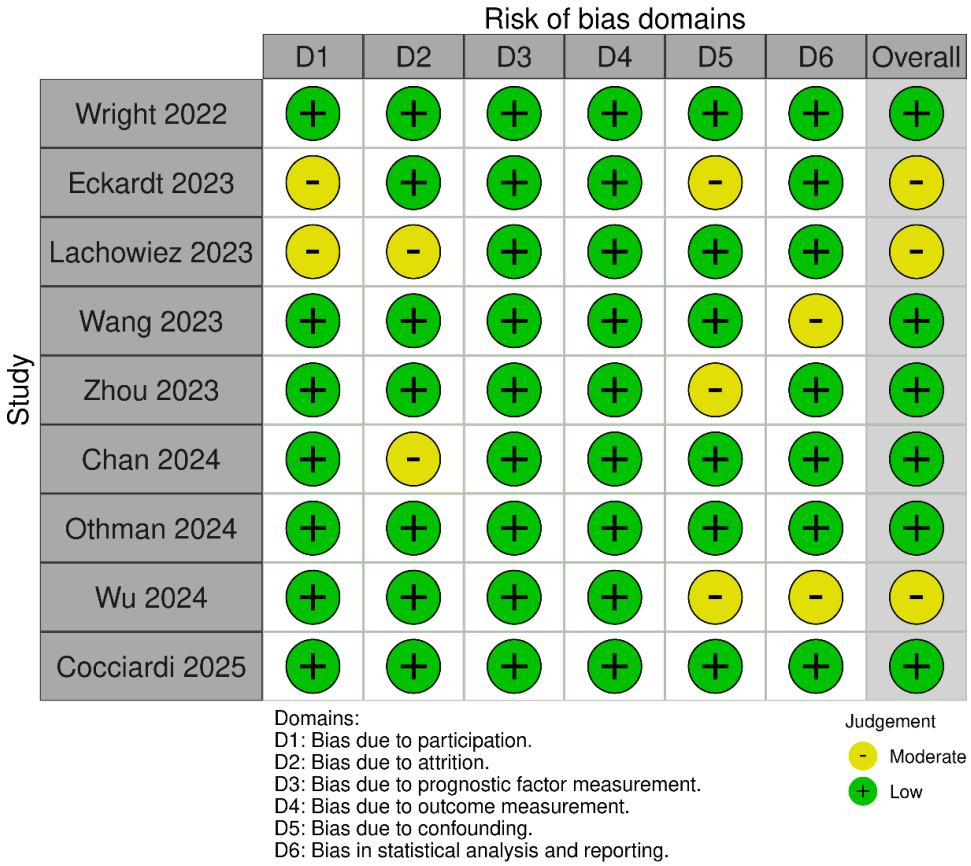
Supplementary Figures

Supplementary Figure 1. Risk of bias assessment using the Quality in Prognosis Studies (QUIPS) tool.

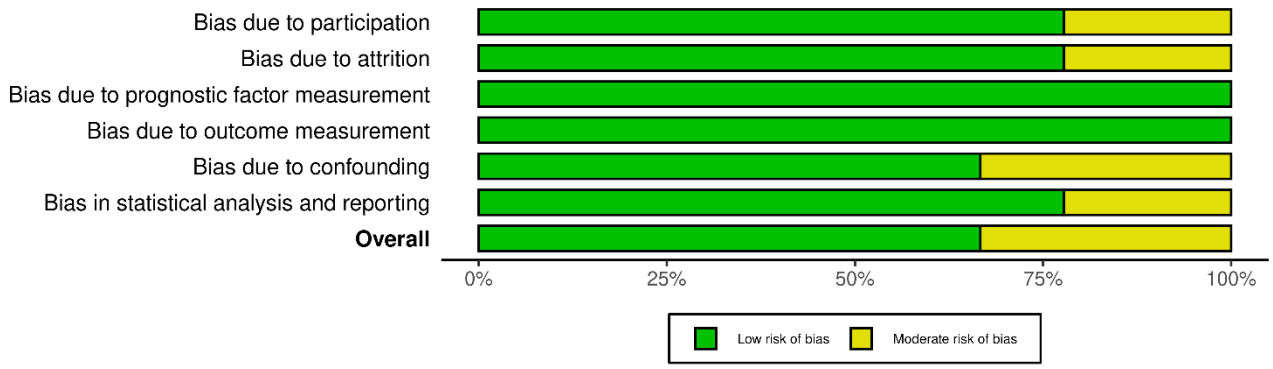
(A) Traffic light plot summarizing the risk of bias across six domains for each included study.

(B) Summary plot displaying the proportion of studies with low, moderate, or high risk of bias across each domain.

(A)



(B)



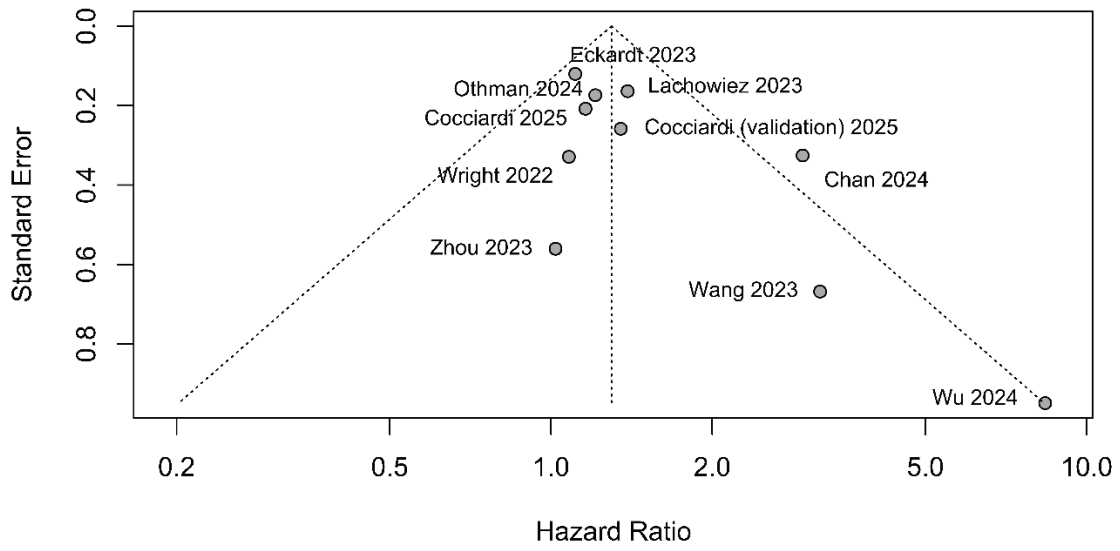
Supplementary Figure 2. Evaluation of publication bias in the analysis of overall survival associated with myelodysplasia-related gene (MRG) mutations in NPM1-mutated (NPM1mut) acute myeloid leukemia (AML).

(A) Funnel plot assessing asymmetry in the included studies.

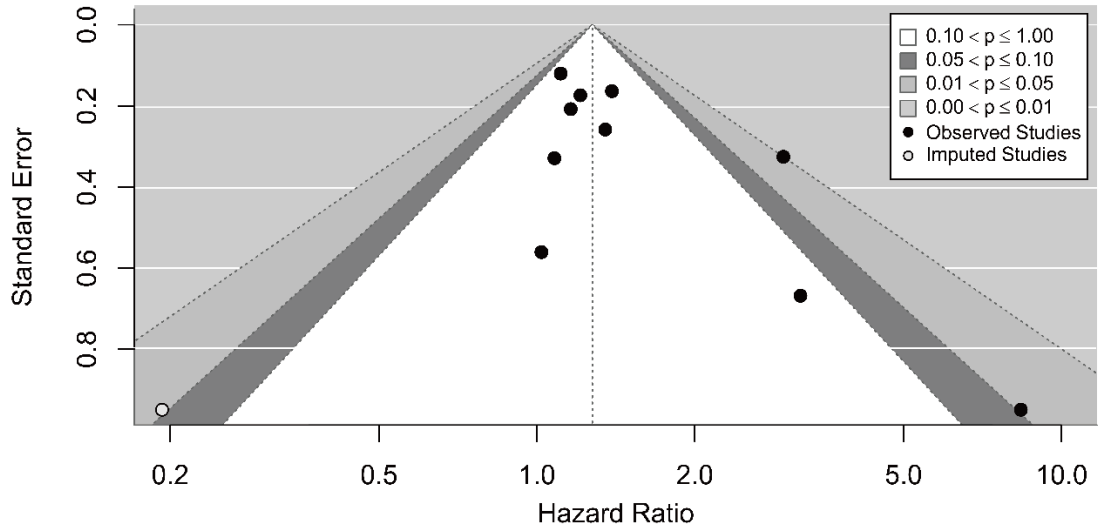
(B) Contour-enhanced funnel plot after trim-and-fill adjustment to evaluate the potential for missing studies.

(C) Forest plot of hazard ratios for overall survival following the trim-and-fill adjustment.

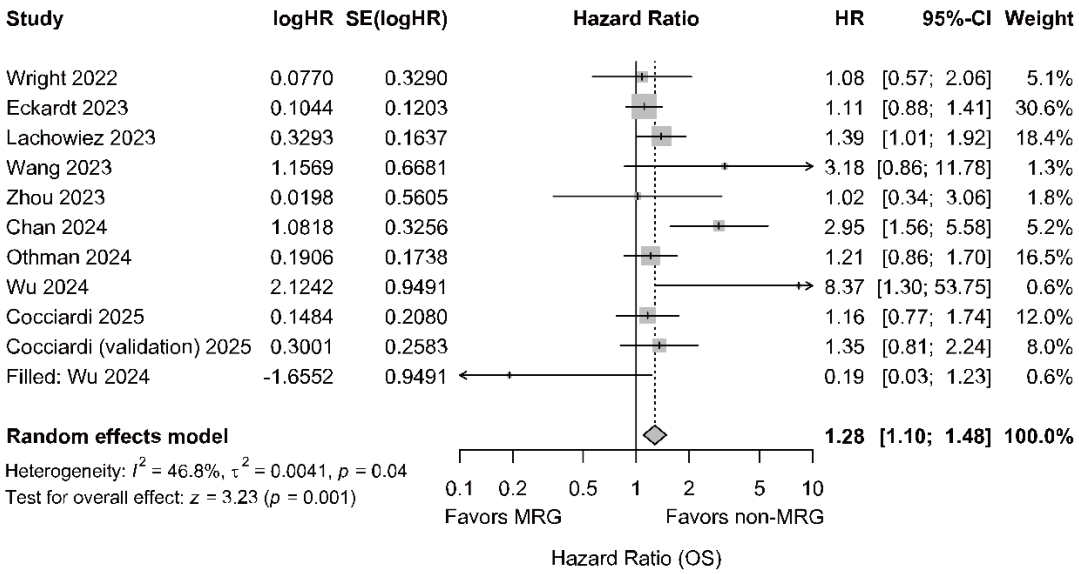
(A)


Standard Error
0.0
0.2
0.4
0.6
0.8
Eckardt 2023
Othman 2024
Lachowiez 2023
Cocciardi 2025
Cocciardi (validation) 2025
Wright 2022
Chan 2024
Zhou 2023
Wang 2023
Wu 2024
0.2
0.5
1.0
2.0
5.0
10.0
Hazard Ratio

(B)


Standard Error
0.0
0.2
0.4
0.6
0.8
0.10 < p ≤ 1.00
0.05 < p ≤ 0.10
0.01 < p ≤ 0.05
0.00 < p ≤ 0.01
Observed Studies
Imputed Studies
0.2
0.5
1.0
2.0
5.0
10.0
Hazard Ratio

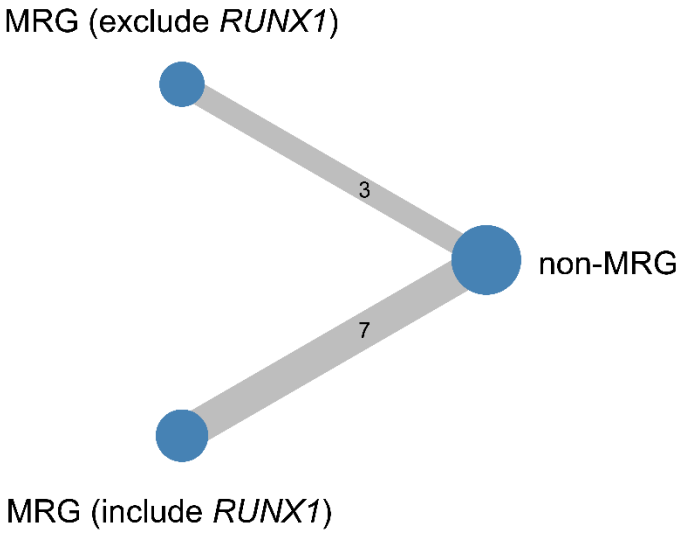
(C)


Study
logHR
SE(logHR)
Hazard Ratio
HR
95%-CI
Weight
Wright 2022 0.0770 0.3290 1.08 [0.57; 2.06] 5.1%
Eckardt 2023 0.1044 0.1203 1.11 [0.88; 1.41] 30.6%
Lachowiez 2023 0.3293 0.1637 1.39 [1.01; 1.92] 18.4%
Wang 2023 1.1569 0.6681 3.18 [0.86; 11.78] 1.3%
Zhou 2023 0.0198 0.5605 1.02 [0.34; 3.06] 1.8%
Chan 2024 1.0818 0.3256 2.95 [1.56; 5.58] 5.2%
Othman 2024 0.1906 0.1738 1.21 [0.86; 1.70] 16.5%
Wu 2024 2.1242 0.9491 8.37 [1.30; 53.75] 0.6%
Cocciardi 2025 0.1484 0.2080 1.16 [0.77; 1.74] 12.0%
Cocciardi (validation) 2025 0.3001 0.2583 1.35 [0.81; 2.24] 8.0%
Filled: Wu 2024 -1.6552 0.9491 0.19 [0.03; 1.23] 0.6%
Random effects model 1.28 [1.10; 1.48] 100.0%
Heterogeneity: I^2 = 46.8%, τ^2 = 0.0041, p = 0.04
Test for overall effect: z = 3.23 (p = 0.001)
0.1
0.2
0.5
1
2
5
10
Favors MRG
Favors non-MRG
Hazard Ratio (OS)

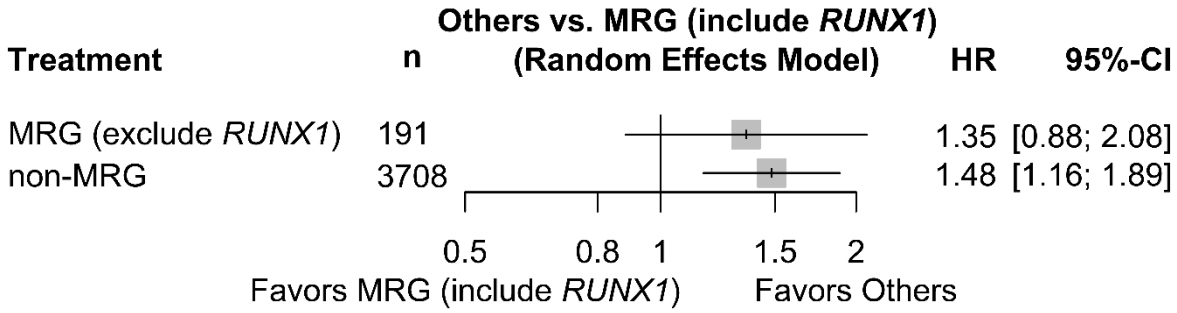
Supplementary Figure 3. Network meta-analysis evaluating the impact of *RUNX1* inclusion on the prognostic significance of MRG mutations.

(A) Network diagram of included studies, using the non-MRG group as the common comparator.
(B) Forest plot showing indirect comparisons of overall survival for MRG (excluding *RUNX1*) and non-MRG groups, with MRG (including *RUNX1*) as the reference.

(A)

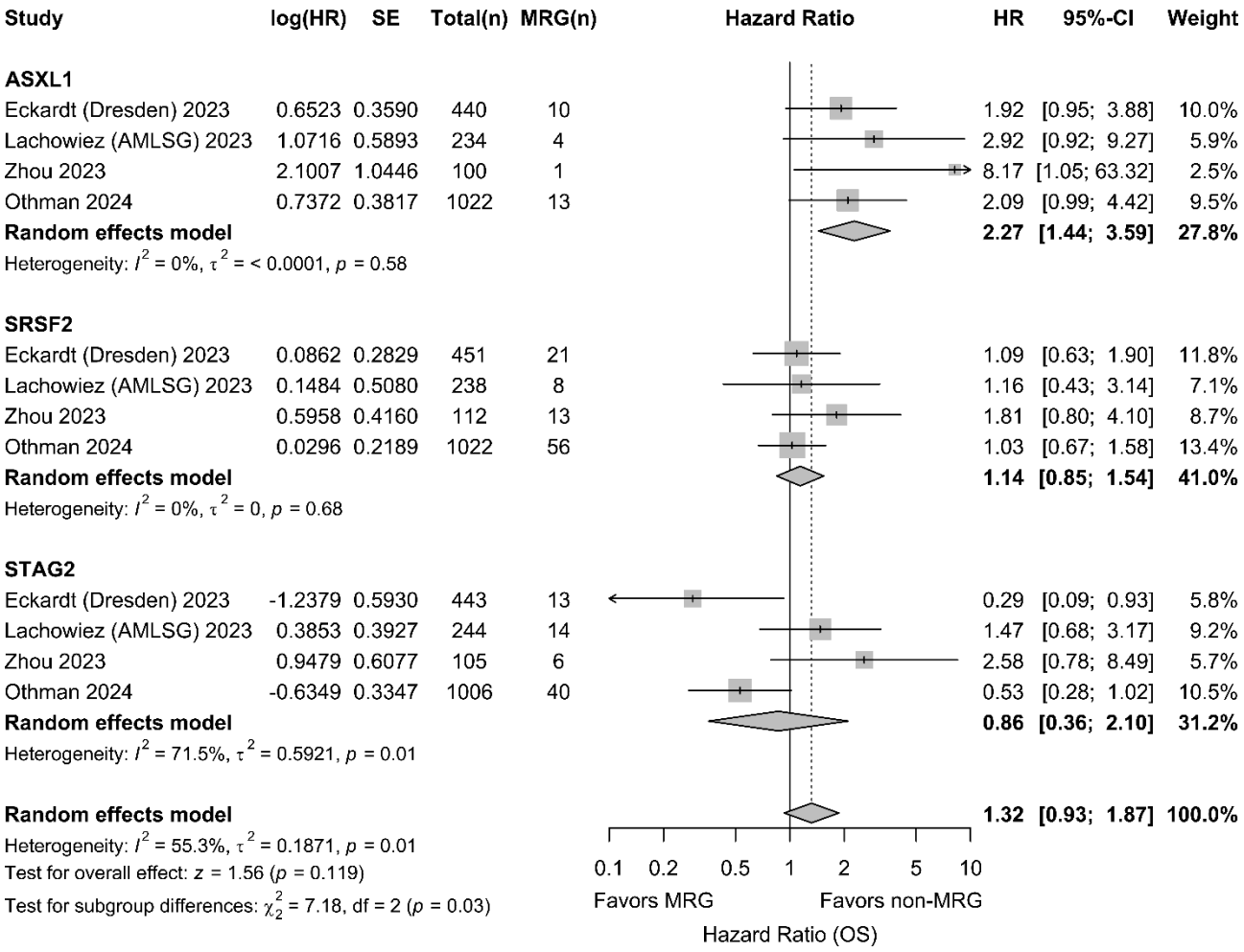


(B)



Supplementary Figure 4. Subgroup analysis of overall survival (OS) in *NPM1*-mutated (*NPM1*^{mut}) acute myeloid leukemia (AML) with myelodysplasia-related gene (MRG) mutations by individual MRG mutations.

Forest plot of hazard ratios for overall survival comparing patients with *ASXL1*, *SRSF2*, or *STAG2* mutations with those lacking MRG mutations.



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