Leukemia Research 80 (2019) 11–18

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Mutations in the DNA methylation pathway predict clinical efficacy to hypomethylating agents in myelodysplastic syndromes: a meta-analysis

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ARTICLE INFO

Keywords:
Myelodysplastic syndromes
DNA methylation pathway
Mutational profile
Hypomethylating agents

ABSTRACT

Purpose: Myelodysplastic syndromes (MDS) are characterized by variable degrees of clinical outcomes. Until now, hypomethylating agents (HMAs) are the only drugs that have been approved by FDA in remedying this complicated prognosis disease, but without satisfactory outcome. So, biomarkers of better clinical outcome are of great significance. Many studies have already reported the potential prognostic value of DNA methylation pathway related gene (TET2/DNMT3 A/IDH) mutations in demethylation therapy patients, with controversial results. Therefore, a meta-analysis was performed to investigate their prognostic impact on HMAs treated MDS.
Methods: Databases, including PubMed, Embase, web of science and the Cochrane Library, were searched for relevant studies published up to 29 May 2018. Overall response rate (ORR) and overall survival (OS) were selected as endpoints. We extracted odds ratio to evaluate the effect of mutations on ORR, and the corresponding hazard ratios and their 95% confidence intervals for OS.
Results: A total of 13 cohort studies, covering 1398 patients with MDS treated by HMAs were included in the final meta-analysis. Our results indicated that DNMT3 A mutations had a favorable impact (P = 0.008) and TET2 mutations, which showed no significance (P = 0.06) in all included patients, could imply good efficacy in some subgroups on ORR. However, none advantages of mutations on ORR translated into a benefit in overall survival.
Conclusions: This meta-analysis indicates one favorable factor, DNMT3 A mutations, on ORR in MDS patients with HMAs therapy. The identification of mutations in DNMT3 A can improve clinical efficacy and help make treatment decisions.

1. Introduction

Myelodysplastic syndromes (MDS) are heterogeneous malignant myeloid stem cell disorders characterized by invalid hematopoiesis, peripheral cytopenia and high risk of progression to acute myeloid leukemia (AML) [1]. At present, the hypomethylating agents, azacitidine (AZA) [2] and decitabine (DAC) [3], are the only approved drugs for treatment of MDS by the Food and Drug Administration (FDA) in 2004 and 2006 respectively; however, only 40–50% of patients respond to the agents, and the effect on overall survival (OS) does not come to agreement, although there is individual variation [4,5]. Effective methods for identifying patients who are the most likely to have better clinical efficacy treated with HMAs would be of key clinical significance. Clinical features and patient characteristics may help stratify patients, but these models are not sufficiently conclusive to deny eligible patients a trial of AZA or DAC based on their predictions alone [6]. Better biomarkers of clinical efficacy to HMAs are needed.

DNA methylation is an epigenetic mechanism that consists in the addition of methyl groups to cytosine residues within CpG-islands, which are located in or near gene promoter regions. Aberrant DNA methylation has been associated with gene silencing, via inhibition of gene transcription [7,8]. DNA methylation mutations [9] (e.g., Tet methylcytosine dioxygenase 2 [TET2], DNA (cytosine-5)-methyltransferase 3 A [DNMT3A] or Isocitrate dehydrogenase 1/2 [IDH1/2]) are candidates to influence OS [10–12], overall response rate (ORR) [12–14], complete remission rate (CR) [15,16], and progression free survival (PFS) [10,15] when treatment with HMAs in MDS, but there is no unanimity between the studies and some data even do not support such associations. Mutations in different genes of the same pathway, interactions between gene mutations, or alterations in diverse populations could also affect the efficacy of HMAs and explain, at least in part, heterogeneity between the studies as well as individual differences.

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https://doi.org/10.1016/j.leukres.2019.03.001
Received 1 December 2018; Received in revised form 22 February 2019; Accepted 1 March 2019
Available online 02 March 2019
0145-2126/

We performed this meta-analysis to evaluate the impact of gene mutations related to DNA methylation on the clinical efficacy of HMAs in MDS patients, to identify biomarkers with prognostic value for individualized therapy.

2. Method

2.1. Literature search

Relevant papers were identified in the electronic databases PubMed, Embase, Cochrane library and Web of Science, using the following keywords (chosen according to the scientific literature): "myelodysplastic syndrome/s", "DNA methylation pathway", "mutation", "hypomethylating agents", as well as any possible combinations of these terms, up to May 29, 2018. (Please refer to the *Supplementary* for details) The title and abstracts of the selected articles were reviewed to determine the primary eligibility for meta-analysis.

2.2. Inclusion and exclusion

The criteria of including a study in the meta-analysis were the following: (i) MDS patients diagnosed by FAB or WHO criterion, who were treated mainly with hypomethylating agents (either azacitidine or decitabine or both); (ii) mutational analysis was assessed on gene sequencing platforms; (iii) Correlation of gene mutations on DNA methylation pathway (TET2/DNMT3A/IDH) with ORR and/or OS was recorded. Exclusion criteria were as follows: (i) Review articles, case reports and laboratory studies; (ii) studies with insufficient data for estimating pooled ORs or HRs; (iii) studies had duplicate data or repeat analysis, in this case, only the most recent or the highest quality studies were included; (iv) more than 20% of patients were therapy related MDS.

Two reviewers (Mengyi Du and Fen Zhou) screened the databases and identified the eligible studies, independently. Disagreements were resolved by discussion.

2.3. Data extraction

We collected the following items from each study, if available: the first author's name, year of publication, publish journal, country of origin, median follow-up, number of patients, age and gender distribution of patients, distribution of relatively high-risk patients by international prognostic scoring system (IPSS) or revised-international prognostic scoring system (IPSS-R) classification and therapy. We selected ORR as the primary endpoint, OS as the secondary endpoint. (ORR, the rate of patients obtaining specific improvements in marrow and peripheral blood measurements according to the IWG 2006 criteria [17], covers complete remission rate, partial remission rate and marrow complete remission rate.) We extracted number of patients with gene mutations, number of patients with a 'wild-type' genotype, ORR of these two types of patients for odds ratio (ORs) to evaluate the effect of gene mutations. Endpoints for OS were defined as either deceased (failure) or alive at last follow-up. We extracted the corresponding hazard ratios (HRs) and their 95% confidence intervals (CIs) for OS from univariate or multivariate Cox proportional hazards models (date from multivariate COX model were preferred) to evaluate the prognostic impact of the gene-mutated patients compared with the unmutated with MDS. If only survival curves were provided, Engauge Digitizer software was applied for data extraction, and the Excel designed by JayneF Tierney [18] was utilized for calculating HRs and 95% CIs.

Two independent reviewers (Mengyi Du and Fen Zhou) extracted the data. Disagreements between reviewers regarding data abstraction were resolved through discussion.

2.4. Quality assessment

Two reviewers independently evaluated the methodological quality of each included study. The quality of cohort studies was evaluated by the Newcastle-Ottawa quality assessment scale (NOS) [19]. The NOS includes a total of 9 points, with 4 points for selection, 2 points for comparability, and 3 points for exposure or outcome. Six or more points in cohort or case-control studies were regarded as high quality. Disagreements were resolved by discussion.

2.5. Statistical analysis

Review Manager software version 5.3 (following the recommendation of the Cochrane Collaboration (http://tech.cochrane.org/revman/download) and STATA statistical software version 12.0 (STATA, College Station, TX) were used to calculate the combined survival impact on gene mutations of DNA methylation. The impress of mutations on ORR and CR was derived by calculating ORs and their 95% CIs. The prognostic effect of gene mutations on OS and PFS was evaluated by calculation of the combined HRs and their 95% CIs with the generic inverse variance method. The result suggested statistical significance if the 95% CI did not overlap 1. Moreover, gene mutations contributed an adverse survival effect compared to unmutated patients when the HR was more than 1 or OR was less than 1.

The heterogeneity of the studies was evaluated through the chi-squared test, with significance set at a p-value of less than 0.10. The statistic I^2 was used to quantify the heterogeneity. I^2 value less than 25% was regarded as low heterogeneity, value between 25 and 50% indicated moderate heterogeneity, and value over 50% suggested high heterogeneity [20]. The random effect model was used if high heterogeneity was observed; otherwise, a fixed effect model was used for the meta-analysis. Subgroup analysis and sensitivity analysis were applied to explore the origin of heterogeneity. Funnel plots, Begg's test and Egger's test were used to screen for potential publication bias concerning the total population. The poor stability resulted from inclusion and exclusion of the studies would be reappraised.

3. Results

3.1. Study identification and selection

As shown in Fig. 1, the initial search revealed 584 studies. After exclusion of 92 duplicates, 492 citations were further reviewed by reading the titles and abstracts, and 474 citations were then excluded for irrelevant subject or content. A total of 18 studies were left for full text review. Among them, 4 studies were excluded because of insufficient data, and 1 were further excluded because the proportion of therapy related MDS was higher than 20%. During revision, none additional citation was included in the final meta-analysis, leaving a total of 13 citations.

3.2. Characteristics of included studies

The 13 included studies were cohort or case control studies and were published between 2011 and 2018 (Table 1). They were conducted in East Asia, Europe or America. The studies included a total of 1398 patients with MDS, in which one third patients harbored a mutation in DNA methylation pathway. Occurrence of mutations of DNMT3 A and IDH1/2 varied approximately between 4% and 26%, and mutations of TET2 were more frequent (9%–32%), especially in Europe-America MDS patients.

3.3. Quality assessment of included studies

As shown in Table 1 and supplementary, the mean overall NOS score was 8 (range 7–9), indicating that the quality of included studies

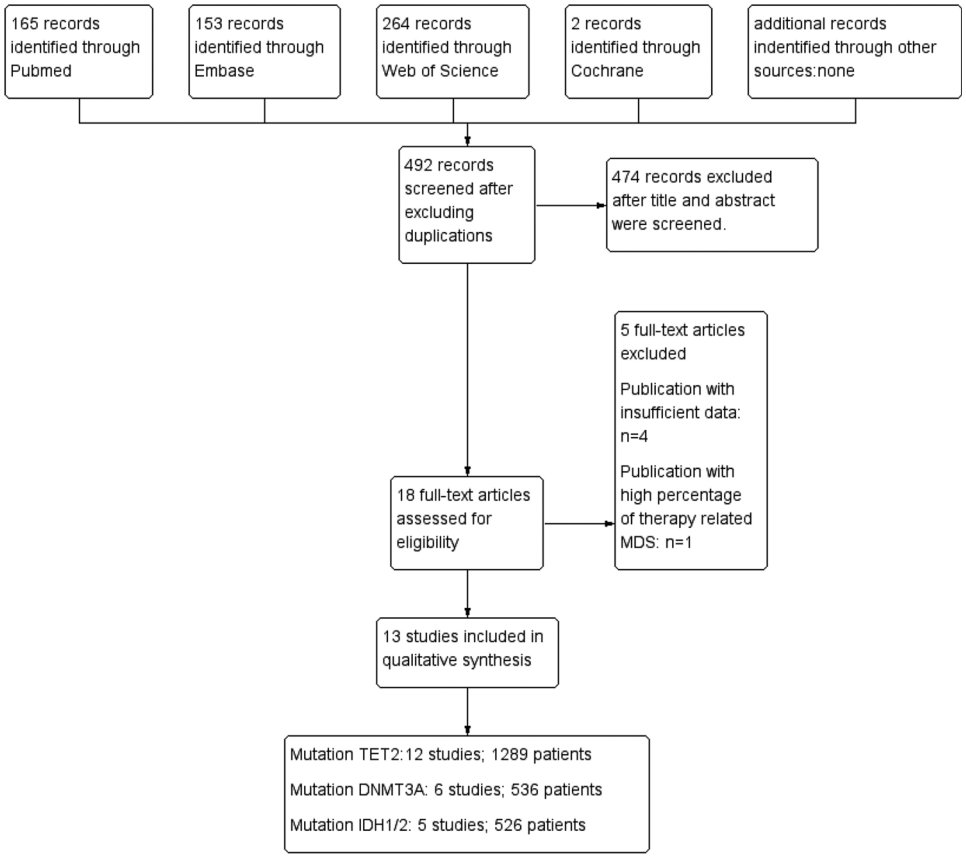

Fig. 1. Flow chart of the included studies.

was high (details in supplementary data).

3.4. Outcome

3.4.1. Primary outcome: Overall response rate (ORR)

Data of the effect of TET2 mutations on ORR were available from 10 studies with a total of 1001 patients. As regards to response rate during study, there was no significant advantages for patients with TET2-mutated (OR = 1.36, 95% CI = 0.99–1.86, P = 0.06, I^2 = 10%), compared to the TET2-wildtype. For more precise assessment, the ten included studies were divided into subgroups by risk level (relatively high-risk patients vs relatively low-risk patients), original country (Asia vs Europe-America), and therapy respectively. Four studies laid emphasis on patients with relatively high-risk MDS harboring TET2 mutations (see in Table 2, percentage of relatively high-risk MDS was higher than 80%). The pooled OR for ORR raised to 2.29 (95% CI: 1.21–4.33, with a p-value of 0.01, I^2 = 27%), for patients with relatively high-risk MDS with TET2 mutations, compared to TET2-unmutated patients (Fig. 2a). Several studies focused on patients with Europe-America population. The pooled OR for ORR was 1.48 (95% CI: 1.05–2.09, with a p-value of 0.03, I^2 = 18%) for patients with TET2 mutations, compared to TET2-unmutated patients (Fig. 2b). There was no evidence for a difference between treatment therapies both on the drug side (AZA or DAC) and on the treatment cycle side. Our results indicated that TET2 mutations did not significantly affect the ORR with HMAs treatment, but the positive effect could be discovered in Europe-America MDS population, and the promising influence would be enlarged in patients with relatively high-risk. (details in Table 2)

536 patients provided data about the effect of DNMT3 A on ORR in six studies, the pooled OR was 2.11 (95% CI = 1.22–3.66, P = 0.008, I^2 = 0%), and the OR raised to 2.49 (95% CI = 1.25–5.00, P = 0.01, I^2 = 0%) when Asia patients excluded (Fig. 2c). Five studies reported data about the effect of IDH1/2 on ORR with 526 patients. There was no evidence for a difference in the pooled analysis (OR = 1.15, 95% CI = 0.63–2.10, P = 0.65, I^2 = 0%) (Fig. 2d), mutations in IDH1/2 might not affect overall response rate.

3.4.2. Secondary outcome: overall survival (OS)

As shown in Table 2, the meta-analysis of the effect of TET2 mutations on OS in MDS patients was performed for 8 studies with a total of 950 patients. Our results indicated that the presence of TET2 mutations might not significantly affect the OS (HR = 1.13, 95% CI: 0.95–1.33, p = 0.16) with a low heterogeneity (I^2 = 24%) (Fig. 3). For more precise assessment of the association between OS and gene mutations, the 8 studies were divided into subgroups by the same principle as the evaluation of relationship between ORR and gene mutation, however, there was no evidence for a difference between TET2-mutated patients and unmutated patients for OS in HMAs treated MDS, even the population was specially restricted.

Four studies provided with data about the effect of DNMT3 A (3 studies) and/or IDH1/2 (2 studies) mutations on OS, but there was high heterogeneity among the analyzed studies, and the reason for exclusion was not abundant, so the identified studies were not meta-analyzed.

3.4.3. Other outcomes: Complete remission rate (CR)

We tried to analyze other two end points —— CR and PFS to investigate the prognostic effect of gene mutations on DNA methylation pathway in MDS patients with HMAs therapy. However, after extracting useful data from the included studies, we were only able to analyze the prognostic impact of TET2 mutations on the CR. We evaluated three studies with a total of 287 patients. The pooled OR for the CR was 1.65 (95% CI = 0.80–3.39, p = 0.17, I^2 = 0%) in hypomethylated MDS patients with TET2 mutations compared to the wild-type. These data indicated that the TET2 mutations did not significantly affect the CR in

Table 1
Main characteristics of all the studies included in the meta-analysis.

Num	Study	Journal	study type	median follow-up	total patients	country	age(years)	male(%)	percentage of relatively high risk*	therapy	median treatment cycle	gene mutation(%)	NOS
1	Voso [43]	Leukemia	cohort study	12(0.7-21) months	38	Italy	64(37-77)	17/38(44.7%)	100%	AZA + others	4(2-30)	TET2 (12/38; 32%)	7
2	Itzykson [14]	Leukemia	cohort study	23.3 months	86	French	71(52-88)	56/96 (65.12%)	83.80%	AZA	6(1-39)	TET2(13/86; 15%)	8
3	Bejar [13]	Blood	cohort study	3.8 years patients remaining alive	213	USA	≥70y n = 103 (48.4%)	155/ 213(73.1%)	53.10%	AZA, DAC, DAC + others	NG	TET2(58/213; 27%)	8
4	Traina [15]	Leukemia	cohort study	NG	92	USA & Brazil	68 (34-81)	68/92 (73.9%)	32.50%	AZA, AZA + others, DAC, DAC + AZA	AZA 6 (1–35), DAC 4 (1-17)	TET2(17/92; 18%) DNMT3 A(8/ 92; 9%) IDH1/2(7/92; 8%) TET2/DNMT3 A/IDH1/2 (28/ 92; 30%)	8
5	Hong [44]	Anticancer Res	cohort study	40 months	58	Korea	67 (26-89)	46/58 (79.3%)	46.6%-81%	DAC	4(1-25)	TET2(5/58; 9%)	9
6	Kim [11]	Bone Marrow Transplantation	cohort study	40.8 (35.8–45.7) months for survivors	52	Korea	52 (18–73)	36/52 (69.1%)	> 65.4%	AZA, DAC, others	AZA 4(1-29), DAC 2(1-5)	TET2 (8/52; 15%)	9
7	Jung [10]	Oncotarget	case control	2.28 years (0.07 to 6.24)	107	Korea	59 (23-76)	67/107 (62.6%)	> 64.5%	AZA, DAC	4 (1-18)	TET2 (17/107; 16%) DNMT3 A(9/107; 8%) IDH(4/ 107; 4%)	9
8	Tobiasson [23]	Oncotarget	cohort study	14 months	134	Sweden & UK	70.5 (35-88)	NG	85.40%	AZA	7 (1-45)	TET2 (26/134; 19%) IDH1/ 2(17/134; 13%) TET2/ DNMT3 A/IDH1/2 (49/134; 37%)	8
9	Cedene [28]	Oncotarget	cohort study	17months (1–93)	84	Spain	69 (49–99)	54/84 (64.3%)	> 52.4%	AZA	6(1-55)	TET2(23/84; 27%) DNMT3 A(16/84; 19%) IDH1/ 2(9/84; 11%) TET2/DNMT3 A/ IDH1/2 (41/84; 49%)	7
10	Chang [45]	British Journal of Haematology	cohort study	37months (25.2–38.8)	109	China	61 (17–85)	71/109 (65.1%)	57.80%	DAC	4 (2–21)	IDH1/2(10/109; 9%)	8
11	Zhao [16]	journal of Zhonghua hematology	cohort study	37months (2 ~ 101)	109	China	61(17 ~ 85)	70/109 (64.2%)	57.80%	DAC	4(2 ~ 11)	TET2(13/109; 12%) DNMT3 A(17/109; 16%) IDH1/ 2(11/109; 10%)	9
12	Sekeres [12]	Journal Of Clinical Oncology	cohort study	23 months (1-43)	277	USA & Canada	70 (28-93)	192/277 (69.3%)	66.80%	AZA, AZA + others	a median of 5.5 cycle	TET2(26/113; 23%) DNMT3 A(12/113; 11%)	9
13	Cabezon [29]	Oncotarget	cohort study	11months (0-68)	39	Spain	71 (55-83)	29/39 (74.4%)	90.00%	AZA	6 (range 1–36)	TET2(5/31; 16%) DNMT3 A(8/ 31; 26%)	7

Abbreviation: NGnot given; AZAazacitidine; DACdecitabine; NOSNewcastle-Ottawa quality assessment scale.
*Relatively high-risk patients corresponds to: 1) the intermediate-risk group plus the very high-risk group of the IPSS; 2) the high-risk and very high-risk groups of the WPSS; and, 3) the intermediate risk, high-risk and very high-risk groups (IPSS-R Intermediate patients were designated as “relatively high-risk” if their score was > 3.5) of the IPSS-R. The other subgroups of the individual systems were combined in the “relatively low-risk” group.

Table 2
Summary of the meta-analysis results.

gene	endpoint	subgroup	group title	No. of studies	No. of participents	Statistical method	Effect size	P	I^2	P (Heterogeneity)	P Begg's Test	P Egger's test
TET2	ORR	no	All	10	1001	M-H,Fix	1.36 [0.99,1.86]	**0.06**	10%	0.35	1	0.976
		race	Asia	2	216	M-H,Fix	0.87 [0.39, 1.90]	0.72	0%	0.71	1	–
			Europe-America	8	785	M-H,Fix	1.48 [1.05, 2.09]	**0.03**	18%	0.03	0.711	0.805
		risk level	lower risk	6	718	M-H,Fix	1.13 [0.78, 1.63]	0.52	0%	0.71	0.707	0.152
			higher risk*	4	283	M-H,Fix	2.29 [1.21, 4.33]	**0.01**	27%	0.25	1	0.869
		drug	AZA	6	480	M-H,Fix	1.44 [0.92, 2.25]	0.11	41%	0.13	1	0.609
			DAC	1	109	M-H,Fix	0.73 [0.22, 2.41]	0.6	–	–	–	–
			AZA & DAC	3	412	M-H,Fix	1.40 [0.87, 2.26]	0.17	0%	0.59	–	–
		cycle	≤4 cycles	3	248	M-H,Fix	1.09 [0.54, 2.19]	0.81	0%	0.42	1	0.42
			> 4 cycles	6	540	M-H,Fix	1.37 [0.89, 2.12]	0.16	35%	0.17	1	0.759
	OS	no	All	8	950	M-H,Fix	1.13 [0.95, 1.33]	0.16	24%	0.24	0.711	0.437
		race	Asia	2	110	M-H,Fix	1.62 [0.76, 3.46]	0.21	82%	0.02	–	–
			Europe-America	6	840	M-H,Fix	1.11 [0.93, 1.31]	0.25	0%	0.74	0.707	0.443
		risk level	lower risk	3	258	M-H,Fix	1.13 [0.95, 1.33]	0.16	55%	0.06	0.462	0.442
			higher risk*	5	692	M-H,Fix	1.07 [0.60, 1.90]	0.83	0%	0.88	1	0.754
		drug	AZA	4	535	M-H,Fix	1.35 [0.89, 2.07]	0.16	0%	0.64	0.734	0.783
			DAC	1	58	M-H,Fix	0.60 [0.20, 1.84]	0.37	–	–	–	–
			AZA & DAC	3	357	M-H,Fix	1.11 [0.92, 1.33]	0.28	64%	0.06	–	–
		cycle	≤4 cycles	3	148	M-H,Fix	1.62 [0.77, 3.41]	0.21	64%	0.06	1	0.953
			> 4 cycles	4	589	M-H,Fix	1.26 [0.89, 1.79]	0.19	0%	0.58	0.734	0.503
DNMT3 A	ORR	no	all	6	536	M-H,Fix	2.11 [1.22, 3.66]	**0.01**	0%	0.84	1	0.979
		race	Asia	2	216	M-H,Fix	1.60 [0.65, 3.95]	0.31	0%	0.94	1	–
			Europe-America	4	320	M-H,Fix	2.49 [1.25, 5.00]	**0.01**	0%	0.7	1	0.802
IDH	ORR	no	all	5	526	M-H,Fix	1.15 [0.63, 2.10]	0.65	0%	0.44	0.462	0.242

Abbreviations: ORR: overall response rate; OS: overall survival; M－H: Mantel–Haenszel; Fix: Fixed-effects model ; AZA: azacitidine; DAC: decitabine.
*Higher-risk patients corresponds to1) the intermediate-risk group plus the very high-risk group of the IPSS; 2) the high-risk and very high-risk groups of the WPSS; and, 3) the intermediate risk, high-risk and very high-risk groups (IPSS-R Intermediate patients were designated as "relatively high-risk" if their score was > 3.5) of the IPSS-R. The other subgroups of the individual systems were combined in the "lower-risk" group.

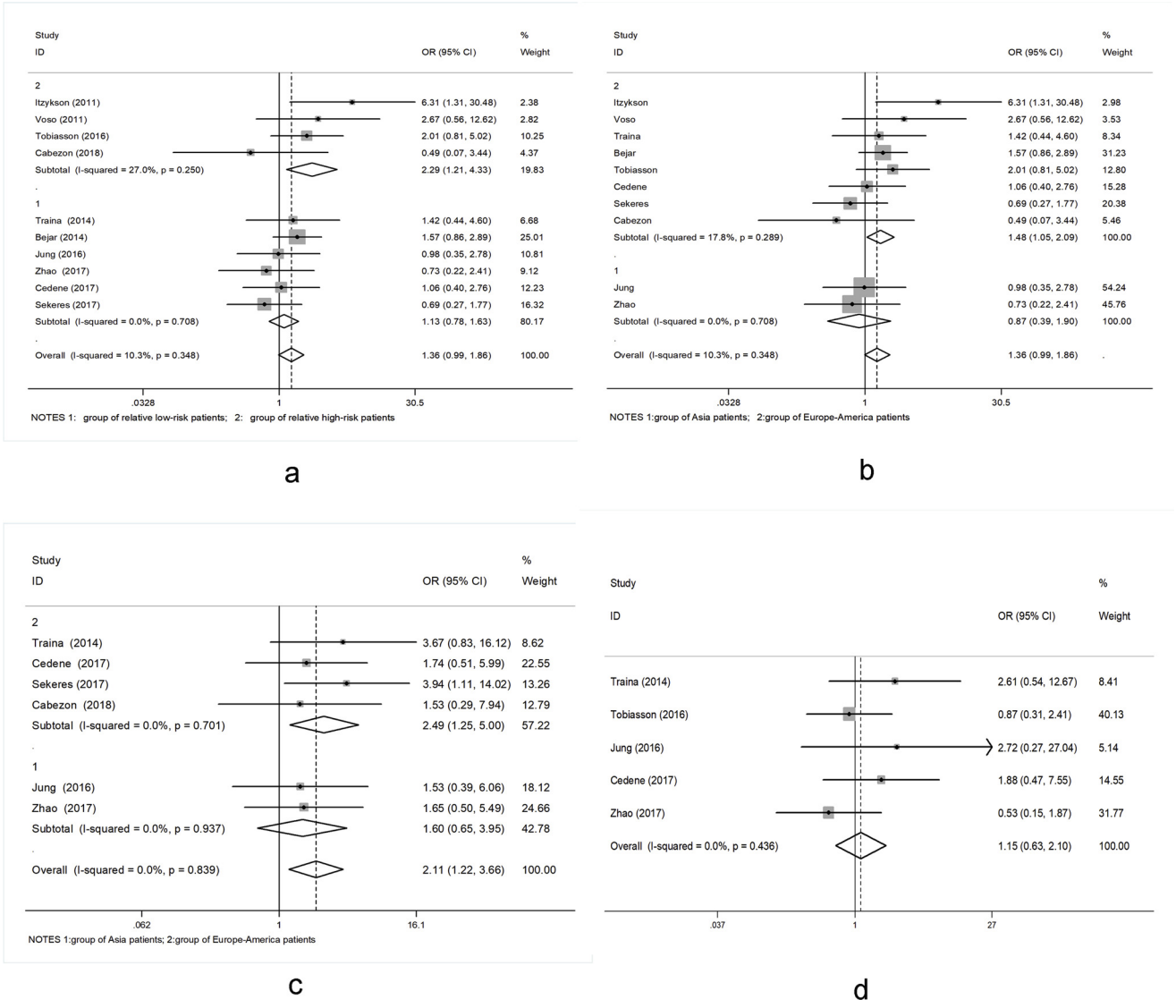

Fig. 2. Forest plots of pooled ORs and 95% CIs assessing the ORR of gene mutations in HMAs treated patients comparing with the unmutated. The size of the blocks or diamonds represents the weight, the length of the straight line represents the width of 95% CI. (a) comparing TET2-mutated patients with TET2-unmutated and subgrouped by risk-level; (b) comparing TET2-mutated patients with TET2-unmutated and subgrouped by race; (c) comparing DNMT3 A-mutated patients with DNMT3A-unmutated and subgrouped by race; (d) comparing IDH-mutated patients with IDH-unmutated.

MDS patients with AZA and/or DAC therapy.

3.5. Sensitive analysis and publication bias

We conducted a sensitivity analysis by excluding one study at a time from the meta-analysis, examining the effect of individual studies on the combined OR and HR. The results indicated that there were no significant effects of individual studies on the combined OR for ORR and HR for OS in the total population (details in supplementary). There was no significant publication bias of ORR and OS of all population, which contained all 13 studies (Table 2; Funnel plot in supplementary).

4. Discussion

Myelodysplastic syndromes (MDS) are a group of clonal neoplasms of the hematopoietic stem cell characterized with variable degrees of clinical outcomes. Until now, azacitidine and decitabine are the only drugs that have been approved by FDA in remedying this complicated prognosis disease. A large number of studies have investigated the prognostic value of TET2/DNMT3 A/IDH in MDS patients treated with hypomethylating agents. But, with less than half responsers and controversial effect on overall survival [21,22], the clinical efficacy of HMAs applied to all patients is not satisfied, so biomarkers of better clinical outcome to AZA or DAC are of great significance.

The pooled meta-analysis of the included studies demonstrated that, in relatively high-risk patients and in Europe-America population, TET2 mutations indicated increased response rates to HMAs compared with wildtype, and this is consistent with many prior studies [13,14,23]. It is easy to understand because AZA and DAC are recommended to remedy patients with higher-risk by NCCN Guideline (Version 1.2019; available at Http://www.nccn.org/) and the efficacy of HMAs has been verified in Europe-America [21,22,24,25] population mostly, with less clinical trials on this topic in Asia. Effective as HMAs are, more appropriate usage and dosage for Asia population should be probed into. As for the treatment cycles of HMAs, there was no relationship between gene mutations and higher response rate, no matter in groups no more than 4 cycles or in groups greater than 4 cycles. Previous studies showed that HMAs began to take effect at 2 cycles, and the effect reached the climax at 4 cycles [26,27]. Nearly all the treatment cycles of included patients were no less than 4, so the number of treatment cycles did not result

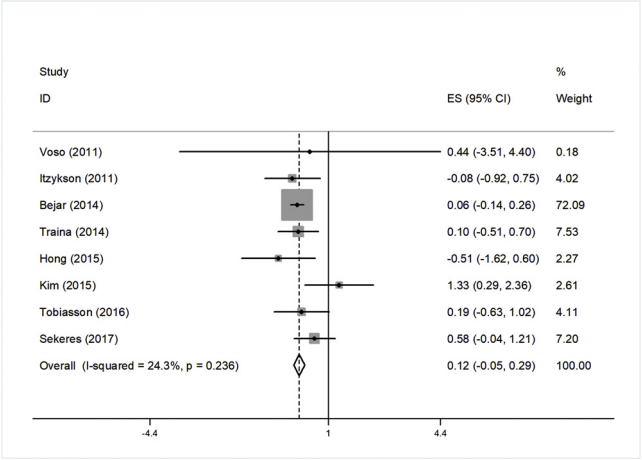

Fig. 3. Forest plots of pooled HRs and 95% CIs assessing the OS of TET2 mutations in HMAs treated patients comparing with unmutated. The size of the blocks or diamonds represents the weight, the length of the straight line represents the width of 95% CI.

into difference. DNMT3 A mutations were correlated with favorable outcomes in ORR, and more obvious distinction was determined in Europe-America patients. This result was derived from four studies [12,15,28,29] with same tendency, and in this paper, the significant diversity ($P < 0.05$) between DNMT3 A-mutated and DNMT3 A-wild-type patients was confirmed in 536 patients leading to a convincing result. Generally, DNMT3 A mutations may be as significant biomarkers in evaluating response to HMAs. Different from DNMT3 A, the impact of IDH mutations on response rate was pretty controversial. While three Europe-America studies showed odds ratios (ORs) more than 1 for the effect of IDH mutations, two Asian studies showed the adverseness. Though the P value of the pooled data from previous studies was greater than 0.05 (indeed 0.65), advanced studies focus on Europe-America populations may throw new light on this argument.

Although the presence of DNMT3 A mutations predicted a higher response rate and TET2 mutations indicated better outcomes of response in some subgroups to HMAs, these did not translate into benefits in overall survival. The result is consistent with some of prior studies [12,13,15]. Experts have tried to combine gene mutation profiles with existing prognostic scoring systems (e.g. IPSS/WPSS/IPSS-R) [30–33] for more precise OS prediction without taking treatment into consideration. Among them, Haferlach [32] and Gangat [33] hold the idea that TET2 is not associated with OS, however Hou [31] takes DNMT3 A and IDH2 as adverse factors in the new prognostic scoring system established by him and his colleagues. DNMT3 A and IDH2 mutations were poor-risk genotypes due to the heterogeneity resulting from patient inclusion criteria, gene sequencing platforms and treatment in Hou's cohort. Except for the heterogeneity, the association with clinical phenotypes, the co-mutation between genes also add up the difficulty of evaluating the real effect of gene mutations on OS.

TET2, DNMT3 A and IDH1/2 are the genes of the highest mutated frequency in the pathway of DNA methylation of MDS patients [9]. TET2 is an alpha-ketoglutarate (αKG) and Fe (II) -dependent oxygenase, an enzyme that converts 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) in DNA. TET2 mutant patients have hypermethylated promoters and hypermethylated enhancers enriched [34,35]. Although TET2 mutations have been shown to be associated with a decrease of 5hmC at the genomic DNA level [36], but it does not correlate with changes in expression of neighbor genes [37]. DNMT3A is a de novo DNA methyltransferase, which is responsible for the covalent linking of methyl groups to the CpG dinucleotide [38]. These mutations always suggest a gain of function effect [39], which results in a hypermethylation. IDH1/2-mutant is associated with more extensive promoter hypermethylation compared to the wild subtypes [34]. Although no agreement on the function and biological consequences of gene mutations in DNA methylation pathway has been reached, these mutations show a tendency to be hypermethylated by main published studies. HMAs are nucleoside analogues that irreversibly inhibit the DNA methyltransferases, leading to progressive loss of methylation and reversal of gene silencing [40–42], with an influence on the clinical outcome of mutant patients. Gene alterations in MDS not only cause initiation and progression, but could be predictors of clinical efficacy.

To date, our paper is the most comprehensive study with 1398 MDS patients treated with hypomethylating agents[10–16,23,28,29,43–45]. And it is the first meta-analysis concerning the controversial prognostic value of three gene mutations in DNA methylation pathway in MDS patients treated with HMAs. Clinical efficacies such ORR, OS, CR and PFS were all taken into consideration, though some meta-analysis were not performed due to the limitation of the number of patients. Besides, there were two other limitations in this study. First, the analysis was based on observational studies rather than on randomized trials or prospective studies. Second, Both Egger's test and Begg's test have relatively lower power when the number of studies included in meta-analysis was less than 10. Hence, the publication bias may not be detected by Egger's and Begg's test.

In conclusion, our meta-analysis indicates one favorable prognostic biomarker, DNMT3 A mutations, on overall response rate in MDS patients by HMAs treatment. Though no gene mutation in DNA methylation pathway is confirmed to have prognostic value to HMAs on OS, MDS Patients with DNMT3A mutations is encouraged to have HMAs as major therapy. Challenges such as how to use the drug more rationally, and how to translate the advantage of overall response rate into a benefit in overall survival should be studied in the future researches.

Acknowledgments

We thank all patients and clinical investigators who were involved in the studies selected for this meta-analysis. This work was financially supported by National Natural Science Foundation of China (Grant No. 81570116; Grant No 81873434).

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