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Prognostic significance of *U2AF1* mutations in myelodysplastic syndromes: a meta-analysis

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Abstract

Introduction: Although the effects of U2 small nuclear RNA auxiliary factor 1 gene (*U2AF1*) mutations on the outcomes of patients with myelodysplastic syndromes (MDS) have previously been investigated, their prognostic significance remains controversial. We performed a meta-analysis to investigate the impact of *U2AF1* mutations on MDS progression.

Methods: Two reviewers independently extracted information such as hazard ratios (HRs) and 95% confidential intervals (CIs) for overall survival (OS) and leukemia-free survival (LFS) as well as the number of surviving patients each year after diagnosis from the included studies.

Results: Thirteen studies with a total of 3038 patients were included. The summary odds ratio (OR) for *U2AF1* mutations with an OS of 5 years was 0.37, the summary HR for *U2AF1* mutations in OS was 1.60, and the summary OR for an OS of 5 years in patients with U2AF1^{S34} and U2AF1^{Q157} was 3.68. There were no significant differences in leukemia-free survival or hypomethylating therapy response between patients with and without *U2AF1* mutations.

Conclusion: *U2AF1* mutations were associated with poor survival in MDS patients, and patients with U2AF1^{Q157} had a worse OS than those with U2AF1^{S34}. Our findings suggest that MDS patients with *U2AF1* mutations could benefit more from hypomethylation therapy.

Keywords

Myelodysplastic syndromes, U2AF1, mutations, meta-analysis, prognosis, hypomethylating therapy

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Introduction

Myelodysplastic syndromes (MDS) are a group of clonal hematopoietic diseases characterized by ineffective hematopoiesis leading to peripheral blood cytopenias, and are likely to evolve into acute myelogenous leukemia (AML).[1–3] The prognosis of MDS is very diverse because of the background of their genetic heterogeneity. Common karyotypic abnormalities have been included in the Revised International Prognostic Scoring System (IPSS-R),[4] and the development of next-generation sequencing (NGS) has revealed that approximately 90% of MDS patients have a somatic mutation in at least one driver gene.[5] The number of these driver genes is large, but they can be organized into a limited number of categories such as RNA splicing genes, epigenetic regulators, transcription factors, cohesin components, DNA damage response, and signal transduction molecules.[6] Some somatic mutations, such as those in *TP53*, *EZH2*, *ETV6*, *RUNX1*, and *ASXL1*, were demonstrated to be associated with poor outcome, and only the *SF3B1* mutation was reported to be associated with a more favorable prognosis.[7] However, the prognostic values of many mutations remain to be confirmed.

U2 small nuclear RNA auxiliary factor 1 (U2AF1) is a member of the SR protein family and a subunit of the U2 small nuclear ribonucleoprotein responsible for recognition of the AG dinucleotide in 30 pre-mRNA splice sites. A total of 5% to 12% patients with MDS carry *U2AF1* mutations,[8–10] and these almost exclusively affect one of two codons, S34 and Q157, which are located in separate conserved zinc finger domains. *U2AF1* mutations can lead to variable post-transcriptional splicing of genomes, including exons and introns that cannot be spliced,[11] which results in the downregulation of many genes.[8] *U2AF1* mutations were also reported to be an early, initiating genetic event in MDS.[12] Previous research found that *U2AF1* mutations are closely related to sole trisomy 8 and isolated del(20q).[13,14] Trisomy 8 was reported not to influence the outcome of MDS patients, while isolated del(20q) is a good factor for prognosis. Some studies showed that *U2AF1* mutations had no impact on the outcomes of MDS patients,[13,15,16] while others revealed them to be negative factors in MDS prognosis.[17–20]

Hypomethylating agents such as azacytidine and decitabine have been shown by randomized phase III trials to decrease the risk of leukemic transformation and, in a portion of patients, to improve survival.[7] Thus, hypomethylating therapy (HTM) is considered a conventional treatment for MDS patients, and *U2AF1* mutations have been reported to affect the response to HTM.[13,17] Therefore, this meta-analysis was conducted to gain a full insight into the prognostic value of *U2AF1* mutations in patients with MDS.

Materials and methods

Study selection

A systematic literature search of Chinese Biological Medical Disc, PubMed, Embase, and the Cochrane library databases was performed by two independent reviewers (B.L. and D.Z.). Relevant papers published between 2013 and 2019 were obtained using the search terms ((MDS) OR (myelodysplastic syndrome) OR (myelodysplasia) OR (preleukemia) AND ((U2AF1) OR (U2 Auxiliary factor 1)) in PubMed and the Cochrane library, and (myelodysplastic AND syndrome OR MDS OR myelodysplasia OR 'preleukemia':af) AND u2af1 AND [2013-2019]/py in Embase. Independent search terms were used to search the Chinese Biological

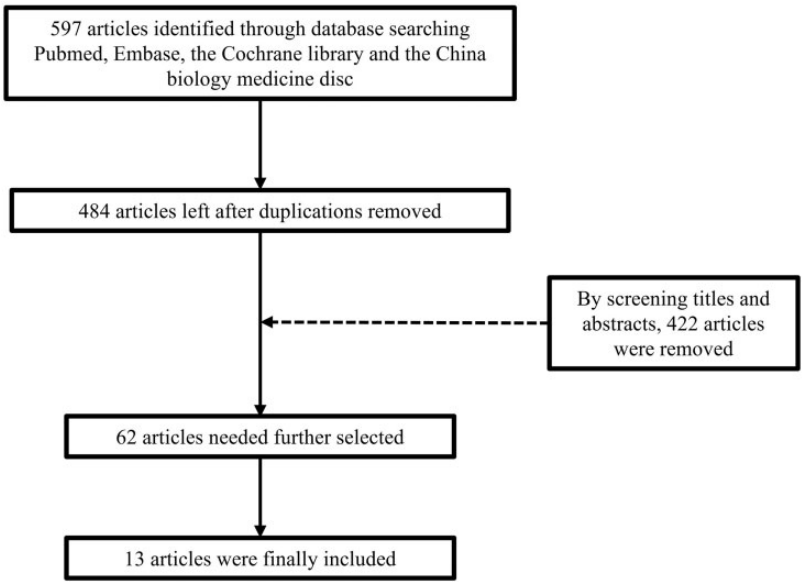

Figure 1. Flow diagram of selection process in the meta-analysis.

Medical Disc database. The search was restricted to human studies with no language limitation. References were also reviewed to obtain missing information.

Both prospective and retrospective research literature was included in the meta-analysis. Inclusion criteria were as follows: (1) published between 2013 and 2019 as original articles; (2) assessed the association between *U2AF1* mutations and outcomes in MDS; and (3) provided detailed survival information of patients with *U2AF1* mutations, including the number of surviving patients every year after diagnosis or a clear survival curve or corresponding hazard rations (HRs), 95% confidence intervals (CIs) and *P*-values. Overall survival (OS) was defined as the length of time from the date of the first sample to the time of death or the last visit. Leukemia-free survival (LFS) was calculated from the date of the first sample to the AML diagnostic time.

Five hundred and ninety-seven studies were identified by the search strategy. By screening titles and reviewing abstracts, animal studies, reviews, case reports, letters to the editor, duplicate publications, and other articles which did not meet the selection criteria were excluded. After this, 62 articles underwent a further screening process which is outlined in Figure 1. Finally, 13 studies were included in the meta-analysis.[13,15–21]

Data extraction

To reduce bias, two reviewers (B.L. and D.Z.) independently extracted the following information from the included studies (Table 1): first author's name, year of publication, journal, region, total number of patients, number of *U2AF1* mutations, age and sex distribution of patients, criteria for classification of MDS and karyotypes, and IPSS-R classification. HRs and 95% CIs for OS and LFS were also extracted from the included studies together with the number of patients which survived every year after diagnosis. If the article provided

Table 1. Summary of the data extracted from the 13 included studies.

Study	Li, 2018[19]	Wu, 2016[20]	Jung, 2016[17]	Hwang, 2016[16]	Kang, 2015[18]	Hong, 2015[15]	Kim, 2017[13]	Tefferi, 2018[21]	Hamilton, 2019[22]	Xu, 2017[26]	Tefferi, 2017[24]	Heuser, 2017[23]	Wu, 2013[25]
Journal	Genes Chromosomes Cancer	Tumour Biol	Oncotarget	J Hematol Oncol	BMC Cancer	Anticancer Res	Leuk Res	Am J Hematol	Bone Marrow Transplant	Sci Rep	Am J Hematol.	Ann Hematol	Am J Hematol
Region	China	China	Korea	Korea	Korea	Korea	Korea	America	America	China	America	Germany	China
Patients (n)	511	304	107	58	129	58	153	357	80	320	179	304	478
U2AF1 mutations (n)	86	26	21	9	10	10	25	52	4	30	28	18	36
Average patient age, years (range)	52 (14–84)	57 (11–89)	≥60 (48)	64 (NR)	64 (NR)	67 (26–89)	65 (18–87)	74 (NR)	52 (12–70)	57 (11–91)	73 (28–96)	58 (19–75)	66 (17–98)
Males, n (%)	308 (60.2)	162 (53.3)	67 (62.6)	37 (63.8)	71 (55.0)	46 (79.3)	97 (63.4)	250 (70.0)	43 (53.8)	178 (55.6)	122 (68.2)	193 (63.5)	290 (60.7)
Criterion	WHO	WHO	WHO	WHO	WHO	FAB	WHO	WHO	NR	FAB	NR	WHO	WHO
Karyotype, n (%)													
Normal	273 (53.4)	124 (40.8)	NR	25 (43.1)	93 (72.1)	28 (48.2)	66 (43.1)	NR	NR	196 (61.2)	72 (40.2)	NR	271 (56.7)
Abnormal	184 (36.0)	180 (59.2)		32 (55.2)	36 (27.9)	30 (51.8)	87 (56.9)			124 (38.8)	107 (59.8)		175 (36.6)
Unknown	54 (10.6)	–		1 (1.7)	–	–	–						32 (6.7)
IPSS -R risk group, n (%)													
Very low	NR	NR	37 (34.6)	4 (6.9)	15 (11.6)	1 (1.7)	6 (3.9)	39 (10.9)	NR	4(1.3)	9 (5.0)	2 (6.6)	13 (2.7)
Low				14 (24.1)	30 (23.3)	10 (17.2)	32 (20.9)	54 (15.1)		63 (19.7)	96 (53.6)	133 (43.8)	107 (22.4)
Intermediate				14 (24.1)	42 (32.6)	20 (34.5)	44 (28.8)	61 (17.1)		127 (39.7)	40 (22.3)	51 (16.8)	110 (23.0)
High			69 (64.5)	14 (24.1)	31 (24.0)	12 (20.7)	37 (24.2)	143 (40.1)		78 (24.4)	9 (5.0)	85 (28.0)	114 (23.8)
Very high				12 (20.7)	11 (8.5)	15 (25.9)	34 (22.2)	57 (16.0)		48 (15)	25 (14.0)	11 (3.6)	102 (21.3)
Unknown			1 (0.9)	–		–		3 (0.8)		–	–	22 (7.2)	32 (6.7)

Abbreviations: WHO, World Health Organization; FAB, French–American–British; U2AF1, U2 small nuclear RNA auxiliary factor 1; NR, Not Reported; IPSS-R, International Prognostic Scoring System.

Table 2. Total NOS score of each study.

Study	Selection	Comparability	Outcome	Score
Li, 2018[19]	****	*	***	8/10
Wu, 2016[20]	***	*	***	7/10
Jung, 2016[17]	***	**	**	7/10
Hwang, 2016[16]	***	*	***	7/10
Kang, 2016[18]	***	*	***	7/10
Hong, 2015[15]	***	*	**	6/10
Kim, 2017[13]	****	**	***	9/10
Tefferi, 2018[21]	****	**	**	8/10
Hamilton, 2019[22]	***	**	***	8/10
Heuser, 2017[23]	****	**	***	9/10
Tefferi, 2017[24]	***	**	**	7/10
Wu, 2013[25]	****	*	***	8/10
Xu, 2017[26]	***	**	***	8/10

Note: studies scored a maximum of one star (*) for meeting each criterion, except comparability (design or analysis) scored a maximum of two stars (**).
NOS, Newcastle–Ottawa quality assessment.

a survival curve, Engauge Digitizer software (Github)[22] was used to extract the number of surviving patients every year after diagnosis. Efforts were made to contact corresponding authors for missing data.

Quality assessment

The quality of the included literature was evaluated by Newcastle–Ottawa quality assessment (NOS). This included 10 items categorized into three major categories: four items for selection, four items for outcome, and two items for comparability, with a total score of 10 (Table 2). We considered a final score of 6 or more as representing a high quality study, and the quality of each included study was high enough for meta-analysis.

Statistical analysis

All statistical analyses were performed using Reviewer Manager Ver5.3 software (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark). For studies that provided a survival curve, ORs and 95% CIs of OS and LFS were used to assess the prognostic effect of *U2AF1* mutations in MDS patients compared with wild-type. For the studies that provided HRs and 95% CIs for OS, we used the O-E and Variance model to perform the meta-analysis. We also performed an OS comparison between $U2AF1^{S34F}$ and $U2AF1^{Q157}$. The statistical heterogeneity of the effect was assessed by I^2 and Q statistics. Data were calculated using fixed effects models when the *P*-value of Q statistics was more than 0.05. Otherwise, the random effects model was used. A two-tailed *P*-value of less than 0.05 was defined as statistically significant. All statistical analyses were performed by B.L. and D.Z. Bubble diagrams were made by R studio, using ggplot2 package.

Results

Characteristics of the selected studies

As shown in Figure 1, 13 studies covering a total of 3038 patients were included in the present meta-analysis. Characteristics of

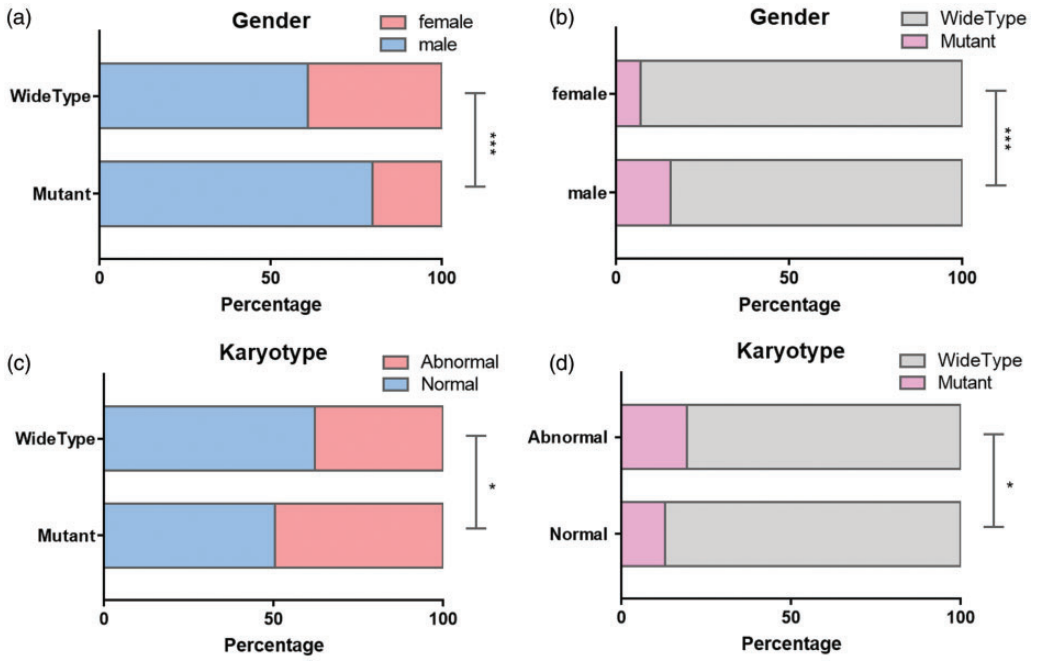

Figure 2. (a) Ratio of males and females for mutant and wild-type *U2AF1*. (b) Ratio of *U2AF1* mutation/wild-type in male and female patients. (c) Ratio of normal and abnormal karyotypes in mutant and wild-type *U2AF1*. (d) Ratio of *U2AF1* mutations in patients with normal and abnormal karyotypes. *: $P< 0.05$, ***: $P < 0.001$.

these studies are shown in Table 1. One study included patients from Germany, three studies included patients from America, four were from China, and five were from Korea. Among the 3038 patients, 355 carried *U2AF1* mutations. Patient ages ranged from 52 to 74 years. Nine studies reported the karyotype of patients, and nine reported the IPSS-R score which was defined as five grades (very low, low, intermediate, high, and very high). The NOS score is shown in Table 2.

Outcome of the meta-analysis

Significantly more males carried *U2AF1* mutations than had the wild-type gene (79.6% vs 60.8%, $P < 0.001$). Moreover, of those patients with *U2AF1* mutations, there were significantly more males than females (15.8% vs. 7.0%, $P < 0.001$). Abnormal karyotypes were encountered significantly more often in patients with than without *U2AF1* mutations (49.6% vs. 37.8%, $P < 0.05$); similarly, patients with abnormal karyotypes were significantly more likely to have *U2AF1* mutations (19.3% vs 12.1%, $P < 0.05$) (Figure 2).

As shown in Figure 3a–d, we analyzed the ORs of OS in MDS patients with *U2AF1* mutations in eight studies;[13,15–21] summary ORs for OS of 1, 2, 3, and 5 years were 0.76 (95% CI: 0.54–1.09), 0.47 (95% CI: 0.35–0.62, $P < 0.001$), 0.43 (95% CI: 0.24–0.78, $P = 0.006$), and 0.37 (95% CI: 0.26–0.51, $P < 0.001$), respectively. In the case of 3-year OS, high heterogeneity was observed with an I^2 of 64% ($P = 0.006$). As shown in Figure 3e, the summary HR of OS in MDS patients with

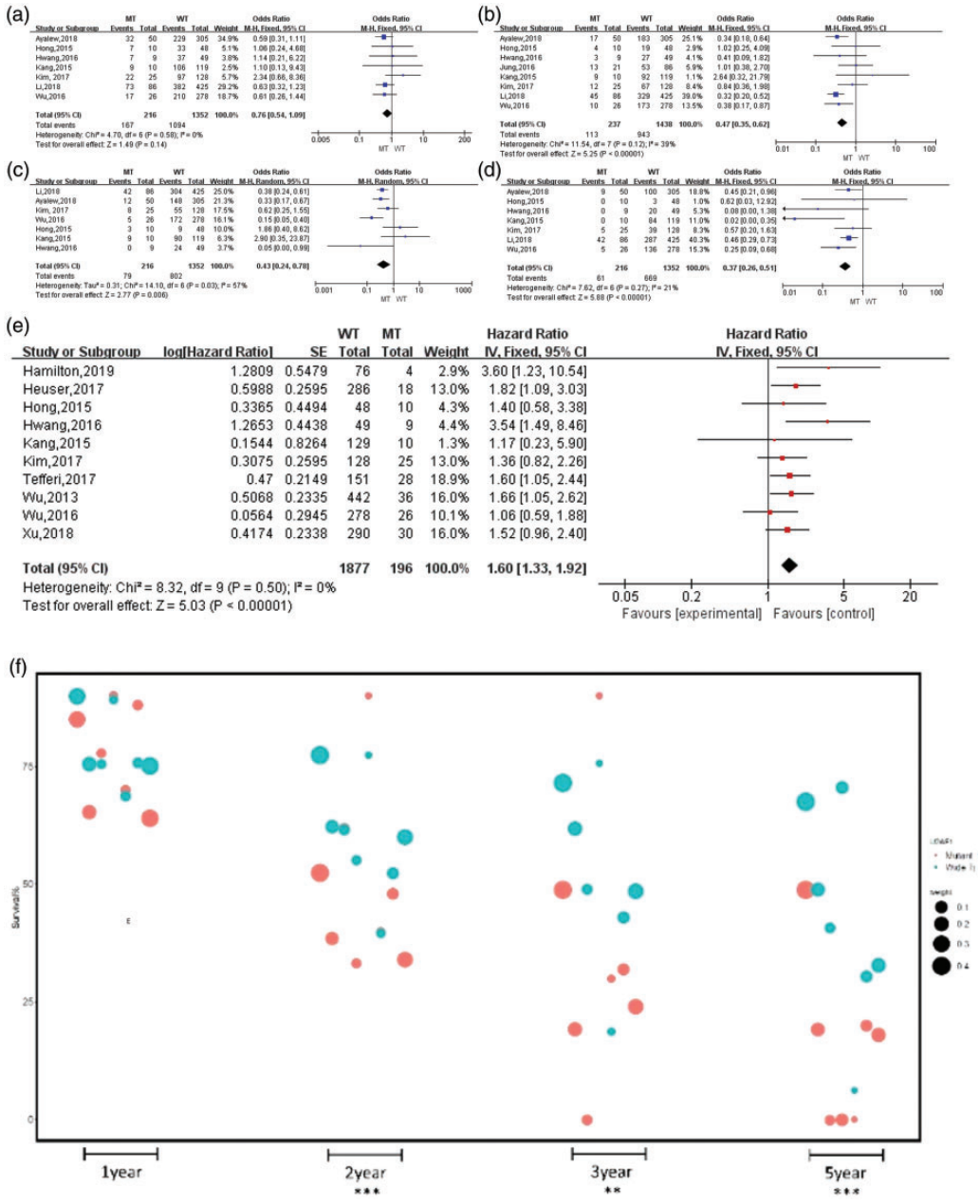

Figure 3. Summary odds ratios (ORs) for overall survival (OS) at 1, 2, 3, and 5 years in MDS patients with and without *U2AF1* mutations. (a) 1 year OS. (b) 2 year OS. (c) 3 year OS. (d) 5 year OS. (e) Summary hazard ratios (HRs) for OS. (f) OS tendency in patients with and without *U2AF1* mutations; green plots represent those without *U2AF1* mutations. The size of each point represents the weight of each study in the meta-analysis.

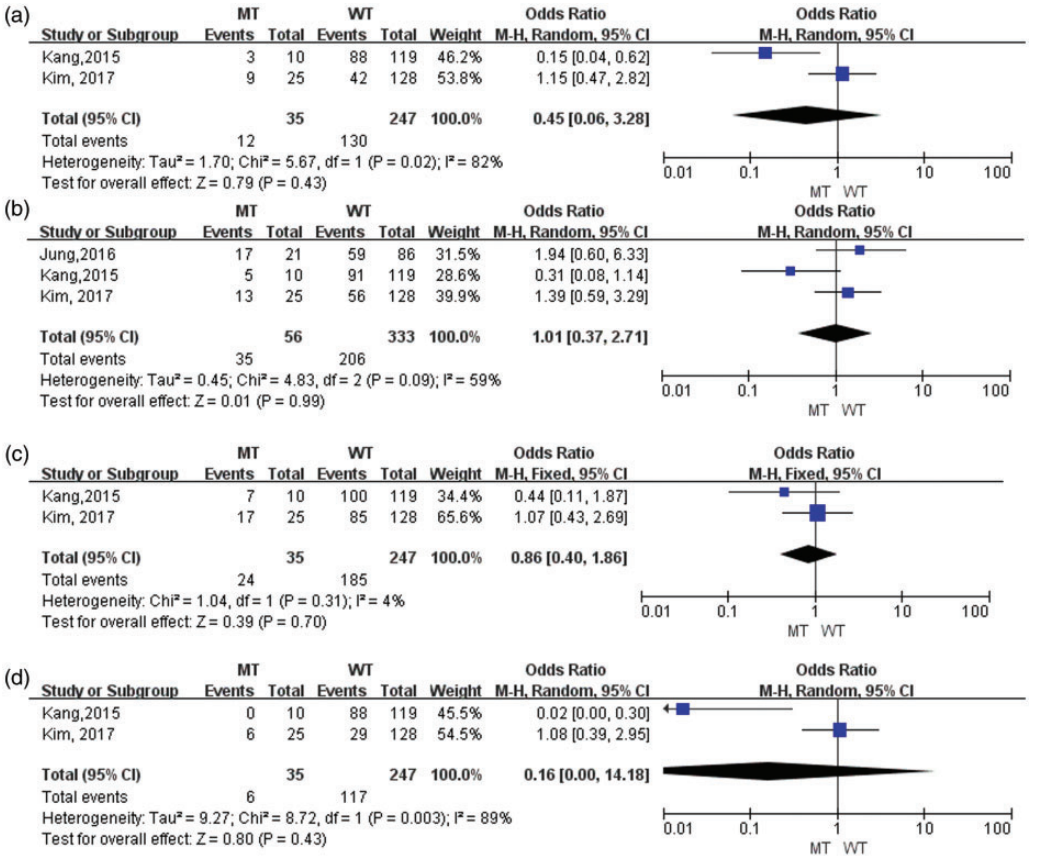

Figure 4. Summary ORs for leukemia-free survival (LFS) at 1, 2, 3, and 5 years in MDS patients with and without *U2AF1* mutations. (a) 1 year LFS. (b) 2 year LFS. (c) 3 year LFS. (d) 5 year LFS.

U2AF1 mutations from 10 studies[13,15,16,18,20,23–27] was 1.60 (95% CI:1.33–1.92, P <0.001); this demonstrated that *U2AF1* mutations were a poor factor for longer-term survival in MDS patients. The tendency for OS in patients with and without *U2AF1* mutations is shown in Figure 3f for each study. Because of a lack of information, LFS data were only collected from three studies [13,17,18]. No differences in LFS were identified between patients with and without *U2AF1* mutations (Figure 4).

In MDS, *U2AF1* mutations predominantly affect S34 and Q157 codons, so these were further analyzed in the meta-analysis. Figure 5a–d shows that summary ORs for OS in patients with $U2AF1^{S34}$ and $U2AF1^{Q157}$ of 1, 2, 3, and 5 years were 0.92 (95% CI: 0.30–7.52), 1.53 (95% CI: 0.68–3.48), 1.56 (95% CI: 0.65–3.76), and 3.68 (95% CI: 1.32–10.25, $P = 0.01$), respectively, indicating that patients with $U2AF1^{Q157}$ had a worse prognosis than those with $U2AF1^{S34}$ for long-term survival. This tendency is also shown in Figure 5e. Our findings also revealed an OR for HTR between patients with and without *U2AF1* mutations of 0.76 (95% CI: 0.24–2.44), demonstrating that the *U2AF1* mutation status does not affect HTR (Figure 6).

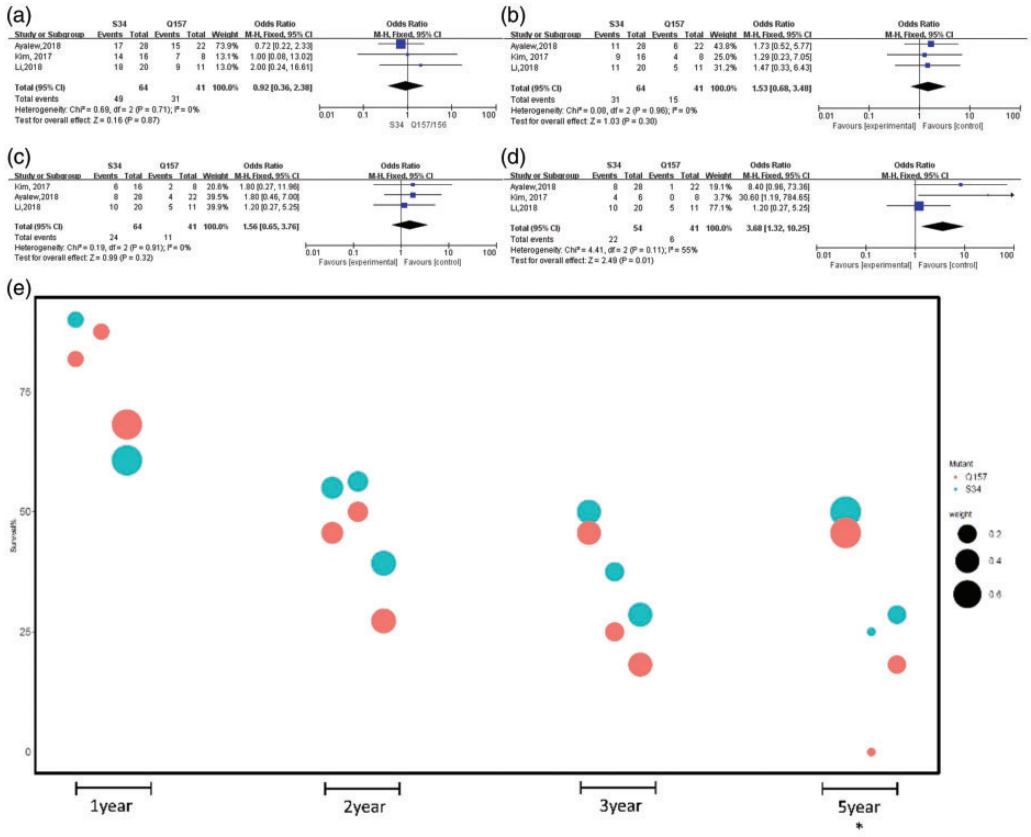

Figure 5. Summary OR of OS in MDS patients with U2AF1^{S34} and U2AF1^{Q157}. (a) 1 year OS. (b) 2 year OS. (c) 3 year OS. (d) 5 year OS. (e) OS tendency in patients with U2AF1^{S34} and U2AF1^{Q157}.

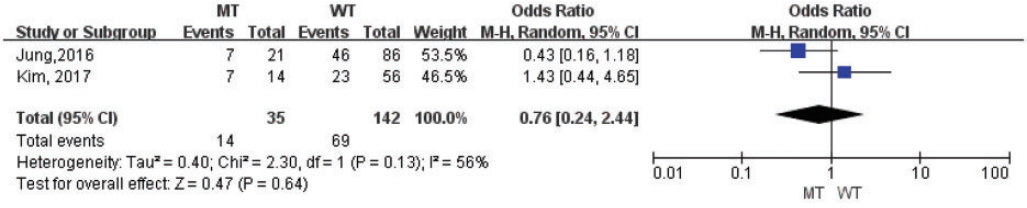

Figure 6. Summary OR for hypomethylating therapy response (HTR) in MDS patients with and without *U2AF1* mutations.

Discussion

Recently, with the development of next-generation sequencing, many more mutations have been identified as being associated with hematological disorders, which may affect disease progression.[28] Patients with *U2AF1* mutations were previously shown to have distinct clinical features, including a younger age, isolated +8 or 20q-, and *ASXL1* mutations[13,14,19]. However, the association between *U2AF1* mutations and MDS outcomes was controversial.

In our analysis, summary ORs for OS of 2, 3, and 5 years as well as the summary HR in all patients demonstrated significant differences in patients with and without *U2AF1* mutations, suggesting that the mutations could have an adverse survival impact. This is despite the association of *U2AF1* mutations with improved prognostic factors such as younger age.[13,14,19] We also found that patients carrying *U2AF1* mutations had a higher risk of abnormal karyotypes, which are indicative of a worse prognosis. Because U2AF1 is a splicing factor, its mutations can cause abnormal splicing and aberrant expression of different genes. Additionally, a previous *in vitro* experiment showed that *U2AF1* mutations affect cell cycle and apoptosis in HeLa cells,[11] indicating that they play an important role in cell proliferation. However, in our meta-analysis, there was no significant difference in LFS between patients with and without *U2AF1* mutations although this may reflect the limited number of cases.

$U2AF1^{S34}$ and $U2AF1^{Q157}$ have been reported as two major mutation types of *U2AF1*.[21] The $U2AF1^{S34}$ mutation tends to splice CAG rather than UAG 3′ splice site sequences,[29] while $U2AF1^{Q157}$ reinforces the preferential recognition of G instead of A at the +1 position.[30] In our meta-analysis, we compared the prognostic values of the two mutation variants, but only three of the included studies had performed an OS comparison of $U2AF1^{S34}$ and $U2AF1^{Q157}$. Nevertheless, we showed that patients with $U2AF1^{Q157}$ had a worse OS than those with $U2AF1^{S34}$ after 5 years, suggesting that mechanistic differences occur between $U2AF1^{S34}$ and $U2AF1^{Q157}$ that should be further studied.

Hypomethylating therapy such as azacitidine and decitabine is a major breakthrough in the treatment of MDS patients which could improve transfusion requirements and change the natural history of the disease.[31] Therefore, we analyzed the impact of *U2AF1* mutations on MDS outcomes under hypomethylating therapy in our meta-analysis. Although there were only two studies reporting the proportion of HTR, we found that the HTR of MDS was independent of the *U2AF1* mutation status, indicating that HTR could still improve the prognosis of MDS patients with *U2AF1* mutations. Further studies are needed to confirm this conclusion.

In our meta-analysis, the analysis of 3-year OS in all patients presented with a high heterogeneity because of a limited number of cases in the original study.[18] If we removed this study, the *P*-value of the Q test became 0.06 and I^2 became 53%, which we can regard as low heterogeneity. This enabled the analysis to be performed using fixed effects models, which gave an OR of 0.38 (95% CI: 0.22–0.66, $P< 0.001$). Similarly, the limited information about HTR also may have caused heterogeneity in the corresponding results.

Although this meta-analysis has a number of limitations, it nevertheless demonstrates that *U2AF1* mutations have a poor impact on survival in MDS, which to the best of our knowledge has not previously been reported. It is conceivable that a new prognostic scoring system for MDS will be developed to include *U2AF1* and other splicing factor gene mutations to replace the traditional risk ones such as IPSS, IPSS-R, and WPSS. However, in the meantime, more research is required to determine the prognostic values of mutations.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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