Hypomethylating agents for elderly patients with acute myeloid leukemia: a PRISMA systematic review and meta-analysis


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Abstract. – OBJECTIVE: Abnormal DNA methylation plays a critical role in acute myeloid leukemia (AML) pathogenesis and hypomethylating agents (HMAs) such as decitabine (5-aza-29-deoxycytidine) and azacitidine (5-azacytidine) are considered efficacious for treating AML. This study aimed to identify if HMAs have therapeutic advantages compared with conventional care regimens (CCR) or placebo in elderly AML patients.

MATERIALS AND METHODS: We systematically searched PubMed, Embase, and Cochrane Central Register of Controlled Trials from inception to November July 15, 2020. Randomized controlled trials that compared the efficacy and adverse events associated with HMAs, CCR, or placebo were searched. RevMan 5.3 software was used to calculate the hazard ratio (HR) and risk ratio (RR) with a 95% confidence interval (CI).

RESULTS: Seven trials with a total of 1966 participants were included. Meta-analyses showed that the overall survival of HMAs was better than that of CCR [HR=0.76, 95% CI (0.69-0.85), (p<0.01)], and the complete remission rate of elderly AML patients was increased by HMAs compared with CCR [RR=1.46, 95%CI (1.08-1.99), p=0.01)]. HMA treatment showed higher incidence of neutropenia [RR=1.30 (95%CI 1.07-1.59, p=0.008)], thrombocytopenia [RR=1.14 (95%CI 1.01-1.59, p=0.04)], and pneumonia [RR=1.37 (95%CI 1.06-1.76, p=0.02)] compared with CCR.

CONCLUSIONS: Although HMAs cause a higher incidence of adverse events such as neutropenia, thrombocytopenia, and pneumonia, demethylation drugs are well-tolerated and effective for treating AML in the elderly.

Key Words: Acute myeloid leukemia, DNA hypomethylating agents, Elderly patients, Systematic review, Meta-analysis.

Abbreviations
CI: Confidence interval; RCT: Randomized controlled trials; RR: Risk ratios; HR: Hazard ratio; AZA: Azacitidine; DAC: Decitabine; OR: Overall survival; CR: Complete response; CCR: Conventional care regimens; IC: Intensive chemotherapy; LDAC: Low-dose cytarabine; BSC: best supportive care; AML: Acute myeloid leukemia; HMAs: hypomethylating agents; MDS: myelodysplastic syndromes.

Introduction

Acute myeloid leukemia (AML) is an aggressive stem cell malignancy characterized by the clonal expansion of abnormal hematopoietic progenitors in the bone marrow. It is mostly seen in elderly individuals, with a median age at diagnosis of 68 years; the age-specific incidence rates are below 10/100,000 among individuals <65 years, while they progressively increase to 28.5/100,000 for individuals aged 80-84 years. Although therapeutic regimens like “3 + 7” chemotherapy, hematopoietic stem cell transplantation, and supportive care show significant effect among patients younger than 60 years, 70%-80% achieve complete remission, and 35%-40% are cured. Nevertheless, because of resistance to conventional chemotherapy, adverse cytogenetics, and frequent comorbidities, the prognosis of elderly patients (aged >65 years) with AML still remains poor, with a median overall survival (OS) time of 9.2 months and a 5-year OS rate of 13.5%. Moreover, primary refractory or resistant AML can hardly be cured by conventional salvage therapy, and therefore new therapeutic approaches are urgently needed for these patients.

Abnormal DNA methylation plays a crucial role in AML pathogenesis and is known to regulate the expression of tumor suppressor genes and oncogenes, promoting dysplasia and blast transformation. With the breakthrough in molecular biology research on the characteris-
tics and pathogenesis of AML, hypomethylating agents (HMAs) such as decitabine (5-aza-29-deoxycytidine) and azacitidine (5-azacytidine) have become research hot spots in treating myelodysplastic syndromes (MDS) and AML. The National Comprehensive Cancer Network (NCCN) recommends HMAs as the preferred treatment option for elderly AML patients with unfavorable cytogentic, poor molecular markers, a history of hematologic disorders, therapy-related AML, or unfit performance status. Over the last few decades, HMAs have been widely used for the treatment of MDS, and studies have also shown that these drugs show certain effects as first-line and rescue treatment for AML. Nevertheless, the survival outcome data with regard to HMAs in elderly AML patients have been inconsistent. Therefore, this systematic review and meta-analysis aimed to evaluate the efficacy of HMAs and their adverse effects when treating older AML patients.

**Materials and Methods**

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Supplementary File 1: PRISMA 2015 Checklist).

**Search Strategies**

Relevant randomized controlled trials (RCTs) that were published electronically in the PubMed, Embase, and Cochrane Central Register of Controlled Trials (Central) databases were searched from inception to July 15, 2020, without any restrictions to language. The search terms were “acute myeloid leukemia”, “azacitidine”, “decitabine”, “elderly patients”, and “randomized controlled trial”. A bibliography of identified articles and other documents from relevant references were manually searched to identify any additional relevant trials. Two study researchers designed and performed the search strategy (Supplementary File 2: Search strategies).

**Eligibility Criteria**

The eligibility criteria were (1) Phase II and III RCTs, (2) with adult patients aged ≥55 years, (3) with morphologically proven diagnosis of AML and with no previous allogeneic stem cell transplantation, (4) treated with HMAs (such as azacitidine, decitabine or guadecitabine) and compared with those of conventional care regimens (CCR) including best supportive care (BSC), low-dose cytarabine or intensive chemotherapy in a setting of first-line treatment, and (5) studies that evaluated complete response (CR) rate or overall survival (OR). The trial data from the most recent publication were used only once in the analysis.

**Exclusion Criteria**

Trials were excluded if any of the following factors were identified: (1) conference abstracts, case reports, editorials, review articles, and cell or animal studies, (2) studies with insufficient information concerning OR or CR, (3) patients diagnosed with myelodysplastic syndrome (MDS), (4) the age of the participants is not limited, and (5) retrospective studies.

**Study Selection**

Two reviewers (AA and ZZ) independently screened the titles and abstracts of all trials and included the trials based on the eligibility criteria. The full-text articles and their relevant references were selected for further assessment. Any disagreements were settled by discussion between the two reviewers, and a third independent reviewer (BB) was invited to participate if necessary.

**Data Extraction**

Two reviewers (AA and ZZ) independently read and extracted the data using a standardized form. The following data were extracted from each study: population size, median age, bone marrow (BM) blast count, cytogenetic risk categories, treatment and dosing regimens, median treatment duration, and adverse events (AEs) of interest. The co-primary endpoints such as OS, CR, neutropenia, leukopenia, thrombocytopenia, anemia, and febrile neutropenia were included. All data were recorded in Excel 2016 (Microsoft, Redmond, WA, USA).

**Assessment of Bias Risk**

The Cochrane Collaboration’s tool was used to evaluate the random sequence generation, allocation concealment, blinding, incomplete outcome data, selective reporting, and other sources of bias. The risk of bias was rated as high, unclear, or low. Two reviewers (WW and YY) independently evaluated the risk of bias in each study, and any disagreements were settled down by discussion with a third independent reviewer (BB).
**Statistical Analysis**

Data analyses were carried out using Review Manager (Version 5.3, The Nordic Cochrane Centre, The Cochrane Collaboration, London, UK). A 95% confidence interval (CI) with risk ratio (RR) or hazard ratio (HR) was used to present the results of the meta-analysis. The Cochrane Q statistic was used to estimate the heterogeneity, and the $I^2$ test was used to quantify the inconsistency. $p>0.10$ and $I^2<50\%$ indicated an acceptable level of heterogeneity, and a fixed-effects model was adopted; otherwise, a random-effects model was adopted. Publication bias was evaluated using a funnel plot for analyses with ≥10 studies. If no publication bias was observed, then a symmetrical scatter forming a triangular “funnel” could be seen. If publication bias exists, then this might suggest missing negative studies and results in an unbalanced shape. Sensitivity analysis was conducted by deleting one study at a time to assess the stability of the results. A two-tailed $p$-value of $<0.05$ was considered statistically significant in all statistical tests.

**Results**

**Literature Search Results**

The literature search yielded 163 potential abstracts, and 105 studies were removed because of duplications. After reviewing the titles and abstracts, 21 studies were reviewed (full-text) for eligibility. Of these, 14 studies were excluded due to duplications, post hoc or without primary endpoints of interest, and the remaining six articles and one abstract were included in this meta-analysis (Figure 1), which included two Phase II trials (n=292) and five Phase III trials (n=1674) published between 2010 and 2019. The characteristics of these trials are summarized in Table I.

**Publication Characteristics**

All trials included patients with morphologically confirmed AML and aged 55 years or more. A total of 1966 patients were included in this analysis. Of these, 976 were treated with either azacitidine (n = 719) or decitabine (n = 257), and the remaining 990 were treated with CCR includ-
<table>
<thead>
<tr>
<th>Reference</th>
<th>HMA</th>
<th>Country</th>
<th>Median age, years</th>
<th>Cytogenetic risk group, n (%)</th>
<th>BM Blast</th>
<th>Median F/U, months</th>
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<tbody>
<tr>
<td>Fenaux et al19</td>
<td>AZA</td>
<td>France, UK, Sweden, Italy, Spain, USA, Germany, Australia</td>
<td>37/18</td>
<td>Intermediate: 81 (71.7)</td>
<td>Intervention: 23.0 (20.0-34.0) %</td>
<td>20.1</td>
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<td>41/17</td>
<td>Normal: 52 (46.0)</td>
<td>Comparison: 23.1(13.0-68.9) %</td>
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<td>70</td>
<td>Poor risk: 27 (23.9)</td>
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<td>Missing: 5 (4.4)</td>
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<tr>
<td>Dombret et al24</td>
<td>AZA</td>
<td>France, Poland, USA, Belgium, Korea, UK, Canada, Italy, Spain, Germany, Australia</td>
<td>139/102</td>
<td>Intermediate: 306 (63.1)</td>
<td>Intervention: 70.0 (2.0-100.0)%</td>
<td>24.4</td>
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<td></td>
<td></td>
<td></td>
<td>149/98</td>
<td>Poor risk: 174 (35.8)</td>
<td>Comparison: 72.0 (2.0-100.0)%</td>
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<tr>
<td>Huls et al25</td>
<td>AZA</td>
<td>Netherlands, Belgium</td>
<td>35/21</td>
<td>Unfavorable risk: 23 (19.8)</td>
<td>NR</td>
<td>41.4</td>
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<td></td>
<td>33/27</td>
<td>intermediate: 93 (80.2)</td>
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<tr>
<td>Seymour et al18</td>
<td>AZA</td>
<td>France, Poland, USA, Belgium, Korea, UK, Canada, Italy, Spain, Germany, Australia</td>
<td>81/48</td>
<td>Intermediate: 124 (47.3)</td>
<td>Intervention: 65.0 (27-99)%</td>
<td>NR</td>
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<td>78/55</td>
<td>Poor risk: 138 (52.7)</td>
<td>Comparison: 70.0 (26-100)%</td>
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<tr>
<td>Wei et al27</td>
<td>AZA</td>
<td>International</td>
<td>238</td>
<td>NR</td>
<td>NR</td>
<td>41.2</td>
</tr>
<tr>
<td>Kantarjian et al24</td>
<td>DAC</td>
<td>International</td>
<td>137/105</td>
<td>Intermediate: 306 (63.4)</td>
<td>20-30%: 123 (25.2%)</td>
<td>NR</td>
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<td>151/92</td>
<td>Poor risk: 174 (36.0)</td>
<td>30-50%: 141 (29.3%)</td>
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<td>73</td>
<td>&gt;50%: 206 (42.7%)</td>
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<td>Jacob et al21</td>
<td>DAC</td>
<td>India</td>
<td>12/3</td>
<td>Unsatisfactory 15 (50)</td>
<td>20-30%: 10 (33.3%)</td>
<td>NR</td>
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<td>12/3</td>
<td>Normal karyotype 10 (33.3)</td>
<td>30-50%: 12 (40%)</td>
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<td>Inv(16) 1 (3.3)</td>
<td>&gt;50%: 8 (26.7%)</td>
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<td></td>
<td>62</td>
<td>Abnormality of chromosome 8 2 (6.7)</td>
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<td>Abnormality of chromosome 7 1 (3.3)</td>
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NR = not reported, AZA = Azacitidine, DAC = Decitabine, HMA = hypomethylating agents, BM = bone marrow. *Abstract.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Treatment regimens</th>
<th>Median treatment duration</th>
<th>OS, months</th>
<th>Adverse event, n(%)</th>
</tr>
</thead>
</table>
| Fenaux et al19     | Intervention: Azacitidine (subcutaneously 75 mg/m²/day for 7 days Q28 days for at least 6 cycles)  
Comparison: CCR (BSC, LDAC 20 mg/m²/day for 14 days Q28 days for at least 6 cycles, IC) | Intervention: 34 (15-79) days       | Intervention: Anemia: 30 (56.6), Neutropenia: 50 (94.3), Thrombocytopenia: 48 (90.6)  
Comparison: Anemia: 36 (67.9), Neutropenia: 44 (83.0), Thrombocytopenia: 44 (83.0) |
| Dombret et al24    | Intervention: Azacitidine (subcutaneously 75 mg/m²/day for 7 days Q28 days for at least 6 cycles)  
Comparison: CCR (BSC, LDAC 20 mg/m²/day for 14 days Q28 days for at least 6 cycles, IC) | Intervention: 6 (1-28) cycles       | Intervention: Anemia: 37 (15.7), Neutropenia: 62 (26.3), Thrombocytopenia: 56 (23.7), Febrile neutropenia: 66 (28.0), Pneumonia: 45 (19.1), Leukopenia: 16 (6.8), Hypokalemia: 12 (5.1)  
Comparison: Anemia: 43 (18.3), Neutropenia: 54 (22.9), Thrombocytopenia: 53 (22.5), Febrile neutropenia: 70 (29.8), Pneumonia: 33 (14.0), Leukopenia: 19 (8.1), Hypokalemia: 18 (7.7) |
| Huls et al25       | Intervention: Azacitidine (50 mg/m² sc for 5 days every 4 weeks for 12 cycles)  
Comparison: Observation (no further treatment) | Intervention: 1-4 cycles: 55  
5-8 cycles: 44; 9-12 cycles: 37  
Comparison: 1-4 months: 60  
5-8 months: 39; 9-12 months: 28 | Intervention: 8.9 (6.9, 12.9)  
Comparison: 4.9 (3.8, 6.5) | Intervention: 0 SAE, 42 (75), 1 SAE, 11 (20), 2 SAE, 2 (3), 3 SAE, 1 (2)  
Comparison: 0 SAE, 56 (93), 1 SAE, 4 (7) |
| Seymour et al26    | Intervention: Azacitidine (subcutaneously 75 mg/m²/day for 7 days Q28 days for at least 6 cycles)  
Comparison: CCR (BSC, LDAC 20 mg/m²/day for 14 days Q28 days for at least 6 cycles, IC) | Intervention: NR                    | Intervention: Anemia: 19 (15), Neutropenia: 28 (22), Thrombocytopenia: 33 (26), Febrile neutropenia: 29 (23), Pneumonia: 24 (19), Leukopenia: 8 (6), Hypokalemia: 9 (7)  
Comparison: Anemia: 21 (16), Neutropenia: 25 (19), Thrombocytopenia: 27 (21), Febrile neutropenia: 43 (33), Pneumonia: 18 (14), Leukopenia: 10 (8), Hypokalemia: 10 (8) |
| Wei et al27        | Intervention: Azacitidine (CC-486 300 mg QD for 14 days, Best supportive care 28-day cycles)  
Comparison: Placebo (QD for 14 days, Best supportive care 28-day cycles) | Intervention: 12 (1-80) cycles       | Intervention: Nausea 152(64), Vomiting 140(59), Diarrhea 117(49), Neutropenia 98(41), Thrombocytopenia 55(23), Anemia 33(14), Infections 40(17)  
Comparison: Nausea 54(23), Vomiting 23(10), Diarrhea 49(21), Neutropenia 56(24), Thrombocytopenia 52(22)  
Anemia 30(13), Infections 19(8)                  | Intervention: 8.9 (6.9, 12.9)  
Comparison: 4.9 (3.8, 6.5) |
| Kantarjian et al28 | Intervention: Decitabine (intravenously 20 mg/m² QD for 5 days, every 4 weeks)  
Comparison: TC (supportive care, or cytarabine 20 mg/m² QD for 10 days, every 4 weeks) | Intervention: 4 (1-29) cycles       | Intervention: Anemia: 15 (6), Neutropenia: 15 (6), Thrombocytopenia: 21 (9), Febrile neutropenia: 57 (24), Pneumonia: 48 (20), Leukopenia 47(20), Hypokalemia 27(11)  
Comparison: Anemia: 12 (5), Neutropenia: 7 (3), Thrombocytopenia: 11 (5), Febrile neutropenia: 33 (14), Pneumonia: 36 (15), Leukopenia 20(8), Hypokalemia 24(10) |
| Jacob et al29      | Intervention: Decitabine (intravenously 20 mg/m² QD for 5 days, every 4 weeks)  
Comparison: Low-dose cytarabine (subcutaneously 20 mg/m² QD for 10 days, every 4 weeks) | Intervention: 4 (1-7) cycles        | Intervention: Anemia: 8 (53.3), Neutropenia: 7 (46.7), Thrombocytopenia: 8 (53.3), Febrile neutropenia: 5 (33.3), Hypokalemia 2 (13.53), Mucositis 4 (26.67), Fatigue 4 (26.67), Hypocalcemia 3 (20.00)  
Comparison: Anemia: 7 (46.7), Neutropenia: 8 (53.3), Thrombocytopenia: 8 (53.3), Febrile neutropenia: 5 (33.3), Hypokalemia 2 (13.53), Mucositis 4 (26.67), Fatigue 5 (33.33), Hypocalcemia 2 (13.33) |

NR = Not reported, OS = Overall survival, BSC = best supportive care, CCR = Conventional care regimens, IC=Intensive chemotherapy, LDAC = Low-dose cytarabine.
ing BSC, low-dose cytarabine (LDAC), intensive chemotherapy (IC), and placebo. The median age of patients on the selected trials ranged from 62 to 76 years. The median follow-up was reported in 4 studies\textsuperscript{19,24,25,27}. Five studies were large, international, multicenter RCTs, with patients from the United States, United Kingdom, France, Italy, Australia, and other countries.

**Risk of Bias**

The bias analysis is shown in Figures 2 and 3. Six studies were open-labeled RCTs\textsuperscript{19,20,24-26,28} and one study was a double-blinded RCT\textsuperscript{27}. All trials reported randomization, one study did not perform adequate random sequence generation\textsuperscript{20}, and four studies did not perform adequate allocation\textsuperscript{19,20,25,27}. The adequacy of blinding of participants and personnel (performance bias) was evaluated using blind methods for the researchers and participants in the study, and the adequacy of outcome assessment blinding was judged by a reviewer who was blind to the patient groups. One study performed blinding of participants\textsuperscript{27}. In three studies, treatment response was assessed by a third-party specialist in related fields\textsuperscript{24,26,28}. Randomization, follow-up, and safety analysis were well-designed and conducted. Thus, attrition bias and reporting bias were unlikely to exist.

**HR of Overall Survival**

All seven studies analyzed the OS between HMA\textsubscript{a}s and control. The OS of the elderly AML patients who received AZA treatment showed significant prolongation compared to those who received traditional therapy [HR=0.73, 95% CI
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**Figure 4.** Forest plot of HR of OS with HMAs vs. CCR or Placebo. CCR=conventional care regimens, HMAs=hypomethylating agents, AZA=Azacitidine, DAC=Decitabine, HR=Hazard Ratio.

(0.64-0.83), (p<0.01), while the group receiving DAC showed also showed significantly prolonged OS in elderly AML patients compared with the CCR group [HR=0.82, 95% CI (0.68-0.98), (p=0.03)] (Figure 4). There was no significant heterogeneity in the OS analyses across AZA studies (I²=29%, p=0.23) and DAC studies (I²=0%, p=0.98). The combined estimate demonstrated an association of HMA treatments with significantly better OS [HR=0.76, 95% CI (0.69-0.85), (p=0.01)], and there was no significant heterogeneity between the subgroups (I²=0%, p=0.33).

**RR of Complete Remission**

Six studies analyzed the CR rate, including five AZA studies and one DAC study. Compared with patients receiving CCR or placebo, the CR rate was significantly increased in elderly AML patients receiving HMA treatment [RR=1.46, 95%CI (1.08-1.99) (p=0.01)] (Figure 5). There was significant heterogeneity in the CR (I²=69%, p=0.006) analyses among studies.

**Adverse Events**

All seven studies reported AEs, but one of them reported only the number of people with different AEs and lacked the number of specific AEs. Thus, six studies were included for analyzing the AEs, in which the hematologic toxicity effects of HMAs for treating the elderly AML patients were compared.

**RR of Neutropenia**

For calculating the RR of neutropenia, six trials with patients receiving HMAs vs.
CCR were analyzed (Figure 6). The pooled analysis showed that the administration of HMAs significantly increased the risk of neutropenia. The RR of neutropenia was 1.30 (95% CI 1.07-1.59, \(p=0.008\)). There was no significant heterogeneity in the RR of the neutropenia (\(I^2=43\%, p=0.12\)) analysis among the studies.

**RR of Thrombocytopenia**

Thrombocytopenia was reported in six studies\(^\text{19,20,24,26-28}\) (Figure 7). The pooled analysis showed that the administration of HMAs significantly increased the risk of developing thrombocytopenia. The RR of thrombocytopenia was 1.14 (95% CI 1.01-1.59, \(p=0.04\)), showing no significant heterogeneity (\(I^2=0\%, p=0.73\)) among the studies.

**RR of Anemia**

For calculating the RR of anemia, six trials\(^\text{19,20,24,26-28}\) with patients who received HMAs vs. CCR were used for analysis (Figure 8). Adminis-
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The concentration of HMAs showed no significant change in the risk of developing anemia. The RR of anemia was 0.96 (95%CI 0.79-1.16, p=0.64), showing no significant heterogeneity ($I^2=0\%$, $p=0.91$) among the studies.

**RR of Febrile Neutropenia**

Febrile neutropenia was evaluated in four studies 20,24,26,28 (Figure 9). The pooled analysis showed that no HMAs increased the risk of developing febrile neutropenia. The RR of febrile neutropenia was 1.04 (95%CI 0.69-1.58, $p=0.84$), and substantial heterogeneity was observed ($I^2=66\%$, $p=0.01$).

**RR of Pneumonia**

For pneumonia, three trials 24,26,28 with patients who received HMAs vs. CCR were used for analysis (Figure 10). The pooled analysis showed that the administration of HMAs significantly increased the risk of developing pneumonia. The RR of pneumonia was 1.37 (95%CI 1.06-1.76, $p=0.02$), and a low heterogeneity ($I^2=0\%$, $p=0.99$) was observed among the studies.

**RR of Leukopenia**

For leukopenia, three trials 24,26,28 with patients who received HMAs vs. CCR were used for analysis (Figure 11). The results showed no significant differences in the risk of developing leukopenia between HMAs and CCR. The RR of leukopenia was 1.25 (95%CI 0.57-2.76, $p=0.57$), showing significant heterogeneity ($I^2=77\%$, $p=0.01$) among the studies.

**RR of Hypokalemia**

For hypokalemia, four trials 20,24,26,28 with patients who received HMAs vs. CCR were used for analysis (Figure 12). The analysis results showed no significant differences in the risk of developing hypokalemia between HMAs and CCR. The RR of hypokalemia was 0.95 (95%CI 0.65-1.37, $p=0.77$), and no significant heterogeneity ($I^2=0\%$, $p=0.74$) was observed among the studies.

**Discussion**

This systematic review and meta-analysis was intended to test whether HMAs have a better effect and milder AEs in elderly patients with AML. The combined analyses revealed statistically significant differences in OS and CR with HMA therapies when compared to control, but the risk of developing neutropenia, thrombocytopenia, and pneumonia was increased, confirming that HMAs are reasonable therapeutic options with a survival benefit.
advantage for elderly AML patients. Meanwhile, to prevent the occurrence of AEs, optimized treatment plans should be selected to achieve better clinical efficacy for demethylation therapy.

DNA methylation catalyzed by DNA methyltransferase (DNMT) is one of the most important epigenetic modifications. In normal and cancer cells, DNA methylation modifies cytosine to regulate gene expression, while the silencing of tumor suppressor genes is related to abnormal promoter DNA methylation. Aberrant DNA methylation is related to the prognosis and pathogenesis of AML and is regarded as the dominant mechanism of MDS progression to AML. Nevertheless, DNA methylation can be reversed during DNA synthesis, making it a potential therapeutic target. Therefore, demethylation therapy has become a routine treatment in MDS and AML. Azacitidine (5-azacytidine) is metabolized to decitabine (5-aza-2′-deoxycytidine), forming a covalent protein-DNA adduct, depleting intracellular methyl-transferase, and leading to a reversal of DNA hypermethylation on tumor suppressor genes and induction of apoptosis.

Elderly AML patients have a poor physical reserve and have more comorbidities. The median OS of patients who can tolerate the CCR is about 6-8 months, and the 5-year OS rate is about 5%-15%. In elderly patients who can tolerate only LDC therapy or hydroxyurea, the median OS is only about 3-4 months, and the 2-year OS rate is about 8%-10%. Related studies suggest that azacitidine (AZA) can prolong OS with mild side effects and is especially suitable for elderly AML patients with poor prognostic karyotypes. AZA is safe and effective for elderly patients with AML and comorbidities, while some studies have shown that AZA significantly reduces the hospitalization rate and AEs compared with CCR. Other studies have shown that the application of decitabine in the treatment of elderly AML has a certain effect and is well-tolerated. The tolerance of elderly patients to demethylation drugs has undoubtedly brought hope to the treatment of elderly patients with AML.

There is currently a lack of prospective head-to-head studies on AZA and decitabine (DAC) in the treatment of AML. A retrospective study showed that AZA could prolong the median OS more significantly than DAC, while the hospitalization rate in the DAC group was higher than that of in the AZA group due to infection or bleeding. This suggests that AZA is more advantageous than DAC in treating AML in elderly patients. According to a study, AZA and DAC in the treatment of MDS showed sim-
Hypomethylating agents for acute myeloid leukemia

Similar overall efficacy results, but in the elderly patients’ group (65 years or older), the survival advantage of AZA was more significant. At the same time, the incidence of AEs, such as grade 3-4 blood cell reduction and infection in the DAC group was higher\(^{25}\). Our research results confirmed that compared with CCR or placebo, AZA and DAC significantly prolonged the OS of elderly patients with AML, and there were no significant differences between the subgroups. Regarding the adverse events, HMAs demonstrated a higher incidence of neutropenia, thrombocytopenia, and pneumonia compared with CCR, which is consistent with the literature\(^{35,34}\). The incidence of anemia, febrile neutropenia, leukopenia, and hypokalemia between HMAs and CCR showed no significant differences.

As demethylation drugs are still under development, there is still a lack of molecular biomarkers that can predict whether patients might benefit from epigenetic therapy. Therefore, this paper did not involve the analysis of the effects of gene mutations and karyotype differences on the efficacy of demethylation drugs. Related research is ongoing, and some experimental studies showed that some biological indicators might be related to the responsiveness of demethylation drugs. Studies have suggested that the relatively high expression of miR-29b, miR-29c, and miR-181 is related to the clinical response rate of decitabine in treating AML in elderly patients\(^{35,56}\). At the same time, Metzeler et al\(^{57}\) showed that AML patients with low DNMT3A expression might benefit from demethylation drug treatment. Furthermore, if leukemia relapses in AML patients or was refractory to HMA, usually HMAs or other low-intensity therapies that have a dismal prognosis were continued. A multicenter historical prospective study found that the addition of venetoclax (a BCL-2 inhibitor) to AML patients who previously failed HMA might overcome resistance\(^{58}\) and demonstrated superior response and prolonged survival\(^{59}\). Especially in FLT3-mutated (FLT3m) AML patients, the combined use of venetoclax and HMAs showed encouraging efficacy\(^{46}\). These might provide new ideas for targeted therapy of epigenetics.

However, there are several limitations in the current analysis. Firstly, significant heterogeneity was observed in the CR analysis \(I^2=69\%\), \(p=0.006\). The primary source of heterogeneity was from Dombret\(^{24}\), and the CR rate in the AZA group was lower than that in the CCR group (19.5% vs. 21.9%), but the morphologic CR with incomplete blood count recovery was higher (8.3% vs. 3.2%) in this study. Secondly, most of the included studies were not blinded, and the allocation concealment was not clear, which led to increased bias. Thirdly, this study lacked multicenter, large-sample studies. Finally, some unpublished negative results were not included.

A second-generation HMA has been developed to reduce the elimination of decitabine by cytidine deaminase, thereby increasing the in vivo exposure of decitabine. A recent clinical trial with Guadecitabine (SGI-110, dinucleotide of decitabine and deoxyguanosine) demonstrated a comparable safety profile to decitabine with a significantly longer half-life\(^{61}\). A phase II clinical trial showed that more than half of the elderly treatment-naive patients with AML achieved a composite CR with guadecitabine and tolerable toxicity\(^{62}\). Double-blind RCTs should be carried out to confirm the toxicity and efficacy of SGI-110 in the future.

Conclusions

In summary, this study aimed to identify if HMAs have therapeutic advantages compared with CCR or placebo in elderly AML patients. The results showed that in the analysis of prospective RCTs in elderly patients with AML, HMAs showed improved response rates and OS in comparison to CCR or placebo. Although HMAs are associated with a higher incidence of AEs such as neutropenia, thrombocytopenia, and pneumonia, demethylation drugs were well-tolerated in the treatment of elderly AML. The factors affecting the reactivity of demethylated drugs need continuous exploration. Therefore, this meta-analysis suggests that although HMAs cause a higher incidence of adverse events such as neutropenia, thrombocytopenia, and pneumonia, demethylation drugs are well-tolerated and effective for treating AML in the elderly.

Author Contributions

(I) Conception and design: Rui-Juan Zhang, Lin-Hua Yang; (II) Administrative support: Lin-Hua Yang; (III) Provision of study materials or patients: Zhi-Juan Zhang; (IV) Collection and assembly of data: Jia-Hong Zhai; (V) Data analysis and interpretation: Mei-Fang Wang, Chun-Xia Dong; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.
Sources of Support
This study was not supported by any financial sources.

Conflict of Interest
The Authors declare that they have no conflict of interests.

Reporting Checklist
The authors have completed the PRISMA 2009 checklist.

Data Sharing Statement
Not applicable.

Acknowledgments
The authors would like to thank all study participants who were enrolled in this study.

Ethical Statement
This study was based on previously published studies; therefore, ethical approval and patient consent are not relevant.

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