# Mutations in the Spliceosomal Machinery Genes *SRSF2*, *U2AF1*, and *ZRSR2* and Response to Decitabine in Myelodysplastic Syndrome

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**Abstract.** *Background: Hypomethylating agents, such as azacitidine and decitabine, now constitute one of the mainstays of myelodysplastic syndrome (MDS) treatment. In recent years, novel recurrent mutations in multiple genes encoding RNA spliceosomal machinery (SRSF2, U2AF1, ZRSR2, SF3B1) were revealed. However, the clinical impact of these mutations on the outcomes of treatment of MDS patients with hypomethylating agents has not been described. Patients and Methods: A total of 58 de novo MDS patients were included in the study who had received first-line decitabine treatment. Polymerase chain reaction (PCR) followed by direct sequencing analyses was performed for the spliceosomal machinery genes including SRSF2, U2AF1 and ZRSR2. Results: In the present analysis of 58 Korean MDS patients, mutations in the splicing machinery genes SRSF2, U2AF1 and ZRSR2 were detected in 5 (8.6%), 10 (17.2%) and 6 (10.3%) patients, respectively, and the incidence of SRSF2 mutation was lower than those of previous series. The overall response rates (ORRs) including complete remission (CR), partial response (PR), and marrow CR (mCR) were 42.9% in the spliceosome wild-type (WT) group and 46.7% in the spliceosome-mutated group (p>0.999). The median OS was 22.0 months in the spliceosome-WT group and 15.9 months in the spliceosome-mutated group (p=0.267) Conclusion: This study firstly reports the impact of mutations of the spliceosomal machinery genes on the outcomes of decitabine treatment in MDS. The mutational status of the SRSF2, U2AF1 and ZRSR2 did not affect the response rate or survival in MDS patients who had received first-line decitabine treatment. Further studies are needed to confirm the prognostic relevance of spliceosome mutations to the clinical outcomes of treatment with hypomethylating agents.*

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*Key Words:* Myelodysplastic syndrome, *SRSF2, U2AF1, ZRSR2*, decitabine.

The myelodysplastic syndromes (MDSs) are a group of clonal disorders of the hematopoietic system characterized by ineffective hematopoiesis, peripheral cytopenia and an increased risk of acute myeloid leukemia (AML). Hematopoietic stem cell transplantation (HSCT) is the curative treatment modality but several issues regarding the use of HSCT to treat MDS remain unresolved, such as treatment-related mortality, graft-*versus*-host disease and the feasibility in the treatment of elderly and frail patients. Thus, hypomethylating agents, such as the DNA methyltransferase inhibitors azacitidine and decitabine now constitute one of the mainstays of MDS treatment. These drugs have favorable response rates and survival benefit in patients with MDS (1-6).

In recent years, the use of next-generation sequencing in patients with MDS has revealed novel recurrent mutations in multiple genes encoding the epigenetic machinery (*TET2, DNMT3A, ASXL1, EZH2, IDH1/2*) (7-12) and RNA spliceosomal machinery (*SRSF2, U2AF1, ZRSR2, SF3B1*) (13-17). Of note, the RNA-splicing process, whereby non-coding sequences called introns are removed from pre-mRNA, is crucial for gene expression and genetic diversity (18, 19). The detailed splicing consequences are complex and the exact mechanism to explain how somatic mutations in spliceosomal machinery genes can affect the pathogenesis of MDS has not been defined. A number of studies have tried to investigate the clinical impact of mutations in spliceosomal machinery genes in MDS but they failed to demonstrate a consistent prognostic relevance. For example, *SRSF2* and *U2AF1* mutations are associated with unfavorable clinical outcomes and a high risk of transformation to AML in some (14, 20) but not in all (21) studies. Some studies have suggested a positive prognostic impact of *SF3B1* mutations in MDS (17, 22), while other studies have reported no prognostic value of these mutations (23, 24). The clinical impact of these mutations on the outcomes of treatment of MDS patients with hypomethylating agents has not been described.

In the era of use of hypomethylating agents in the treatment of MDS, we aimed to investigate the prevalence and prognostic impact of mutations in the spliceosome machinery genes *SRSF2, U2AF1* and *ZRSR2* on the outcomes of first-line decitabine treatment in patients with MDS.

## Patients and Methods

*Patients*. Between June 2008 and December 2011, a total of 58 *de novo* MDS patients were included in the study who had received 1st-line decitabine treatment and had adequate genomic DNA from pre-treated bone marrow samples. The patients were diagnosed with MDS according to French-American-British (FAB) classification at the Samsung Medical Center. Among them, the 48 patients fulfilled the criteria of MDS according to World Health Organization (WHO) 2008 classification. Clinical information was obtained by reviewing the medical record of each patient. Reviewed clinical parameters were as follows: age, sex, complete blood count, bone marrow blast count, cytogenetics, International Prognostic Scoring System (IPSS) risk category, Revised International Prognostic Scoring System (IPSS-R) risk category, response to decitabine, leukemia-free survival (LFS) and overall survival (OS). All patients signed an informed consent form for sample collection. This study was approved by the Institutional Review Board of the Samsung Medical Center, Seoul, Korea, and the protocol protected the confidentiality of all patients.

*Gene mutation analysis*. Genomic DNA was extracted from bone marrow (BM) aspirate samples using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to manufacturer's recommendation. Polymerase chain reaction (PCR) followed by direct sequencing analyses was performed for the following genes including *SRSF2* (targeted, exon 1), *U2AF1* (targeted, exon 3 and exon 7), *ZRSR2* (all exons), *TET2* (targeted, exon 3~11), *TP53* (targeted, exon 2~11), *NRAS* (targeted, exon 2 and exon 3). Sequencing analyses were performed by the BigDye Terminator Cycle Sequencing Ready Reaction kit on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA). Mutations were detected by using the Sequencher program (Gene Codes Corp., Ann Arbor, MI, USA).

*Treatment and responses*. All patients received decitabine as 1st-line treatment according to 5-day dosing regimen approved by the US Food and Drug Administration (FDA) (20 mg/m$^2$ per day intravenously, for 5 days every 4 weeks). BM examination was performed before the first administration of decitabine and responses were evaluated every 2 or 3 cycles. Overall responses were defined according to the modified International Working Group (IWG) 2006 criteria for MDS (25), including complete remission (CR), partial response (PR), marrow CR (mCR) or stable disease (SD) with hematologic improvement (HI). Responders were to continue treatment until disease progression or unacceptable toxicity.

*Statistical analysis*. Descriptive statistics were reported as proportions and medians. Inter-group comparisons were performed with Fisher's exact test for categorical variables and the Mann-Whitney test for age. LFS was calculated from the first day of decitabine treatment to the day of diagnosis with leukemic transformation, death as a result of any cause or last date of follow-up. Overall survival (OS) was calculated from the first day of decitabine treatment to death or to the last date of follow-up. Survival curves were generated by Kaplan-Meier methods and survival was compared using the log-rank test. Univariate analysis was performed using Cox regression analysis. Statistical analyses were performed using the statistical software package IBM PASW, version 18.0 (SPSS Inc., Chicago, IL, USA).

## Results

*Patients' characteristics*. The baseline characteristics of the 58 patients are shown in Table I. The median age of the patients was 67 years (range=26-89) and the male:female ratio was 3.8:1.0. Forty-nine patients had MDS and 9 patients had chronic myelomonocytic leukemia (CMML). The International Prognostic Scoring System (IPSS) risk category (26) was low in 1 patient (1.7%), intermediate-1 in 33 patients (56.9%), intermediate-2 in 18 patients (31.0%) and high in 6 patients (10.3%). We categorized the patients into two groups. Patients having no spliceosomal machinery gene mutations were classified into the spliceosome wild-type (WT) group (n=38, 65.5%); patients having ≥1 mutation in the spliceosomal machinery genes *SRSF2, U2AF1* and *ZRSR2* were classified into the spliceosome-mutated group (n=20, 34.5%). In this small population of patients, the baseline clinical characteristics, demographics, cytogenetic risk group, IPSS score and IPSS-R risk score did not differ significantly between the two groups (Table I).

*Mutation status of spliceosomal machinery genes*. As shown in Table I, mutations in the splicing machinery genes *SRSF2, U2AF1* and *ZRSR2* were detected in 5 (8.6%), 10 (17.2%) and

Table I. *Baseline characteristics of study participants.*

| | Total (n=58) | | Spliceosome WT (n=38) | | Spliceosome Mutated (n=20) | | *p*-Value |
|---|---|---|---|---|---|---|---|
| Age, years | | | | | | | 0.777 |
| Median (Range) | 67 | (26-89) | 67 | (26-83) | 66 | (27-89) | |
| Gender, n(%) | | | | | | | 0.187 |
| Male | 46 | (79.3) | 28 | (73.7) | 18 | (90.0) | |
| Female | 12 | (20.7) | 10 | (26.3) | 2 | (10.0) | |
| Lab data, n(%) | | | | | | | |
| Neutropenia (ANC < 1800) | 41 | (70.7) | 26 | (68.4) | 15 | (75.0) | 0.764 |
| Anemia (Hb <10 g/dl) | 42 | (72.4) | 27 | (71.1) | 15 | (75.0) | >0.999 |
| Thrombocytopenia (PLT <100k) | 36 | (62.1) | 24 | (63.2) | 12 | (60.0) | >0.999 |
| FAB subtype, n(%) | | | | | | | 0.528 |
| RA/RARS | 20 | (34.5) | 15 | (39.5) | 5 | (25.0) | |
| RAEB/RAEBT | 29 | (50.0) | 18 | (47.4) | 11 | (55.0) | |
| CMML | 9 | (15.5) | 5 | (13.2) | 4 | (20.0) | |
| Karyotype risk, n(%) | | | | | | | 0.177 |
| Good (0) | 28 | (48.3) | 16 | (42.1) | 12 | (60.0) | |
| Intermediate | 16 | (27.6) | 10 | (26.3) | 6 | (30.0) | |
| Poor (1) | 14 | (24.1) | 12 | (31.6) | 2 | (3.4) | |
| IPSS risk, n(%) | | | | | | | 0.354 |
| Low | 1 | (1.7) | 1 | (2.6) | 0 | (0.0) | |
| Intermediate-1 | 33 | (56.9) | 23 | (60.5) | 10 | (50.0) | |
| Intermediate-2 | 18 | (31.0) | 9 | (23.7) | 9 | (45.0) | |
| High | 6 | (10.3) | 5 | (13.2) | 1 | (5.0) | |
| IPSS-R risk, n(%) | | | | | | | 0.962 |
| Very low | 1 | (1.7) | 1 | (2.6) | 0 | (0.0) | |
| Low | 10 | (17.2) | 7 | (18.4) | 3 | (15.0) | |
| Intermediate | 20 | (34.5) | 13 | (34.2) | 7 | (35.0) | |
| High | 12 | (20.7) | 7 | (18.4) | 5 | (25.0) | |
| Very high | 15 | (25.9) | 10 | (26.3) | 5 | (25.0) | |
| BM blast, n(%) | | | | | | | >0.999 |
| <5% | 27 | (46.6) | 19 | (50.0) | 8 | (40.0) | |
| 5-9% | 15 | (25.9) | 12 | (31.6) | 3 | (15.0) | |
| 10-19% | 16 | (27.6) | 7 | (18.4) | 9 | (45.0) | |
| Mutations, n(%) | | | | | | | |
| *SRSF2* | 5 | (8.6) | 0 | (0.0) | 5 | (25.0) | 0.003 |
| *U2AF1* | 10 | (17.2) | 0 | (0.0) | 10 | (50.0) | <0.001 |
| *ZRSR2* | 6 | (10.3) | 0 | (0.0) | 6 | (30.0) | 0.001 |
| *TET2* | 5 | (8.6) | 2 | (5.3) | 3 | (15.0) | 0.328 |
| *TP53* | 7 | (12.1) | 7 | (18.4) | 0 | (0.0) | 0.083 |
| *NRAS* | 3 | (5.2) | 3 | (7.9) | 0 | (0.0) | 0.544 |

WT; Wild type, ANC; absolute neutrophile count, Hb; hemoglobin, PLT; platelet, RA; refractory anemia, RARS; refractory anemia with ringed sideroblast, RAET; refractory anemia with excess blast, RAEBT; refractory anemia with excess blast in transformation, CMML; chronic myelomonocytic leukemia, FAB; French–American–British, IPSS; International Prognostic Scoring System, IPSS-R; Revised International Prognostic Scoring System, BM; bone marrow.

6 (10.3%) patients, respectively. The genomic changes and mutation patterns of 20 patients in the spliceosome-mutated group are summarized in Table II. There were 16 missense mutations, 3 frameshift mutations and 2 splicing mutations. We identified only 1 patient (patient #15) with concomitant *U2AF1* and *ZRSR2* mutations (Table II). Mutations in the *TET2, TP53* and *NRAS* genes were found in 5 (8.6%), 7 (12.1%) and 3 (5.2%) patients, respectively. *TP53* and *NRAS* mutations were found only in the spliceosome WT group (Table I).

*Impact of spliceosome mutations on the efficacy outcomes of decitabine*. The efficacy outcomes of decitabine according to the spliceosome mutations are summarized in Table III. The median number of cycles of decitabine treatment was 4 in both the spliceosome-WT and spliceosome-mutated groups. The overall response rates (ORRs) including CR, PR and mCR were 42.9% in the spliceosome WT group and 46.7% in the spliceosome-mutated group ($p>0.999$). The ORRs, including CR, PR, mCR and SD with hematological

Table II. *Characteristics of 20 patients with mutations in spliceosomal machinery genes.*

| | Gender/Age | Diagnosis | Karyotype | BM blast | Mutated gene | Genomic change | Mutation type |
|---|---|---|---|---|---|---|---|
| 1 | M/71 | RAEB | 46,XY | 7.29 | *SRSF2* | c.284C>G (p.Pro95Arg) | Missense mutation |
| 2 | M/64 | RAEB | 46,XY | 8.06 | *SRSF2* | c.284C>A (p.Pro95His) | Missense mutation |
| 3 | M/74 | RAEB | 46,XY | 8.2 | *SRSF2* | c.284C>A (p.Pro95His) | Missense mutation |
| 4 | M/64 | RAEB | 46,XY | 16.08 | *SRSF2* | c.284C>A (p.Pro95His) | Missense mutation |
| 5 | M/80 | RAEB | 46,XY | 14.87 | *SRSF2* | c.284C>A (p.Pro95His) | Missense mutation |
| 6 | M/65 | RAEB | 46,XY | 3.64 | *U2AF1* | c.470A>C (p.Gln157Pro) | Missense mutation |
| 7 | M/31 | RARS | 46,XY,i(21)(q10)[30] | 3.72 | *U2AF1* | c.101C>A (p.Ser34Tyr) | Missense mutation |
| 8 | M/27 | RARS | 47,XY,+8[6]/46,XY[14] | 0.84 | *U2AF1* | c.101C>T (p.Ser34Phe) | Missense mutation |
| 9 | M/57 | CMML | 46,XY,del(7)(q22)[9] | 2.13 | *U2AF1* | c.470A>G (p.Gln157Arg) | Missense mutation |
| 10 | M/68 | RAEB | 45,XY,-7[20] | 11.07 | *U2AF1* | c.470A>G (p.Gln157Arg) | Missense mutation |
| 11 | M/71 | CMML | 46,XX | 12 | *U2AF1* | c.101C>A (p.Ser34Tyr) | Missense mutation |
| 12 | M/89 | RA | 47,XY,+8[4]/46,XY[7] | 0.94 | *U2AF1* | c.101C>A (p.Ser34Tyr) | Missense mutation |
| 13 | M/67 | RAEB | 46,XX | 1.23 | *U2AF1* | c.101C>A (p.Ser34Tyr) | Missense mutation |
| 14 | M/28 | CMML | 47,XY,+8,i(20)(q10)[20] | 12 | *U2AF1* | c.101C>A (p.Ser34Tyr) | Missense mutation |
| 15 | M/62 | RA | 46,XY,der(1)?t(1;3)(p34.3;q21), dup(1)(p34.1p36.1)[20] | 1.85 | *U2AF1* | c.101C>T (p.Ser34Phe) | Missense mutation |
| | | | | | *ZRSR2* | c.1294G>C (p.Asp432His) | Missense mutation |
| 16 | M/67 | RAEB | 46,XY | 13.62 | *ZRSR2* | c.772-3T>G | Splicing mutation |
| 17 | M/72 | CMML | 46,XY | 0.94 | *ZRSR2* | c.325delG (p.Glu109Asnfs*56) | Frameshift mutation |
| 18 | M/73 | RAEB | 46,XY | 11.01 | *ZRSR2* | c.45-4C>A | Splicing mutation |
| 19 | M/53 | RAEB | 46,XY | 14 | *ZRSR2* | c.1343_1344insGAGCCG | Frameshift mutation |
| 20 | M/48 | RAEB | 46,XY,inv(3)(q21q26.2)[13]/46,XY[7] | 12 | *ZRSR2* | c.1207delA (p.Arg403Glyfs*114) | Frameshift mutation |

BM; Bone marrow, RA; refractory anemia, RARS; refractory anemia with ringed sideroblast, RAET; refractory anemia with excess blast, RAEBT; refractory anemia with excess blast in transformation, CMML; chronic myelomonocytic leukemia.

improvement (SD with HI), did not differ significantly between the groups (71.4% *vs*. 60.0%, $p$=0.507).

At a median follow-up of 40 months, 16 (27.6%) leukemic transformations and 49 (84.5%) deaths were documented. The median LFS and OS were 17.9 months and 18.8 months, respectively (Figure 1A). In patients classified according to their IPSS scores, patients with low and intermediate-1 risk had significantly better OS compared to patients with intermediate-2 and high risk ($p$=0.014) (Figure 1B). The median LFS did not significantly differ between the spliceosome-WT group and spliceosome-mutated group (20.9 months *vs*. 15.9 months, respectively; $p$=0.251). The median OS was 22.0 months in the spliceosome-WT group and 15.9 months in the spliceosome-mutated group ($p$=0.267) (Figure 1C). The 1-year expected OS rate did not differ significantly between the two groups (71.0% *vs*. 66.7%, $p$=0.929). No survival differences were observed in relation to the *SRSF2, U2AF1* and *ZRSR2* mutational status (Figure 1D, E, F). Sub-group analysis of patients with low, intermediate-1 and intermediate-2 risk (excluding patients with high risk) showed a trend of poor LFS and OS of spliceosome-mutated group compared to spliceosome-WT group but failed to show statistical significance (LFS=22.0 months *vs*. 15,9 months, $p$=0.20 and OS=15.9 months *vs*. 24.1 months, $p$=0.22) (Figure 2 A,B).

Table IV shows the results of univariate analysis of LFS and OS. IPSS risk (intermediate-2 or high) and IPSS-R risk (high or very high) were significant negative prognostic factors for LFS. The respective hazard ratios (HRs) were as follows: for LFS, HR=2.4 (95% confidence interval (CI)=1.3-4.4, $p$=0.016) and 2.1 (95% CI=1.1–3.9; $p$=0.026); for OS, HR=2.2 (95% CI=1.2-4.3; $p$=0.017) and HR=1.9 (95% CI=1.0-3.6; $p$=0.044). However, the spliceosomal mutations and respective mutational status (*SRSF2, U2AF1, ZRSR2, TET2, TP53* and *NRAS*) did not affect OS.

## Discussion

In this analysis of 58 Korean MDS patients, the incidence of *SRSF2* mutation (8.6%) was lower than those of previous series (12.4-14.6%) and the mutational status of the spliceosome genes *SRSF2, U2AF1* and *ZRSR2* did not affect the response rate or survival in MDS patients who had received first-line decitabine treatment (20, 21).

Decitabine (5-aza-2'-deoxycytidine) and azacitidine (5-azacytidine) allow for treatment of elderly and frail MDS patients, achieve hematological improvement and transfusion independency and have overall survival benefit (1-6). In 2006, the US Food and Drug Administration approved these hypomethylating agents for the treatment of all subtypes of MDS. Hypomethylating agents now constitute to offer an essential option in the treatment of MDS. Itzykson *et al*. demonstrated that previous treatment with low-dose cytosine

Table III. *Efficacy outcomes of decitabine according to spliceosome mutations.*

| | Total (n=58) | | Spliceosome WT (n=38) | | Spliceosome Mutated (n=20) | | *p*-Value |
|---|---|---|---|---|---|---|---|
| Response, n(%) (n=46)[#] | | | | | | | 0.549 |
| CR | 6 | (14.0) | 4 | (14.3) | 2 | (13.3) | |
| CRm | 10 | (23.3) | 5 | (17.9) | 5 | (33.3) | |
| PR | 3 | (7.0) | 3 | (10.7) | 0 | (0.0) | |
| SD with HI | 10 | (23.3) | 8 | (28.6) | 2 | (23.3) | |
| SD without HI | 8 | (18.6) | 4 | (14.3) | 4 | (26.7) | |
| Progression | 6 | (14.6) | 4 | (14.3) | 2 | (13.3) | |
| Overall response, n(%) (n=46)[#] | | | | | | | |
| CR, PR, CRm | 19 | (44.2) | 12 | (42.9) | 7 | (46.7) | >0.999 |
| CR, PR, CRm, SD with HI | 29 | (67.4) | 20 | (71.4) | 9 | (60.0) | 0.507 |
| Leukemic transformation, n(%) | 16 | (27.6) | 8 | (21.1) | 8 | (40.0) | 0.138 |
| Suvival, months (n=46) * | | | | | | | |
| LFS, median (95% CI) | 17.9 | (10.5-25.4) | 20.9 | (12.9-28.9) | 15.9 | (5.5-26.4) | 0.251 |
| OS, median (95% CI) | 18.8 | (10.9-26.7) | 22.0 | (12.8-31.3) | 15.9 | (3.4-28.4) | 0.267 |
| 1 year expected OS rate | 63.0% | | 71.0% | | 66.7% | | |
| Decitabine cycles, n(range) | 4 (1-25) | | 4 (1-25) | | 4 (1-17) | | |

WT; Wild type, CR; complete remission, CRm; marrow CR, PR; partial remission, SD; stable disease, HI; hematological improvement, LFS; leukemia-free survival, OS; overall survival. [#]12 patients who have not available clinical data for response evaluation were excluded. *12 patients who received salvage therapy of allogeneic stem cell transplantation were excluded.

Table IV. *Univariate analysis for leukemia-free survival and overall survival.*

| | Leukemia-free survival | | | Overall survival | | |
|---|---|---|---|---|---|---|
| Variables | HR | (95% CI) | *p*-Value | HR | (95% CI) | *p*-Value |
| Age > 60 | 1.3 | (0.5-3.0) | 0.605 | 1.5 | (0.6-3.6) | 0.352 |
| Male gender | 1.4 | (0.7-3.0) | 0.333 | 0.7 | (0.3-1.4) | 0.679 |
| IPSS risk (Intermediate-2, high) | 2.4 | (1.2-4.3) | 0.016 | 2.2 | (1.2-4.3) | 0.017 |
| IPSS-R risk (high, very high) | 2.1 | (1.1-3.9) | 0.026 | 1.9 | (1.0-3.6) | 0.044 |
| Any spliceosomal mutations (*SRSF2, U2AF1, ZRSR2*) | 1.5 | (0.8-2.8) | 0.254 | 1.4 | (0.8-2.7) | 0.270 |
| *SRSF2* mutation | 1.1 | (0.4-2.8) | 0.843 | 1.1 | (0.4-2.7) | 0.901 |
| *U2AF1* mutation | 1.4 | (0.6-3.2) | 0.418 | 1.4 | (0.6-3.1) | 0.454 |
| *ZRSR2* mutation | 1.1 | (0.4-3.2) | 0.821 | 1.1 | (0.4-3.1) | 0.872 |
| *TET2* mutation | 0.6 | (0.2-1.7) | 0.352 | 0.6 | (0.2-1.8) | 0.372 |
| *TP53* mutation | 1.4 | (0.6-3.2) | 0.431 | 1.6 | (0.7-3.6) | 0.268 |
| *NRAS* mutation | 1.6 | (0.4-6.5) | 0.549 | 1.6 | (0.4-6.7) | 0.536 |

HR; Hazard ratio, CI, confidence interval; IPSS; International Prognostic Scoring System, IPSS-R; Revised International Prognostic Scoring System.

arabinoside, bone marrow blast percentage >15%, circulating blasts, complex karyotype and red blood cell transfusion dependency were significant clinical parameters associated with lower response rates and/or worse OS in MDS patients treated with decitabine (27). Follo *et al*. suggested phosphoinositide-phospholipase C beta 1 hypomethylation as a favorable predictive factor to azacitidine treatment (28). In this era of novel mutations in MDS, the identification of subgroups who benefit most to hypomethylating agents is needed in terms of mutational status. However, there are few data on the molecular predictors of the response to hypomethylating agents in MDS patients.

Recently identified novel recurrent mutations in epigenetic machinery (*TET2, DNMT3A, ASXL1, EZH2, IDH1/2*) (7-12) and RNA spliceosomal machinery (*SRSF2, U2AF1, ZRSR2, SF3B1*) (13-17) have led to considerable progress in understanding the molecular mechanisms involved in the development of MDS. However, the precise impact of these mutations and their accuracy as molecular predictors of clinical outcomes have not been established fully and remain controversial (14, 17, 20-24). Clinical data on the impact of these mutations on the response to hypomethylating agents are also scarce. One French report, by Itzykson *et al*., showed a

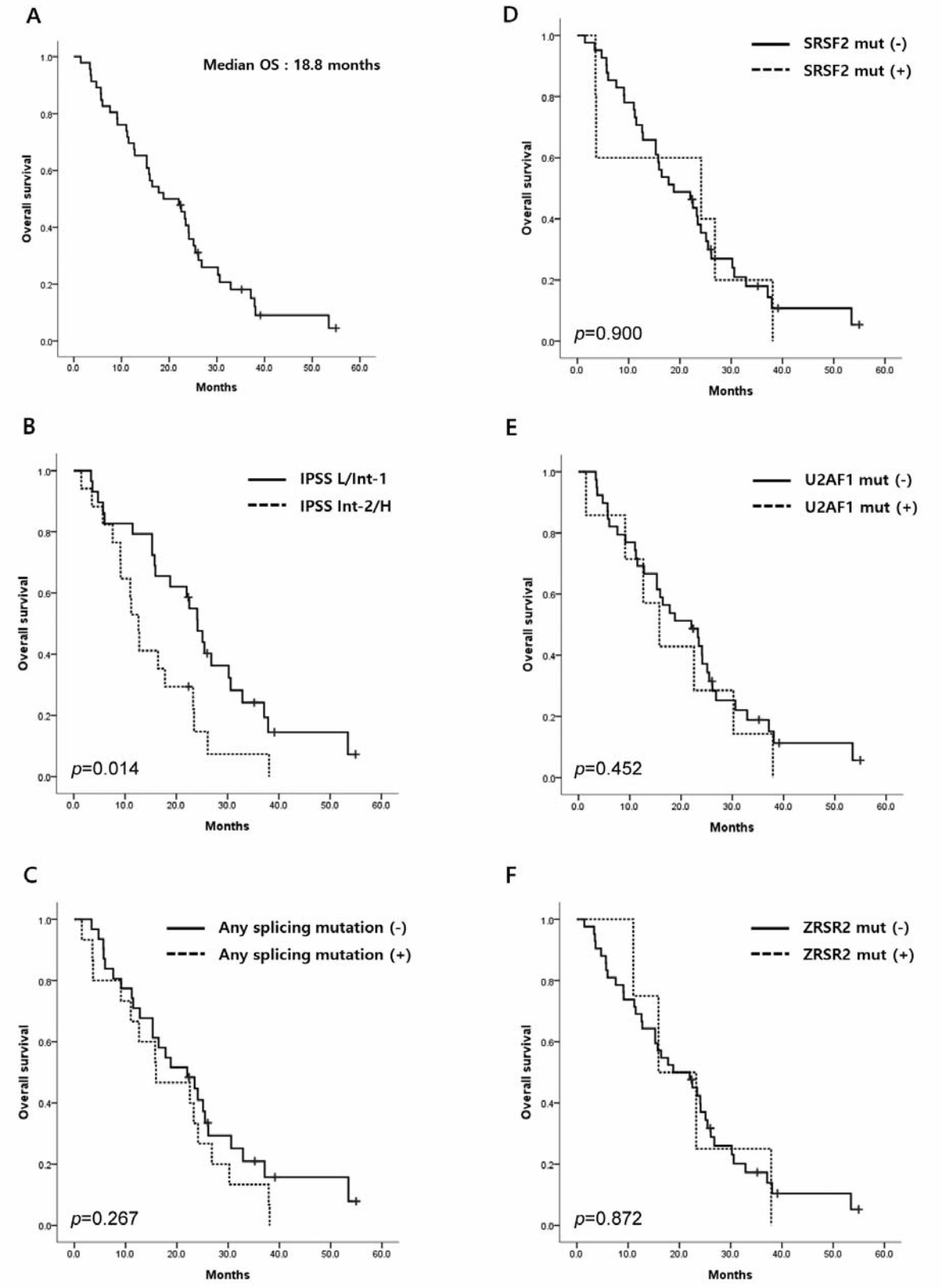


Figure 1. *Kaplan-Meier curves for overall survival (A) all patients, (B) IPSS risk, (C) any mutations of spliceosomal machinery, (D) SRSF2 mutation, (E) U2AF1 mutation, (F) ZRSR2 mutation.*

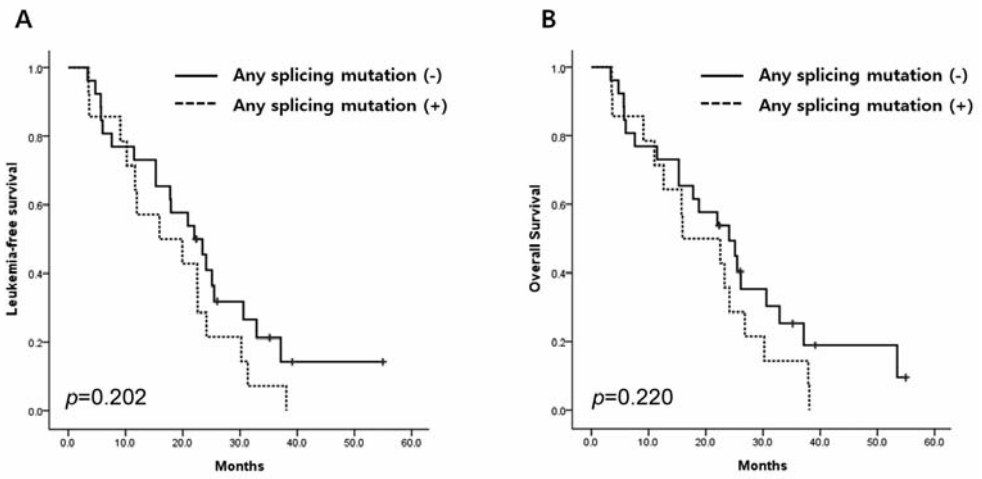


Figure 2. *Sub-group analysis of Kaplan-Meier curves in patients with low, intermediate-1, intermediate-2 IPSS risk according to any mutations of spliceosomal machinery. (A) Leukemia-free survival and (B) overall survival.*

correlation between the epigenetic machinery (*TET2*) mutation and poor clinical response to azacitidine, even though there was no impact on OS (29). The functional consequences of these mutations on spliceosomal machinery genes are not well defined. Some reports have suggested that these mutations result in increased or decreased RNA splicing, whereas others have suggested that these genes down-regulate key gene networks, including the core mitochondrial pathway (13, 16, 17). Recent reports notably suggest that mutations on spliceosomal machinery genes probably play a role in the MDS initiation but not disease progression and evolution to AML (21, 30, 31). Actually, among previous studies, that showed prognostic impact of *SRSF2* mutations in MDS, *SRSF2* mutation showed more prominent prognostic impact in patients with lower IPSS risk groups. (20, 21) In this study, we also performed a subgroup analysis excluding the patients with high IPSS risk group. The results showed a trend of poor LFS and OS of spliceosome-mutated group compared to spliceosome WT group but failed to show statistical significance as shown in Figure 2A and B. Considering previous findings and our results, we suggest that the prognostic impact of spliceosome mutations on the clinical outcomes of decitabine treatment needs to be investigated further in terms of MDS risk groups.

Previous studies on the prognostic impact of spliceosomal mutations in MDS have included heterogeneous populations in terms of treatment modality, such as the use of hypomethylating agents and previous treatment history. The clinical impact of spliceosomal machinery genes on the response to hypomethylating agents has not been explored systemically. Our findings are based on a relatively small number of patients and, therefore, need to be interpreted cautiously. Nevertheless, this study has several strengths. To our knowledge, this is the first study to evaluate the relationship between spliceosomal machinery gene mutations and response to hypomethylating agents in MDS patients. We included patients who had received decitabine treatment as the only first-line treatment. Thus, this study included a relatively homogeneous group of patients in terms of their MDS duration and previous treatment history.

In summary, this study demonstrated that the spliceosome mutations *SRSF2, U2AF1* and *ZRSR2* did not affect the clinical outcomes in response to decitabine treatment. In the era of the use of hypomethylating agents, prospective studies with larger populations are needed to confirm the prognostic relevance of spliceosome mutations to the clinical outcomes of treatment with hypomethylating agents, especially in terms of risk groups.

## Conflicts of Interest

The Authors declare no competing financial interests.

## Trial Registration

This study is registered with ClinicalTrials.gov, number NCT02060409.

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