Abstract: DNA (cytosine-5)-methyltransferase 3 alpha (DNMT3A) mutations were widely believed to be independently associated with inferior prognosis in acute myeloid leukemia (AML) patients. As dominant missense alterations in DNMT3A mutations, R882 mutations cause the focal hypomethylation phenotype. However, there remains debate on the influence of R882 mutations on AML prognosis. Thus, this meta-analysis aimed at further illustrating the prognostic power of DNMT3A R882 mutations in AML patients.

Eligible studies were identified from 5 databases containing PubMed, Embase, Web of Science, Clinical Trials, and the Cochrane Library (up to October 25, 2015). Effects (hazard ratios [HRs] with 95% confidence interval [CI]) of relapse-free survival (RFS) and overall survival (OS) were pooled to estimate the prognostic power of mutant DNMT3A R882 in overall patients and subgroups of AML patients.

Eight competent studies with 4474 AML patients including 694 with DNMT3A R882 mutations were included. AML patients with DNMT3A R882 mutations showed significant shorter RFS (HR = 1.40, 95% CI = 1.24–1.59, \( P < 0.001 \)) and OS (HR = 1.47, 95% CI = 1.17–1.86, \( P = 0.001 \)) in the overall population. DNMT3A R882 mutations predicted worse RFS and OS among the subgroups of patients under age 60 (RFS: HR = 1.44, 95% CI = 1.25–1.66, \( P < 0.001 \)); OS: HR = 1.48, 95% CI = 1.15–1.90, \( P = 0.002 \)), over age 60 (RFS: HR = 2.03, 95% CI = 1.40–2.93, \( P < 0.001 \); OS: HR = 1.85, 95% CI = 1.36–2.53, \( P < 0.001 \)), cytogenetically normal (CN)-AML (RFS: HR = 1.52, 95% CI = 1.26–1.83, \( P < 0.001 \); OS: HR = 1.67, 95% CI = 1.16–2.41, \( P = 0.006 \)), and non-CN-AML (RFS: HR = 1.96, 95% CI = 1.20–3.21, \( P = 0.006 \); OS: HR = 2.51, 95% CI = 1.52–4.15, \( P = 0.0038 \)).

DNMT3A R882 mutations possessed significant unfavorable prognostic influence on RFS and OS in AML patients.

INTRODUCTION

Acute myeloid leukemia (AML) is a clinical and biological heterogeneous clonal stem cell disorder characterized by clonal and aggressive expansion of myeloid progenitor cells or “blast” cells in bone marrow.1,2 AML usually presents with a broad spectrum of prognosis-related cytogenetic abnormalities, genetic mutations, and aberrant expression of genes.3,4 Currently, AML is healed in 35% to 40% among younger patients with age <60, and 5% to 15% among older patients with age ≥60.5 The huge molecular heterogeneity of AML has become growingly distinct over the past 15 years, despite the cytogenetic heterogeneity of the disease has been realized for over 30 years.6 The prognostic significance of this biological heterogeneity is well-accepted, but there remains a need to identify better and more precise predictors of disease outcome.

Recently, genetic mutations and epigenetic alterations have been identified in the bone-marrow leukemogenesis and are reported to be associated with AML outcomes.6,7 Previous studies have suggested that internal tandem duplication in fms-related tyrosine kinase 3 (FLT3-ITD), mutations in nucleophosmin (NPM1), and CCAAT/enhancer binding protein alpha (CEBPA) can be used to stratify risk among patients with normal karyotype.8 Later reports have identified novel prognosis-related mutations in AML patients, which include mutational isocitrate dehydrogenase 2 (IDH2), additional sex combs like 1 (ASXL1), and DNA (cytosine-5)-methyltransferase 3 alpha (DNMT3A).9 RNAs related to DNMT3A in AML have been elucidated to enhance the understanding of AML biology and clinical treatment.9

A meta-analysis of R882 mutations in AML patients is required to evaluate their significant impact on prognosis. To our knowledge, there is no comprehensive analysis been done to evaluate R882 mutations in AML. Therefore, this study aimed to evaluate the prognostic impact of R882 mutations in AML.
DNMT3A is responsible for de novo methylation of genome DNA during mammalian development, and DNMT3A alterations are thought to play important roles in etiology of various diseases including AML.10 DNMT3A is one of the most frequently mutated genes in AML patients, being found mutated in approximately 20% of the patients.11,12 DNMT3A somatic mutation was first identified by whole-genome sequencing in an AML patient with normal karyotype,11 which was associated with worse clinical outcomes.13,14 Overall, mountains of studies have declared that DNMT3A could be a prognostic indicator in AML patients.

With the announcement of the Precision Medicine Initiative in USA, it is urgent to find out the function of more and finer biomarkers, thus to generate knowledge applicable to the whole range of health and disease.16,17 And AML is no exception. In AML patients with DNMT3A mutations, about 60% patients exhibit heterozygous mutations at Arginine 882 (R882), which results in loss-of-function effect and disruption of normal methylation function.18–20 Four R882 mutations included R882C (arginine → cysteine), R882H (arginine → histidine), R882S (arginine → serine), and R882P (arginine → phenylalanine) are reported.14,21 Therefore, DNMT3A mutations are usually classified as R882 mutations and non-R882 mutations.22 However, there existed an inconsistent opinion on whether DNMT3A R882 mutations have the potential to predict AML prognosis. For example, Renneville and colleagues reported that patients with R882 mutations showed shorter RFS and OS in cytogenetically normal (CN)-AML23 while some studies showed negative findings on OS time.24–26 So, this meta-analysis was aimed at systematically elaborating the prognostic values of DNMT3A R882 mutations in AML patients, in order to guide precisely clinical decision-making even to improve the prognosis of the patients.

**MATERIALS AND METHODS**

**Literature Search**

Literature search was conducted in PubMed, Embase, Web of Science, ClinicalTrials, and the Cochrane Library with the following search terms: “AML,” “acute myeloid leukemia,” “Leukemia, Myeloid, Acute,” “acute myelogenous leukemia,” “acute myelogenous leukemia,” “DNMT3A,” “DNMT3A1,” “DNMT3A2,” “DNA (cytosine-5)-methyltransferase 3 alpha,” “DNA methyltransferase 3A,” “DNA methyltransferase 3 alpha,” “DNA (cytosine-5)-methyltransferase 3 alpha,” “DNA (cytosine-5)-methyltransferase 3A,” “DNMT3A,” “DNA methyltransferase 3A,” “DNA methyltransferase 3 alpha,” “DNMT3A,” “DNMT3A1,” “DNMT3A2,” “DNA (cytosine-5)-methyltransferase 3 alpha,” “DNA (cytosine-5)-methyltransferase 3A,” “DNA methyltransferase 3A,” “DNA methyltransferase 3 alpha.”

**Search Results**

A total of 50 publications were identified by the systematic literature search, of which 1 review was excluded and 13 duplicates were removed, resulting in 36 publications. Also, 28 articles were removed in view of relevance, design, and suitable outcome data through the title, abstract, and full-text screening regarding the aforementioned inclusion criteria (Figure 1). Ultimately, 8 publications were included in the meta-analysis.

**Characteristics and Bias Risk of the Included Studies**

Eight studies containing a total of 4474 subjects (694 with DNMT3A R882 mutations) were included in the meta-analysis. The principal features of these subjects with or without DNMT3A R882 mutations are displayed in Tables 1 and 2, respectively, and the accessional characteristics are shown in Tables S1 and S2, http://links.lww.com/MD/A935, respectively. Meanwhile, sample size of the studies ranged from 67 to 1770 patients. Of these studies, 5 studies originated from...
Europe, 1 from Asia, and 2 from USA. The frequency of \textit{DNMT3A} R882 mutations ranged from 7.46% to 24.39%.

Risk of bias (see quality assessment in the part of methods) was evaluated based on 9 assessment items of NOS. The qualities of 7 studies (87.5%) were regarded as high, and the rest 1 study (12.5%) was treated as moderate. Relevant details are presented in Table 3.

Prognostic Power of \textit{DNMT3A} R882 Mutations in Total Population

1. RFS
Data were extracted from 6 studies, totaling 3915 AML patients, containing 631 patients with \textit{DNMT3A} R882 mutations and 3284 without R882 mutations. As presented in Figure 2, results showed no distinct heterogeneity ($P = 0.21$, $I^2 = 30\%$). With a fixed-effect model, a significant shorter RFS was observed in AML patients with \textit{DNMT3A} R882 mutations compared with those without R882 mutations in total population (HR = 1.40, 95% CI = 1.24–1.59, $P < 0.001$).

2. OS
Data were derived from 8 studies, totaling 4474 AML patients, containing 694 patients with \textit{DNMT3A} R882 mutations and 3780 without R882 mutations. With a random-effect model, AML patients with the \textit{DNMT3A} R882 mutations presented an evident shorter OS time than those without R882 mutations in total population (HR = 1.47, 95% CI = 1.17–1.86, $P = 0.001$, Figure 3). These results suggested that \textit{DNMT3A} R882 mutations could predict inferior clinical outcomes in AML patients.

Prognostic Power of \textit{DNMT3A} R882 Mutations in Different Subgroups

Prognostic Power of \textit{DNMT3A} R882 Mutations Stratified by Age ($< 60$ and $\geq 60$)

Age is the most important factor influencing the prognosis of AML; and adults with age older than 60 have a shorter OS than adults with age younger than 60. $^{30,31}$ Meanwhile, based on the NCCN Clinical Practice Guidelines in Acute myeloid leukemia (available free of charge on the NCCN web site: http://www.nccn.org/ and in the NCCN Guidelines for Acute Myeloid Leukemia), we divided AML patients into different subgroups containing different age ($< 60$ and $\geq 60$) to further validate the prognostic ability of \textit{DNMT3A} R882 mutations. The incorporative results of HRs for RFS and OS among AML patients of different age ($< 60$ and $\geq 60$) were presented in fixed- and random-effect model, respectively (Tables 4 and 5). Also, the matching forest plots are shown in Figures S1 and S2, http://links.lww.com/MD/A935. With a fixed-effect model, significant shorter RFS or/and OS were observed in AML patients with the \textit{DNMT3A} R882 mutations in comparison with those without R882 mutations in both subgroups of age $< 60$ (RFS: HR = 1.44, 95% CI = 1.25–1.66, $P < 0.001$) and age $\geq 60$ (RFS: HR = 2.03, 95% CI = 1.40–2.93, $P < 0.001$; OS: HR = 1.85, 95% CI = 1.36–2.53, $P < 0.001$). With a random-effect model, significant shorter OS was observed in AML patients with the \textit{DNMT3A} R882 mutations.
### TABLE 1. Clinical and Laboratory Characteristics of AML Patients With DNMT3A R882 Mutations From the 8 Included Studies

| First Author | Year | Region | N   | n   | Sex (Male/Female) | Median Age, y (Range) | M0 | M1 | M2 | M3 | M4 | M5 | M6 | M7 | Unknown | Favorable | Intermediate | Adverse | Unknown |
|--------------|------|--------|-----|-----|------------------|-----------------------|----|----|----|----|----|----|----|----|---------|-----------|-------------|---------|---------|
| Ley          | 2010 | USA    | 281 | 37  | 17/20            | 53 (21–81)            | 1  | 7  | 5  | 14 | 9  | 0  | 0  | 0  | 0       | 34        | 2           |         |         |
| Thol         | 2011 | Germany| 489 | 58  | —                | —                     | —  | —  | —  | —  | —  | —  | —  | —  | —       | —         | —           |         |         |
| Renneville   | 2012 | France | 123 | 30  | —                | —                     | —  | —  | —  | —  | —  | —  | —  | —  | —       | —         | —           |         |         |
| Marcucci     | 2012 | USA    | 415 | 92  | —                | —                     | 1  | 9  | 11 | 10 | 23 | 0  | 2  | 2  | 10      | 51        | 4           | 3       | 0       |
| Ribeiro      | 2012 | Netherlands | 415 | 58  | 31/27            | 51 (18–60)            | 1  | 9  | 11 | 10 | 23 | 0  | 2  | 2  | —       | —         | —           |         | —       |
| Gaidzik      | 2013 | Germany| 1770| 239 | 102/137          | 49.9 (18–60)          | 1  | 9  | 11 | 10 | 23 | 0  | 2  | 2  | 10      | 51        | 4           | 3       | 0       |
| Park         | 2015 | Korea  | 67  | 5   | 2/3              | 59 (18–77)            | —  | —  | —  | —  | —  | —  | —  | —  | —       | —         | —           |         | —       |
| Gale         | 2015 | UK     | 914 | 175 | 76/99            | 47 (20–67)            | 0  | 20 | 34 | 67 | 44 | 0  | 3  | 0  | 0       | —         | —           | —       | —       |

FAB = the French-American-British, N = number of patients in total, n = number of patients with R882 mutations, — = there is no corresponding data presented.

### TABLE 2. Clinical and Laboratory Characteristics of AML Patients Without DNMT3A R882 Mutations From the 8 Included Studies

| First Author | Year | Region | N   | n   | Sex (Male/Female) | Median Age, y (Range) | M0 | M1 | M2 | M3 | M4 | M5 | M6 | M7 | Unknown | Favorable | Intermediate | Adverse | Unknown |
|--------------|------|--------|-----|-----|------------------|-----------------------|----|----|----|----|----|----|----|----|---------|-----------|-------------|---------|---------|
| Ley          | 2010 | USA    | 281 | 244 | 24/180           | 38 (20–59)            | 19 | 52 | 51 | 12 | 3  | 3  | 3  | 0  | 79      | 132       | 28          | —       |
| Thol         | 2011 | Germany| 489 | 431 | —                | —                     | —  | —  | —  | —  | —  | —  | —  | —  | —       | —         | —           |         |         |
| Renneville   | 2012 | France | 123 | 93  | —                | —                     | —  | —  | —  | —  | —  | —  | —  | —  | —       | —         | —           |         |         |
| Marcucci     | 2012 | USA    | 415 | 323 | —                | —                     | —  | —  | —  | —  | —  | —  | —  | —  | —       | —         | —           |         |         |
| Ribeiro      | 2012 | Netherlands | 415 | 357 | 179/178          | 46 (15–60)            | 15 | 78 | 93 | 69 | 77 | 6  | 19 | —  | 57      | 225       | 66          | 9       |
| Gaidzik      | 2013 | Germany| 1770| 1531| 305/726          | 49.4 (18–60)          | —  | —  | —  | —  | —  | —  | —  | —  | 256     | 848       | 309         | 118     |
| Park         | 2015 | Korea  | 67  | 62  | 3/27             | 56 (11–83)            | —  | —  | —  | —  | —  | —  | —  | —  | —       | —         | —           |         | —       |
| Gale         | 2015 | UK     | 914 | 739 | 363/376          | 45 (15–68)            | 32 | 166| 202| 170| 90 | 22 | 5  | —  | —       | —         | —           | —       | —       |

FAB = the French-American-British, N = number of patients in total, n = number of patients without DNMT3A R882 mutations, — = there is no corresponding data presented.
patients with the \textit{DNMT3A} R882 mutations in comparison with those without R882 mutations in subgroup of age < 60 (OS: HR = 1.48, 95% CI = 1.15–1.90, \( P = 0.002 \)). Meanwhile, similar results were also observed in the other model. This suggested that the \textit{DNMT3A} R882 mutations could predict shorter RFS and OS in AML patients regardless of patient age.

\textbf{Prognostic Power of \textit{DNMT3A} R882 Mutations in the Population of CN-AML and Non-CN-AML}

Cytogenetic influenced dramatically the clinical outcome of AML patients, so subgroup analysis was also carried out in CN-AML and non-CN-AML patients, respectively. Tables 4 and 5 show the pooled results of HRs for RFS and OS among AML patients in subgroups of CN-AML and non-CN-AML in fixed- and random-effect model, respectively. Also, the matching forest plots in subgroups of CN-AML are presented in Figure S3, http://links.lww.com/MD/A935. With a fixed-effect model, a significant shorter RFS was observed in AML patients with the \textit{DNMT3A} R882 mutations compared with those without R882 mutations in subgroup of CN-AML (HR = 1.52, 95% CI = 1.26–1.83, \( P < 0.001 \)). With a random-effect model, a significant shorter OS was observed in AML patients with the \textit{DNMT3A} R882 mutations compared with those without R882 mutations in subgroup of CN-AML (HR = 1.67, 95% CI = 1.16–2.41, \( P = 0.006 \)). Similarly, the consistent results were seen in the other effect model.

RFS and OS in non-CN-AML patients were analyzed by using the original data of Ley study. As presented in Figure 4, remarkable shorter RFS and OS was shown in AML patients with the \textit{DNMT3A} R882 mutations than those without R882 mutations in the non-CN-AML patients (RFS: HR = 1.96, 95% CI = 1.20–3.21, \( P = 0.006 \); OS: HR = 2.51, 95% CI = 1.52–4.15, \( P = 0.0038 \)). In brief, \textit{DNMT3A} R882 mutations may act as a poor prognostic indicator in both CN-AML and non-CN-AML patients.

\textbf{Comparison of Prognostic Power Between \textit{DNMT3A} R882 Mutations and Non-R882 Mutations}

(1) \textbf{RFS}

Data were derived from 3 researches summing up 465 AML patients with \textit{DNMT3A} mutations, including 306 \textit{DNMT3A} R882 mutations and 159 \textit{DNMT3A} non-R882 mutations. As shown in Figure 5, the results showed no visible heterogeneity (\( P = 0.47 \), \( I^2 = 0 \% \)). With a fixed-effect model, there was no obvious difference in RFS time between the group of \textit{DNMT3A} R882 mutations and those without R882 mutations in the non-CN-AML patients (RFS: HR = 1.96, 95% CI = 1.20–3.21, \( P = 0.006 \); OS: HR = 2.51, 95% CI = 1.52–4.15, \( P = 0.0038 \)). In brief, \textit{DNMT3A} R882 mutations may act as a poor prognostic indicator in both CN-AML and non-CN-AML patients.

(2) \textbf{OS}

Data were extracted from 4 studies, totaling 954 AML patients with \textit{DNMT3A} mutations, including 364 \textit{DNMT3A} R882 mutations and 590 \textit{DNMT3A} non-R882 mutations. With a random-effect model, there was no distinct difference in OS time between the group of \textit{DNMT3A} R882 mutations and non-R882 mutations (Figure 6; HR = 0.95, 95% CI = 0.59–1.54, \( P = 0.84 \)). These results suggested that \textit{DNMT3A} R882 mutations may not differentiate the prognosis of AML patients at least on OS with other \textit{DNMT3A} mutations.
Meta-Regression, Publication Bias, and Sensitive Analysis

Meta-regression analysis was analyzed by using the software Stata 12.0. Fixed-effect meta-regression analysis was applied in RFS study due to its relatively low heterogeneity ($P = 0.21 > 0.1$ and $I^2 = 30\% < 50\%$), while random-effect model was applied in OS study. Fixed-effects meta-regression analysis showed that none of the following covariates affected the prognostic values of R882 mutations on RFS in AML patients (Table 6 and Figure S4, http://links.lww.com/MD/A935): publication year (coefficient $= -0.0939653$, $P = 0.835$), region (coefficient $= -0.1422338$, $P = 0.923$), R/N (the ratio of patients’ number with DNMT3A R882 mutations to the all AML patients’ number, coefficient $= 0.3002279$, $P = 0.986$), sex (the ratio of males’ number to females’ number, coefficient $= 0.3981209$, $P = 0.986$), median age (coefficient $= 0.0685541$, $P = 0.936$), median follow-up time (months, coefficient $= -0.0023704$, $P = 0.873$), NOS (Newcastle-Ottawa-Scale for assessment of quality, coefficient $= -0.1587112$, $P = 0.884$), median percentage of BM blast (coefficient $= -0.0224812$, $P = 0.926$), and median WBC (coefficient $= 0.0005318$, $P = 0.991$). Furthermore, the random-effects meta-regression analysis showed that none of the following covariates affected the prognostic values of R882 mutations on OS in AML patients (Table 7 and Figure S5, http://links.lww.com/MD/A935): publication year (coefficient $= -0.080685$, $P = 0.834$), region (coefficient $= 0.0374755$, $P = 0.973$), R/N (coefficient $= -0.9386456$, $P = 0.941$), sex (coefficient $= 0.4083431$, $P = 0.927$), median age (coefficient $= 0.0652603$, $P = 0.771$), median follow-up time (months, coefficient $= -0.0026942$, $P = 0.827$), NOS (coefficient $= -0.219313$, $P = 0.759$), median percentage of BM blast (coefficient $= -0.0486064$, $P = 0.808$), median WBC (coefficient $= 0.008405$, $P = 0.848$), and platelet count (coefficient $= 0.0117103$, $P = 0.867$).

Publication bias was analyzed by using RevMan 5.3.5 software. The funnel plot of RFS outcomes showed that the points were evenly distributed, and most of the points were

![FIGURE 2](image1.png) Forest plots of the HRs with 95% CI for RFS in overall AML patients. The size of the blocks or diamonds represents the weight for the fixed-effect model in the meta-analysis. HR $>1$ indicates that the presence of DNMT3A R882 mutations is associated with a shorter relapse-free survival (RFS).

![FIGURE 3](image2.png) Forest plots of the HRs with 95% CI for OS in overall AML patients. The size of the blocks or diamonds represents the weight for the random-effect model in the meta-analysis. HR $>1$ indicates that the presence of DNMT3A R882 mutations is associated with a shorter overall survival (OS).

TABLE 4. Outcomes of Subgroups Analysis in Fixed-Effect Models

| Subgroup      | N of S | R/A | $I^2$ (%) | HR (95% CI) | $P$   | N of S | R/A | $I^2$ (%) | HR (95% CI) | $P$   |
|---------------|-------|-----|-----------|-------------|-------|-------|-----|-----------|-------------|-------|
| Younger (<60) | 6     | 576/3028 | 18 | 1.44 (1.25–1.66) | <0.00001 | 7     | 634/3459 | 67 | 1.31 (1.16–1.49) | <0.0001 |
| Older (≥60)   | 2     | 55/314   | 0  | 2.03 (1.40–2.93) | 0.0002  | 2     | 55/314   | 0  | 1.85 (1.36–2.53) | 0.0001  |
| CN-AML        | 4     | 373/1986 | 34 | 1.52 (1.26–1.83) | <0.00001 | 5     | 378/2048 | 67 | 1.37 (1.16–1.63) | 0.0002  |
| Non-CN-AML    | 1     | 25/230   | —  | 1.96 (1.20–3.21) | 0.006   | 1     | 25/230   | —  | 2.51 (1.52–4.15) | 0.0038  |

AML = acute myeloid leukemia, CI = confidence interval, CN = cytogenetically normal, HR = hazard ratio, N of S = number of studies, OS = overall survival, R/A = the ratio of patients’ number with DNMT3A R882 mutations to the patients without R882 mutations, RFS = relapse-free survival, — = there is no corresponding data presented.
within 95% CI. This may indicate no obvious publication bias in RFS analysis, and thus, the corresponding results of the study were credible. Although the shape of these funnel plot in OS studies did not amount to gross asymmetry except for the OS outcome in the population of age ≥60, these results merit consideration. In addition, the z-value of 4.54 or something like that and a corresponding 2-tailed $P$-value of <0.001 on OS between the group of \( \text{DNMT3A} \) R882 mutations and without R882 mutations in total patients is also worthy of our attention.

**DISCUSSION**

DNA methylation is a key mechanism of epigenetic regulation in eukaryotes. As one of the enzymatically active mammalian DNA methyltransferases (DNMTs), \( \text{DNMT3A} \) can regulate gene expression and maintain cellular homeostasis by mediating the de novo methylation of DNA.\(^{32}\) Recently, with the ever-accelerated development of cancer genome sequencing, \( \text{DNMT3A} \) was exposed as one of the most frequently mutated genes, raising questions concerning the prominent part of \( \text{DNMT3A} \) mutations in AML patients.\(^{14,33}\) As dominant missense alterations in \( \text{DNMT3A} \) mutations, R882 mutations cause directly the focal hypomethylation phenotype.\(^{20}\) In addition, \( \text{DNMT3A} \) R882 mutations are frequent in AML, but rare in other hematological diseases,\(^{34}\) suggesting that \( \text{DNMT3A} \) R882 mutations own the potential to act as an

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**TABLE 5. Outcomes of Subgroup Analysis in Random-Effect Models**

| Subgroup      | N of S | R/A | $I^2$ (%) | HR (95% CI)       | $P$   |
|---------------|--------|-----|-----------|-------------------|-------|
| Younger (<60) | 6      | 576/3028 | 18        | 1.46 (1.25–1.72) | <0.00001 |
| Older (≥60)   | 2      | 55/314     | 0         | 2.03 (1.40–2.93) | 0.0002 |
| CN-AML        | 4      | 373/1986   | 34        | 1.59 (1.24–2.04) | 0.0003 |
| Non-CN-AML    | 1      | 25/230     | —         | 1.96 (1.20–3.21) | 0.006  |

*AML = acute myeloid leukemia, CI = confidence interval, HR = hazard ratio, N of S = number of studies, OS = overall survival, R/A = the ratio of patients’ number with \( \text{DNMT3A} \) R882 mutations to the patients without R882 mutations, RFS = relapse-free survival, CN = cytogenetically normal, — = there is no corresponding data presented.*

**FIGURE 4.** Kaplan–Meier estimates of RFS and OS in the non-CN-AML patients. The relapse-free survival [RFS] (A) and overall survival [OS] (B) of \( \text{DNMT3A} \) R882 mutations were shown in noncytogenetically normal (CN)-AML patients, including 25 with \( \text{DNMT3A} \) R882 mutations and 205 without R882 mutations (n = 230). The median survival of RFS: \( \text{DNMT3A} \) R882 mutations vs without R882 mutations = 8.8 vs 24.2, $P$ = 0.006; and the median survival of OS: \( \text{DNMT3A} \) R882 mutations vs without R882 mutations = 12.3 vs 32.5, $P$ = 0.0038.

**FIGURE 5.** Forest plots of the HRs with 95% CI for RFS in AML patients with \( \text{DNMT3A} \) mutations. The size of the blocks or diamonds represents the weight for the fixed-effect model in the meta-analysis. HR >1 indicates that the presence of \( \text{DNMT3A} \) R882 mutations is associated with a shorter relapse-free survival (RFS).
independent prognostic marker in AML. This systematic meta-analysis showed that mutant DNMT3A R882 was associated with poor prognosis in AML patients.

In this meta-analysis, 8 studies containing a total of 4474 AML patients were included, which included 694 AML patients with DNMT3A R882 mutations and 3780 AML patients without R882 mutations. And we found that AML patients with the DNMT3A R882 mutations presented significant shorter RFS and OS than those without R882 mutations in overall AML patients. Although there was a considerable but acceptable heterogeneity in those of OS study except for the population over age 60, the outcomes still deserve being considered. And various possible reasons contributed to the production of heterogeneity. First of all, the constituent ratio of patients’ age and cytogenetic abnormalities were diverse in each study. For example, 7 studies just or almost included patients under age 60,14,15,23,24,35–37 and 4 studies merely included CN-AML patients.23–25,35 Secondly, in consideration of less numbers of AML patients with R882 mutations in few included studies, we cannot further reckon the RFS and OS of AML patients.

### TABLE 6. Fixed-Effects Meta-Regression Analysis for RFS Studies

| Covariates                  | N of S | Coefficients | 95% CIs          | P   |
|-----------------------------|--------|--------------|------------------|-----|
| Year                        | 6      | -0.0939653   | -1.267064 to 1.079133 | 0.835 |
| Region                      | 6      | -0.1422338   | -3.982368 to 3.6979  | 0.923 |
| R/N                         | 6      | 0.3002279    | -43.59531 to 44.19577 | 0.986 |
| Sex (male/female)           | 4      | 0.3981209    | -18.61113 to 19.40737 | 0.936 |
| Median age                  | 4      | 0.0685541    | -1.475903 to 1.613011 | 0.866 |
| Median follow-up time (months) | 6      | -0.0023704   | -0.0409793 to 0.0362386 | 0.873 |
| NOS                         | 6      | -0.1587112   | -2.983917 to 2.666494 | 0.884 |
| Median percentage of BM blast | 3      | -0.0224812   | -2.471879 to 2.426916  | 0.926 |
| Median WBC                  | 5      | 0.0005318    | -0.1419846 to 0.1430483 | 0.991 |
| Platelet count              | 2      | —            | —                 | —    |

BM = bone marrow, CI = confidence interval, N of S = number of studies, NOS = Newcastle-Ottawa-Scale for assessment of quality, R/N = the ratio of patients’ number with DNMT3A R882 mutations to the all AML patients’ number, RFS = relapse-free survival, WBC = white blood cell, — = there is no corresponding data presented (insufficient observations).

### TABLE 7. Random-Effects Meta-Regression Analysis for OS Studies

| Covariates                  | N of S | Coefficients | 95% CIs          | P   |
|-----------------------------|--------|--------------|------------------|-----|
| Year                        | 8      | -0.080685    | -0.9832531 to 0.8218831 | 0.834 |
| Region                      | 8      | 0.0374755    | -2.607138 to 2.682089  | 0.973 |
| R/N                         | 8      | -0.9386456   | -30.75976 to 28.88247  | 0.941 |
| Sex (male/female)           | 5      | 0.4083431    | -12.56981 to 13.38649  | 0.927 |
| Median age                  | 5      | 0.0652603    | -0.5859805 to 0.716501  | 0.771 |
| Median follow-up time (months) | 8      | -0.0026942   | -0.0316496 to 0.0262612 | 0.827 |
| NOS                         | 8      | -0.219313    | -1.889979 to 1.451353  | 0.759 |
| Median percentage of BM blast | 4      | -0.0486064   | -0.8026127 to 0.7053999 | 0.808 |
| Median WBC                  | 6      | 0.008405     | -0.1061058 to 0.1229158 | 0.848 |
| Platelet count              | 3      | 0.0117103    | -0.6882703 to 0.7116908  | 0.867 |

BM = bone marrow, CI = confidence interval, N of S = number of studies, NOS = Newcastle-Ottawa-Scale for assessment of quality, OS = overall survival, R/N = the ratio of patients’ number with DNMT3A R882 mutations to the all AML patients’ number, WBC = white blood cell.
when they were included. For example, only 5 AML patients with R882 mutations was appeared in K-M curves of RFS and OS in Park study, which can not accurately even not roughly calculate the values of HRs and 95% CI. They are likely to be a source of heterogeneity. Thirdly, there was a lot of between-study heterogeneity on some other hands, such as the time of follow-up, region origin of patients among the 8 studies. At last, general information of individual patient just like Ley study did was not available for other studies, which also led to the heterogeneity of our analysis.

AML is a clonal disorder of hemopoietic stem cells. The survival of AML is influenced by factors such as age, cytogenetics, somatic mutations, etc. Age is the most vital prognostic factor for AML patients, and adults with age older than 60 have a shorter OS in comparison with adults with age younger than 60. Meanwhile, cytogenetic normality or abnormality influenced clinical outcome of AML dramatically. For these reasons, we stratified AML patients into different subgroups including age (<60 and ≥60) and cytogenetics (CN-AML and non-CN-AML) to further validate the prognostic effect of mutant DNMT3A R882 in AML patients.

Our findings showed shorter RFS and OS in AML patients with DNMT3A R882 mutations compared with those without R882 mutations in subgroups of age <60, age ≥60, CN-AML, and non-CN-AML, respectively. These results indicated that DNMT3A R882 mutations may act as a poor prognostic indicator in AML patients, which is independent of the age and cytogenetics. While, a relatively considerable heterogeneity remained in OS study, except for the subgroup of age ≥60. This may result from the small number of literatures included in the OS study of age ≥60 AML patients. Or else, it suggested that the DNMT3A R882 mutations may be particularly appropriate for predicting the clinical outcome OS in the AML population of age ≥60.

Presently, there is still a controversy on prognostic effect of R882 mutations compared with DNMT3A non-R882 mutations (DNMT3A mutations affecting other codons). Ley et al suggested no difference in the OS between the 2 groups. Meanwhile, Marcucci et al reported that DNMT3A R882 mutations had no prognostic value in younger patients whereas were independently associated with worse outcome in older patients. Yet, Gaidzik et al's findings showed unfavorable for DNMT3A R882 mutations on RFS while favorable for non-R882 mutations on OS in a cohort study. While in our meta-analysis, we observed no difference in either RFS or OS between AML patients with the DNMT3A R882 mutations and those with non-R882 mutations in patients positive for DNMT3A mutations. What merit our concern is that the P-value coincidently was 0.05, and this makes it essential do more studies with larger sample size to validate the OS significance of DNMT3A non-R882 mutations in AML patients. Our results were in lines with the studies of Ley and Marcucci, while were partly inconsistent with Gaidzik’s study. The inconsistency was possibly due to the differences in biometrical analysis, such as selection bias and variances in model building. In Gaidzik’s study, a potential selection bias may exist because of the high percentage of patients was selected for the analysis in relation to the whole study populations with 90%, while low percentage of patients was selected for the analysis in relation to the whole study populations with 6% in Marcucci study and 18% in Ley study. Anyway, DNMT3A R882 mutations could predict shorter RFS and OS in total AML population or in patients with DNMT3A mutations, especially for RFS.

Three main limitations should be considered in our meta-analysis. First, there may be language bias, because the included studies were totally published in English. Second, selectional reporting was existed in some studies, such as the incorporated HRs for RFS were displayed in relative fewer studies than those for OS, and certain subgroups, like the older and non-CN-AML patients, were not analyzed in most of the studies, which led to the unavailability of useful information. Third, if aforementioned authors could offer complete patient data, like Ley, our paper would have been quite more flawless.

In conclusion, our meta-analysis presented definitively an independent inferior prognostic effect of mutant DNMT3A R882 on the RFS and OS in AML patients. This was true also for AML patients in subgroups of age <60, age ≥60, CN-AML, and non-CN-AML. These results of meta-analysis may provide an insight for the prognostic prediction of AML patients, as well as infuse a drop into the ocean of precision prediction. Further studies with larger sample size and open individual data of patients are needed to validate the prognostic significance of mutant DNMT3A R882 in AML patients.

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