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*Original Article*

# Co-occurrence of RUNX1 and ASXL1 mutations underlie poor response and outcome for MDS patients treated with HMAs

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**Abstract:** The molecular determinants of the clinical response to Hypomethylating agents (HMAs) in patients with myelodysplastic syndromes (MDS) are unclear. We analyzed 84 adult patients with MDS who received hypomethylating agents (HMAs) and identified somatic mutations and their relationship to clinical response and survival. The results showed in the MDS patients with ASXL1 mutations,the most frequent co-occurring mutations were RUNX1 mutations, with a significant higher frequency of 43% compared to 17% in wild-type ASXL1 (P = 0.032). ASXL1 mutation demonstrated a significant negative overall response rate (8% vs. 29.4%, $x^2 = 5.228$, P = 0.022), particularly when co-occurring with RUNX1 mutations (P = 0.008). And all patients with RUNX1 and ASXL1 mutations died with a shorter median overall survival of only 14 months (P = 0.002). Moreover, TP53 mutations were associated with unfavorable-risk cytogenetic changes, and responded well to HMAs, with the exception of one case with RUNX1 and ASXL1 gene mutation. In a word, RUNX1 mutations are frequently found in MDS patients with ASXL1-mutations, and Co-occurrence of RUNX1 and ASXL1 mutations are associated with poor response to HMAs and inferior survival.

**Keywords:** Mutations, response, overall survival, myelodysplastic syndrome, hypomethylating agents

## Introduction

Myelodysplastic syndrome (MDS) is a highly heterogeneous disorder that shares the hallmark of variable cytopenia in the setting of dysplastic and cellular bone marrow [1]. Hypomethylating agents (HMAs), including decitabine (DAC) and azacitidine (AZA), are the only class of drugs approved for the treatment and can improve outcomes of patients with higher-risk MDS [2-8]. However, not all patients benefit from this type of therapy, as few as 10 to 15% of patients treated with these agents experience complete responses (CRs), and hematologic improvement (HI) occur in 40 to 50% of cases [9]. In addition, no reliable prognostic tool, including the International Prognostic Scoring System (IPSS), WHO classification-based Prognostic Scoring System (WPSS), or revised International Prognostic Scoring System (IPSS-R), could predict the differential likelihood of benefit from HMAs [10]. Outcomes are different even for patients with the same type of risk classification who receive HMAs. Moreover, four to six cycles are known to be required to achieve a response, and even a small fraction of patients have responded after greater than ten cycles of treatment. Therefore, selecting patients who are not likely to benefit from HMAs at baseline or shortly after the initiation of therapy has become a major clinical and research priority.

Recent advances in molecular technologies, such as epigenetic modification, transcription factors, RNA splicing, and signal transduction, have greatly expanded our understanding of the genetic landscape of MDS and provided insight into the pathogenesis of MDS treatment, natural history, and prognostication of clinical MDS [11], yet the roles of some mutations in patients with MDS remain unclear. In view of this information, we aimed to investigate the molecular, genetic, and clinical variables of patients newly diagnosed with MDS receiving hypomethylating agents (HMAs) and their effect on clinical outcome and treatment response rates. We report that ASXL1 muta-

**Table 1.** Baseline characteristics of the patients according to IWG response criteria

| | Total | Respond | Non-respond | P |
|---|---|---|---|---|
| N | 84 | 50 | 34 | |
| Treatment | | | | 0.96 |
| AZA | 20 | 12 | 8 | |
| DAC | 64 | 38 | 26 | |
| HMAs cycles | | 6 (2-23) | 4 (2-19) | 0.026 |
| Sex | | | | 0.610 |
| Male | 57 | 35 | 22 | |
| Female | 27 | 15 | 12 | |
| Age | | | | 0.153 |
| >65 | 19 | 14 | 5 | |
| ≤65 | 65 | 36 | 29 | |
| Disease status at diagnosis | | | | 0.99 |
| RAEB-I | 24 | 14 | 10 | |
| RAEB-II | 45 | 27 | 18 | |
| CMML | 15 | 9 | 6 | |
| Cytogenetics | | | | 0.955 |
| Good | 51 | 30 | 21 | |
| Intermedian | 15 | 9 | 6 | |
| Poor | 15 | 9 | 6 | |
| Failed | 3 | 0 | 3 | |

tions are frequently found in MDS patients with RUNX1-mutations, and co-occurrence of RUNX1 and ASXL1 mutations are associated with poor response to HMAs and inferior survival.

## Methods

*Patients, treatment, and response criteria*

According to FAB criteria, adults with a diagnosis of MDS were enrolled who were referred to Guangdong Provincial People's Hospital/Guangdong Academy of Medical Sciences/Guangdong Provincial Geriatrics Institute in this study. A total of 84 newly diagnosed patients with MDS received AZA or DAC (3-23 cycles, median number of cycles: 5) between November 2009 and November 2017.

Twenty patients were given the approved schedule for AZA (75 mg/m$^2$ for 7 days per cycle), and 64 patients received DAC (mostly 20 mg/m$^2$ for 5 days per cycle). All patients with MDS received AZA or DAC as a first-line treatment. Unless a patient died or experienced unacceptable adverse events, at least two courses of treatment were advised. To access the therapeutic outcome, bone marrow biopsy smear were examined every two courses, and response assessments were conducted according to IWG (International Working Group) response criteria for myelodysplasia. At the discretion of the treating physician, GSF (granulocyte stimulating factor), prophylactic antimicrobials and other supportive care were administered.

*Sample collected, DNA extracted and mutation analysis*

In this study, bone marrow (BM) and peripheral blood (PB) samples were collected before treatment with HMAs. Following red cell lysis, white blood cells were collected from samples and then total DNA was extracted. By using a next-generation sequencing approach, 13 genes (RUNX1, ASXL1, EZH2, TET2, IDH1, IDH2, JAK2, NRAS, TP53, DNMT3A, CBL, SRSF2 and SF3B1) were detected. Testing was confined to somatic mutations. The germline polymorphisms were excluded from analysis, including previously reported in population databases such as ExAC and dbSNP and identified in >20% of in-house patient population.

*Statistical analysis*

Statistical analysis was performed using SPSS version 22.0 for Windows. The Pearson Chi-square and Fisher's exact test were used to compare the different patients characteristics and gene mutations between responders and non-responders to HMAs. The method of Kaplan-Meier was was used to estimate survival time and statistical differences were analyzed by using log-rank analysis. Statistically significant was defined as *P* values less than or equal to 0.05.

## Results

*Patient characteristics*

We examined samples collected from 84 patients with MDS before treatment with HMAs, 64 patients received the approved schedule for DAC (mostly 20 mg/m$^2$ for 5 days per cycle), and 20 patients who received AZA (75 mg/m$^2$ for 7 days per cycle). Baseline patient characteristics are shown in **Table 1**. The median age of the 84 patients was 60 years (range: 19-79),

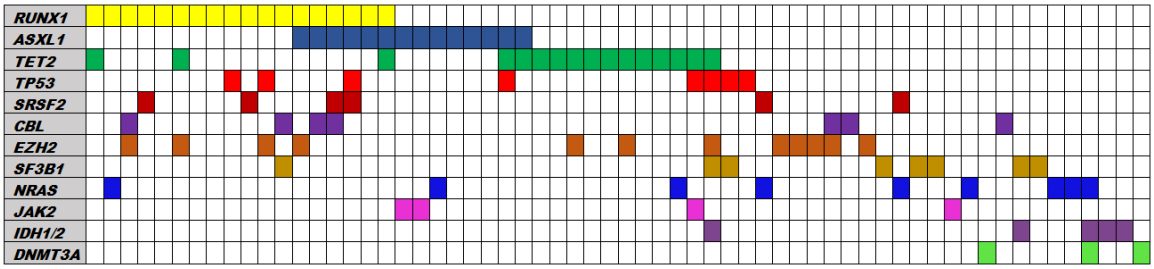



**Figure 1.** Spectrum of mutations in 84 patients in select MDS-associated genes.

**Table 2.** Clinical characteristics of MDS patients receiving HMAs according to RUNX1 mutation status

| | RUNX1$^{mut}$ (n = 18) | RUNX1$^{wt}$ (n = 66) | P |
|---|---|---|---|
| Age, year | 55 (36-65) | 61 (19-79) | 0.009 |
| >65 | 0 | 19 | |
| ≤65 | 18 | 47 | |
| Sex | | | 0.489 |
| Male | 11 | 46 | |
| Female | 7 | 20 | |
| Platelet count, × $10^9$/L | 53 (5-273) | 48 (6-608) | 0.676 |
| Disease status at diagnosis | | | 0.859 |
| RAEB-I | 5 | 19 | |
| RAEB-II | 9 | 36 | |
| CMML | 4 | 11 | |
| Cytogenetics | | | 0.394 |
| Good | 9 | 42 | |
| Intermedian | 4 | 11 | |
| Poor | 5 | 10 | |
| Failed | 0 | 3 | |

and the median number of cycles was 5 (range: 2-23). The WHO diagnoses were RAEB-I, RAEB-II, and CMML for 24, 45, and 15 cases, respectively. According to the International Prognosis Scoring System (IPSS), the cytogenetic risk was good for 51, intermediate for 15, and poor for 15 cases, respectively.

*Spectrum of gene mutations and pretreatment patient characteristics*

Frequently mutated regions in 13 genes were detected, including the most frequently transcription factors, mutated splicing factors, kinases and epigenetic regulators including RUNX1, ASXL1, EZH2, TET2, IDH1, IDH2, JAK2, NRAS, TP53, DNMT3A, CBL, SRSF2, and SF3B1. In total, 75% (63/84) of the patients had a mutation in at least one recurrently mutated gene. The most frequently mutated genes were RUNX1 (21%), TET2 (19%), ASXL1 (15%), EZH2 (14%), NRAS (11%), SF3B1 (10%), TP53 (10%), CBL (8%), and SRSF2 (7%) followed by IDH1/IDH2 (6%), JAK2 (5%) and DNMT3A (1%) (**Figure 1**). The frequency of mutations identified was largely similar to results from prior studies.

In the MDS patients with ASXL1 mutations, we found that the most frequent co-occurring mutations were RUNX1 mutations, with a significant higher frequency of 43% compared to 17% in wild-type ASXL1 (P = 0.032). And there was no other mutation positively associated with mutations in ASXL1. SRSF2 and TP53 mutations also both frequently occurred in 14% of patients with a ASXL1 mutation compared to 6% in wild-type ASXL1 (P = 0.57) and 8% in wild-type ASXL1 (P = 0.868). In addition, TET2 occurred at a frequency of 21% in ASXL1 mutants compared to 19% in wild-type ASXL1 (P = 1.0), consistent with SF3B1 mutations (P = 0.341). Moreover, we also analyzed a number of other gene mutations and showed varying associations with mutant RUNX1. Except for ASXL1, RUNX1 mutations were positively associated with mutations in SRSF2 (P = 0.022) and CBL (P = 0.054). **Table 2** summarizes the clinical variables evaluated with respect to the impact of the RUNX1 mutational status, and we found there was no significant difference between mutated RUNX1 MDS patients receiving HMAs and sex (P = 0.489), platelet count (P = 0.676), disease status (P = 0.859), or cytogenetics (P = 0.394). Interestingly, when comparing the patient groups ≤65 and 66-79 years old, we found that the younger cases had a higher frequency of RUNX1 mutations (28.8% vs. 0%, P = 0.009).

**Table 3.** Univariate analysis of the association between the most common gene mutations and overall response

| Mutated genes | OR (95% CI) | P |
|---|---|---|
| $ASXL1^{mt}$ VS. $ASXL1^{wt}$ | 0.209 (0.059, 0.736) | 0.022 |
| $RUNX1^{mt}$ VS. $RUNX1^{wt}$ | 0.561 (0.195, 1.612) | 0.280 |
| $TP53^{mt}$ VS. $TP53^{wt}$ | 5.535 (0.646, 47.186) | 0.176 |
| $SRSF2^{mt}$ VS. $SRSF2^{wt}$ | 0.66 (0.125, 3.48) | 0.951 |
| $EZH2^{mt}$ VS. $EZH2^{wt}$ | 0.636 (0.187, 2.17) | 0.468 |
| $TET2^{mt}$ VS. $TET2^{wt}$ | 0.452 (0.15, 1.364) | 0.153 |
| $RUNX1^{mt}$ with $ASXL1^{mt}$ VS. other | 2.786 (2.071, 3.747) | 0.008 |
| $RUNX1^{mt}$ with $ASXL1^{mt}$ VS. both WT | 2.762 (1.963, 3.887) | 0.01 |

Furthermore, TP53 mutations occurred in 10% of the patients and were associated with unfavorable risk cytogenetic changes (P = 0.044).

*Association of gene mutations and response to HMAs*

According to the IWG criteria revised in 2006, 50 patients responded for an overall response rate of 59.5%. This response rate demonstrated a significant and positive association with the number of HMA cycles (odds ratio [OR] = 2.817; 95% confidence interval [CI]: 1.118-7.097; P = 0.026), but there was no significant difference in response by sex (P = 0.61), age (P = 0.153), disease status (P = 0.99), cytogenetics (P = 0.955) or treatment regimen (P = 0.96).

Univariate analysis of the association between the most common gene mutations and overall response is shown in **Table 3**. In the cohort, the presence of ≥1 gene mutation tended to associated with overall response (OR: 0.366, 95% CI 0.12-1.123, P = 0.072). ASXL1 mutation alone demonstrated a significant and negative overall response rate (8% vs. 29.4%, $x^2$ = 5.228, P = 0.022), particularly when co-occurring with the RUNX1 gene (P = 0.008). Moreover, all of the patients with TP53 mutations responded well to HMAs, with the exception of one case that had co-occurring RUNX1 and ASXL1 mutations, and demonstrated only a trend toward an increased response rate compared with WT (P = 0.083). However, in this cohort, there was no relation between TET2 mutations and response to HMA treatment (P = 0.153), and the results remained unchanged when analysis was limited to TET2 mutations with wild-type ASXL1 cases (P = 0.285).

*Correlation between biomarkers and survival*

We explored the relationship between mutation status and overall survival in the subset of patients with available survival data. Of the 84 patients in our cohort, 52 died, and 2 cases were lost during follow-up. The median follow-up for patients was 39.5 (6-90) months.

In our cohort, neither ASXL1 nor RUNX1 mutation alone was associated zwith overall survival (15 months VS. 25 months, P = 0.327; 17 months vs. 25 months, P = 0.141), but patients with both a RUNX1 and a ASXL1 mutation died and had a shorter median overall survival of only 14 months (P = 0.002) (**Figure 2**). Furthermore, we observed the only gene significantly associated with OS in univariate analysis was EZH2 (14 months vs. 24 months, P = 0.028), and no correlation between overall survival and SRSF2 mutation (17 months vs. 24 months, P = 0.761) or CBL mutation (20 months vs. 25 months, P = 0.18) alone (**Figure 3**). In contrast, in the presence of mutant RUNX1, we found that there was no association between survival and co-occurrence of CBL or SRSF mutations.

## Discussion

Treatment approaches for patients with MDS have significantly improved in the post-epigenetic therapy era. Hypomethylating agents have improved transfusion requirements and quality of life while prolonging survival and decreasing leukemic transformation. Azacitidine and decitabine are the same classic agents currently available for the treatment of higher risk MDS. However, there is no useful prognostic tool for tailoring hypomethylation treatment and assessing azacitidine and decitabine responses. With the next generation sequencing, the analysis of detailed mutational patterns of patients with MDS may identify those who benefit from HMAs, guiding treatment and prognosis.

In our study, we were able to identify mutations in approximately three-quarters of the patients and identified 20% of patients with a mutated RUNX1, and RUNX1 mutations were positively associated with mutations in ASXL1, which is similar to that found in prior studies [11-13]. Biologically, ASXL1 loss is associated with increased self-renewal, hematopoietic transformation and a higher risk of secondary AML for patients with CMML [14]. Recent studies have reported a similar adverse impact of ASXL1 mutations in HMAs treatment responses in ch-

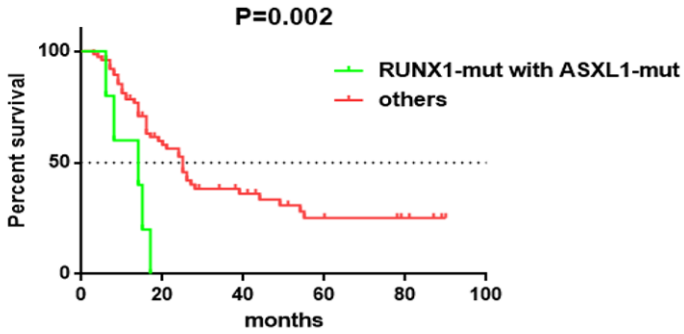
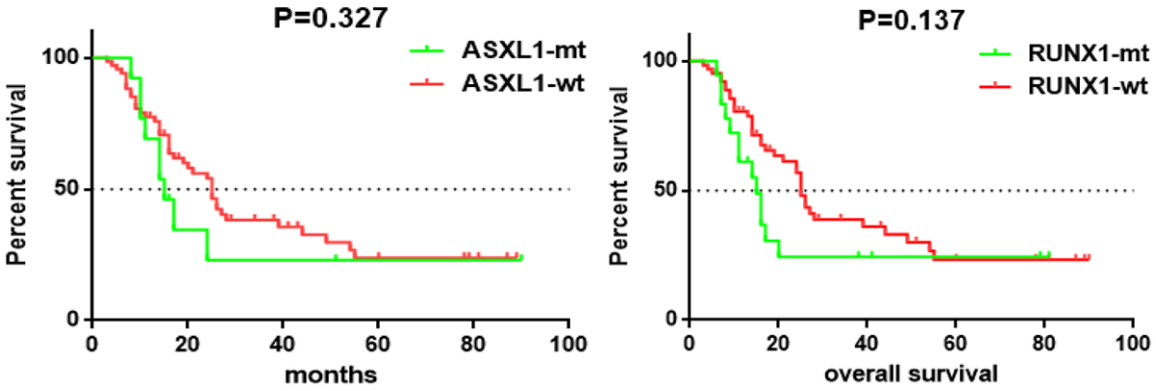



Figure 2. Kaplan-Meier curves for overall survival and ASXL1with or without RUNX1 mutation in the 84 patients.



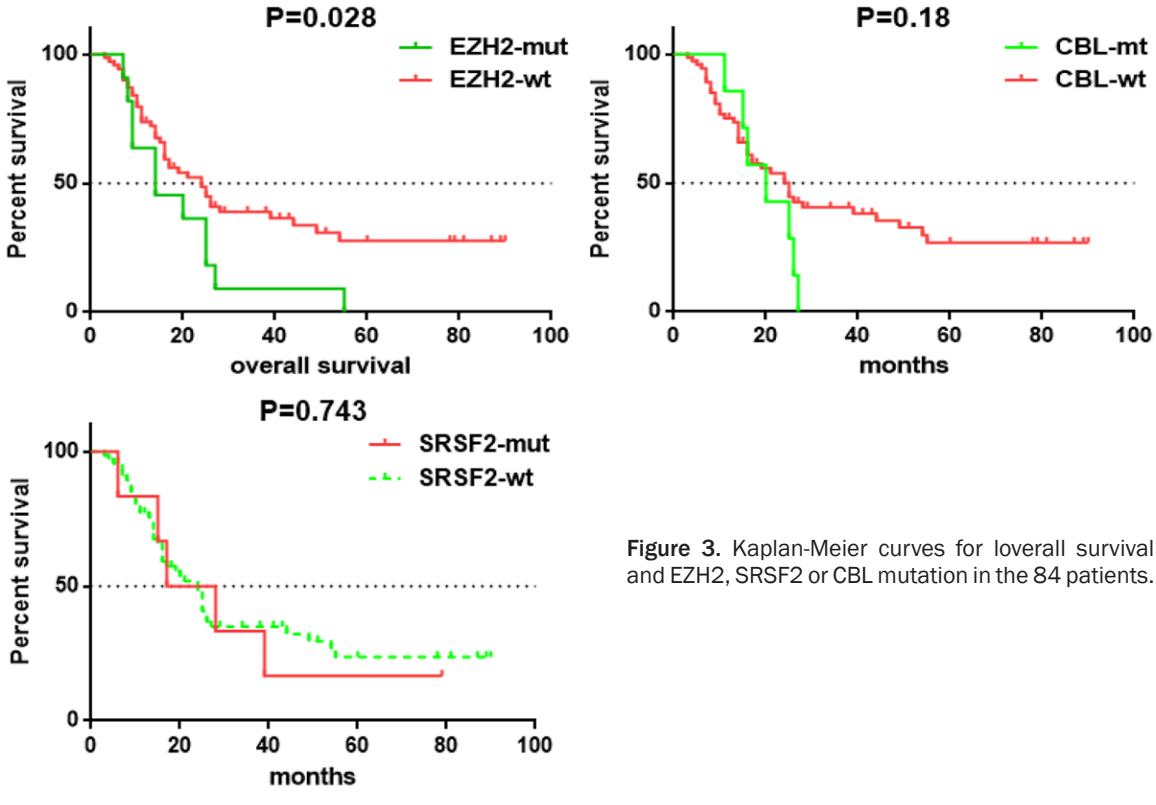




Figure 3. Kaplan-Meier curves for loverall survival and EZH2, SRSF2 or CBL mutation in the 84 patients.

ronic myelomonocytic leukemia [15] and MDS [16]. Of interest, we found ASXL1 mutations in patients with MDS that predict an inferior response to treatment with HMAs, particularly those with co-occurring RUNX1 mutations. The presence of both ASXL1 and RUNX1 mutations

is associated with a more significant poor response to HMAs and shorter survival. Differences in outcomes between patients may be related to the distribution of cytogenetic abnormalities and co-existing mutations that may mitigate or exacerbate mutational effects. The mechanism by why co-occurring of RUNX1 and ASXL1 mutations influence response to HMAs and OS is unclear, but prior studies have reported clonal expansion of ASXL1 mutant cells as a frequent event in age-related clonal hematopoiesis, and subsequent RUNX1 mutations may be involved in the malignant progression of these individuals [17]. Moreover, both ASXL1 and RUNX1 can increase BCL-2 expression in high-grade MDS [18]. And in high-risk MDS and secondary AML, an acquired resistance was associated with an increase in expression of the anti-apoptotic BCL-2 protein [19]. Recent studies have showed that the combination of hypomethylating agents and the Bcl-2 inhibitor venetoclax results in high rates of complete remission (CR) both in the first line and relapsed AML settings, suggesting synergy between these two agents [20]. Moreover, in elderly patients with AML, azacitidine or decitabine plus venetoclax demonstrated a favorable overall response rate and tolerable safety [21].

Similar to previous studies [22-26], in our cohort, the presence of TP53 mutations also strongly correlated with unfavorable karyotypes, and all patients with TP53 mutations responded well to HMAs with the exception of one case with RUNX1 and ASXL1 mutation. In addition, mutations in the epigenetic regulator gene EZH2 are frequently observed in patients with myelodysplastic disease and are associated with poor outcome. However, we found no association between TET2 mutations with or without ASXL1 and response to HMA treatment, which is similar to that found in previous studies [16].

With the technique of deep-sequencing, comprehensive molecular genetic profiling is a highly promising approach for being conducive to the diagnostic accuracy, biologic sub-classification, pathogenesis, risk stratification, and prognostication for patients with MDS. This approach may predict benefit from HMAs therapy, decreased costs, help avoid exposing patients with a low probability of benefit to ineffective treatment, and allow earlier consideration for clinical trials. Molecular profiling of multiple target genes can be integrated in individualized therapeutic decision making for patients with MDS in the near future.

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## Disclosure of conflict of interest

None.

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