# Clinical Implications of Copy Number Alteration Detection Using Panel-Based Next-Generation Sequencing Data in Myelodysplastic Syndrome

**Yoo-Jin Kim**
Department of Hematology, Seoul St. Mary's Hematology Hospital, College of Medicine, The Catholic University of Korea

**SeungHyun Jung**
Department of Biochemistry, College of Medicine, The Catholic University of Korea, Seoul, Korea

**Eun-Hye Hur**
Department of Hematology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

**Eun-Ji Choi**
Department of Hematology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

**Kyoo-Hyung Lee**
Department of Hematology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

**Hyeon-Chun Park**
Integrated Research Center for Genome Polymorphism, Precision Medicine Research Center, Department of Microbiology, College of Medicine, The Catholic University of Korea, Seoul, Korea

**Hye-Jung Kim**
Department of Hematology, Seoul St. Mary's Hematology Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea

**Yong-Rim Kwon**
Department of Hematology, Seoul St. Mary's Hematology Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea

**Silvia Park**
Department of Hematology, Seoul St. Mary's Hematology Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea

**Sug Hyung Lee**
Department of Pathology, College of Medicine, The Catholic University of Korea, Seoul, Korea

**Yeun-Jun Chung** ( yejun@catholic.ac.kr )
Integrated Research Center for Genome Polymorphism, Precision Medicine Research Center, Department of Microbiology, College of Medicine, The Catholic University of Korea, Seoul, Korea
https://orcid.org/0000-0002-6943-5948

**Je-Hwan Lee**
Department of Hematology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

**Research**

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# Abstract

**Background:** Recent advancements in next-generation sequencing (NGS) technologies allow the simultaneous identification of targeted copy number alterations (CNAs) as well as somatic mutations using the same panel-based NGS data. We investigated whether CNAs detected by the targeted NGS data provided additional clinical implications, over somatic mutations, in myelodysplastic syndrome (MDS).

**Methods:** Targeted deep sequencing of 28 well-known MDS-related genes was performed for 266 patients with MDS.

**Results:** Overall, 215 (80.8%) patients were found to have at least one somatic mutation; 67 (25.2%) had at least one CNA; 227 (85.3%) had either a somatic mutation or CNA; 160 had somatic mutations without CNA; and 12 had CNA without somatic mutations. Considering the clinical variables and somatic mutations alone, multivariate analysis demonstrated that sex, revised International Prognostic Scoring System (IPSS-R) and *NRAS* and *TP53* mutations were independent prognostic factors for overall survival. For AML-free survival, these factors were sex, IPSS-R, and mutations in *NRAS*, *DNMT3A*, and complex karyotype/ *TP53* mutations. When we consider clinical variables along with somatic mutations and CNAs, genetic alterations in *TET2*, *LAMB4*, *U2AF1*, and *CBL* showed additional significant impact on the survivals.

**Conclusions:** Our study suggests that the concurrent detection of somatic mutations and targeted CNAs may provide clinically useful information for the prognosis of MDS patients.


# Background

Myelodysplastic syndrome (MDS) is a heterogeneous group of clonal myeloid stem cell disorders, which are characterized by varying degrees of persistent cytopenias without other causes, presence of bone marrow dysplasia, and an increased risk of progression to acute myeloid leukemia (AML). Recurrent somatic mutations are found in about 90% of the MDS patients, and some of the mutations are known to have a prognostic value at various clinical stages of MDS, i.e., at diagnosis [1, 2] and before the initiation of hypomethylating therapy (HMT) [3–5] or allogeneic hematopoietic cell transplantation (HCT) [6–8]. These mutations include single-nucleotide variants (SNVs) and short insertions/deletions (indels). Copy number alterations (CNAs) represent a large fraction of human genetic variations, with many studies showing their prognostic value in MDS [9–11]. However, there are no specific guidelines for the assessment of CNAs, and the CNA test is not included in standard practice in the diagnosis and prognostication of MDS [12, 13]. Thus, there is a need to evaluate whether the detection of CNAs, in addition to genetic mutations, has clinical significance.

Targeted gene panel sequencing using next-generation sequencing (NGS) technology has rapidly become a routine clinical tool to detect somatic mutations in patients with MDS [9, 14–16]. Although microarray comparative genomic hybridization can detect CNAs with high accuracy, its application in a clinical setting has been limited due to high cost and the requirement of trained personnel for operation and data

interpretation. Recent advancements in NGS technologies have led to the development of more cost-effective and rapid methods which allow the simultaneous identification of targeted CNAs as well as somatic mutations using the same panel-based NGS data [17].

In this study, we have investigated the importance of CNAs, detected by targeted NGS, in the context of providing additional clinical implications over somatic mutations.

## Methods

# Patients

This study involved patients with MDS, including chronic myelomonocytic leukemia (CMML) at two Korean Institutes (Seoul St. Mary's Hematology Hospital, Seoul, Korea, and Asan Medical Center, Seoul, Korea). The bone marrow samples of all patients were available for molecular analysis. The patients in this study partly overlap with those in our two prior studies: one was about somatic mutations in MDS patients receiving HMT [5], and the other was about *TP53* mutations in HCT for *de novo* MDS [6]. We collected clinical and laboratory data at the time of diagnosis of MDS and calculated the revised International Prognostic Scoring System (IPSS-R) scores. This study was approved by the institutional review board at each institute. Each one of the patients gave written informed consent for molecular analysis.

# Targeted deep sequencing

We performed targeted deep sequencing as previously described [5, 6]. In brief, an MDS panel targeting 28 well-known MDS-related genes (*NRAS, DNMT3A, SF3B1, IDH1, TET2, NPM1, LAMB4, EZH2, JAK2, CBL, ETV6, KRAS, FLT3, IDH2, PRPF8, TP53, NF1, SRSF2, SETBP1, DNMT1, ASXL1, RUNX1, U2AF1, ZRSR2, ATRX, STAG2, MMP8*, and *ARID2*) was used for targeted deep sequencing. This MDS panel consisted of 1,088 amplicons covering 98.4% of all coding exons in 26 target genes. Sequencing libraries were generated using the AmpliSeq Library Kit 2.0 (Life Technologies, Carlsbad, CA, USA) and subsequently sequenced using the Ion Torrent Proton system (Life Technologies) according to the manufacturer's instructions. Sequencing reads were aligned to UCSC hg19 reference genome and genomic variant calling was performed using the Torrent Suite 5.2 (Thermo Fisher Scientific, Waltham, MA, USA). Stringent post-filtering processes were performed in order to achieve reliable and robust mutation calling. In the first step, we selected the functional variants (non-silent) in coding exons. The known polymorphic sites for East Asians (> 1% of minor allele frequency) in public databases (dbSNP137, ESP6500, and the 1000 genomes project) were filtered out as germline polymorphisms. The variants with > 1% minor allele frequency in our in-house normal database (comparison with 38 whole genome and 2,283 whole exome sequencing data from Koreans) were also filtered out. The remaining variants were considered as candidate somatic mutations.

# DNA copy number analysis

DNA copy number was estimated using the targeted deep sequencing data. The multiscale reference module and Rank Segmentation statistical algorithm in NEXUS software v9.0 (BioDiscovery, El Segundo, CA, USA) were used to define the CNAs for each sample, as previously described [18]. The CNA segments were classified as gains and losses when the $\log_2$ ratios were greater than 0.25 and less than − 0.25, respectively. All the identified CNA events were manually curated in terms of depth ratio.

# Statistical analysis

The purpose of this study was to determine the prognostic value of CNAs, in addition to the clinical implications of somatic mutations, using the same targeted NGS data. The endpoints included overall survival (OS) and AML-free survival (AFS). The survivals were calculated from the date of diagnosis of MDS to the date of death from any cause (OS) or to the date of AML progression (AFS).

We used the chi-square test to compare categorical variables and the Mann–Whitney U test or *t*-test to compare continuous variables. Survival was estimated using the Kaplan–Meier method, and the differences in survival were compared using the log-rank test. For each survival, multivariate analysis was performed using two Cox proportional hazards models. Essentially, one included clinical variables and genetic aberrations (somatic mutations only), while the other included clinical variables and genetic aberrations (both somatic mutations and CNAs). The data were analysed using SPSS software version 21 (IBM, Armonk, NY, USA).

## Results

# Patient characteristics

This study involved 266 patients, including 167 men and 99 women. For all patients, the genetic aberration results of both somatic mutations and targeted CNAs using the same panel-based NGS data were available. Table 1 describes the clinical and laboratory characteristics of patients at the time of MDS diagnosis and data for treatment. The median age of the patients was 50 years (range, 7–76). Six patients had therapy-related MDS. The IPSS-R risk category was very low or low in 49 (19.4%) patients, intermediate in 75 (29.6%), and high or very high in 129 (51.0%).

Table 1
Patient characteristics.

| Characteristic | | No. of patients (%) |
|---|---|---|
| Sex | | |
| Male | | 167 (62.8) |
| Female | | 99 (37.2) |
| Age, year | 50 (7–76) | |
| Median (range) | | |
| Age, year | | |
| < 50 | | 137 (51.5) |
| ≥ 50 | | 129 (48.5) |
| WHO type | | |
| MDS-(RS)-SLD/MDS U | | 26 (9.8) |
| MDS-(RS)-MLD | | 97 (36.5) |
| MDS-EB-1 | | 66 (24.8) |
| MDS-EB-2 | | 71 (26.7) |
| CMML | | 6 (2.3) |
| MDS presentation | | |
| De novo | | 244 (91.7) |
| Prior hematological disease | | 16 (6.0) |
| Therapy-related | | 6 (2.3) |
| Hemoglobin, g/dL (N/A in 5 patients) | | |
| ≥ 10 | | 53 (20.3) |
| 8-<10 | | 83 (31.8) |
| < 8 | | 125 (47.9) |

*Cytogenetic risk: Very good = -Y, del(11q); Good = normal, del(5q), del(12p), del(20q), double including del(5q); Intermediate = del(7q), + 8, +19, i(17q), any other single or double independent clones; Poor = -7, inv(3)/t(3q)/del(3q), double including − 7/del(7q), complex (3 abnormalities); Very poor = complex (> 3 abnormalities)

WHO, World Health Organization; MDS, myelodysplastic syndrome; RS, ring sideroblasts; SLD, single lineage dysplasia; U, unclassifiable; MLD, multilineage dysplasia; EB, excess blasts; CMML, chronic myelomonocytic leukemia; N/A, not available; ANC, absolute neutrophil count; BM, bone marrow; IPSS-R, Revised International Prognostic Scoring System

| Characteristic | No. of patients (%) |
|---|---|
| ANC, /μL (N/A in 8 patients) | |
| ≥ 800 | 153 (59.3) |
| < 800 | 105 (40.7) |
| Platelet count, /μL (N/A in 5 patients) | |
| ≥ 100,000 | 88 (33.7) |
| 50,000-<100,000 | 72 (27.6) |
| < 50,000 | 101 (38.7) |
| BM blast percentage (N/A in 5 patients) | |
| 0–2 | 83 (31.8%) |
| > 2-<5 | 45 (17.2) |
| 5–10 | 74 (28.4) |
| > 10 | 59 (22.6) |
| Cytogenetic risk* (N/A in 10 patients) | |
| Very good | 4 (1.6) |
| Good | 145 (56.6) |
| Intermediate | 62 (24.2) |
| Poor | 19 (7.4) |
| Very poor | 26 (10.2) |

*Cytogenetic risk: Very good = -Y, del(11q); Good = normal, del(5q), del(12p), del(20q), double including del(5q); Intermediate = del(7q), + 8, +19, i(17q), any other single or double independent clones; Poor = -7, inv(3)/t(3q)/del(3q), double including − 7/del(7q), complex (3 abnormalities); Very poor = complex (> 3 abnormalities)

WHO, World Health Organization; MDS, myelodysplastic syndrome; RS, ring sideroblasts; SLD, single lineage dysplasia; U, unclassifiable; MLD, multilineage dysplasia; EB, excess blasts; CMML, chronic myelomonocytic leukemia; N/A, not available; ANC, absolute neutrophil count; BM, bone marrow; IPSS-R, Revised International Prognostic Scoring System

| Characteristic | No. of patients (%) |
|---|---|
| IPSS-R risk category | |
| Very low | 6 (2.4) |
| Low | 43 (17.0) |
| Intermediate | 75 (29.6) |
| High | 78 (30.8) |
| Very high | 51 (20.2) |
| *Cytogenetic risk: Very good = -Y, del(11q); Good = normal, del(5q), del(12p), del(20q), double including del(5q); Intermediate = del(7q), + 8, +19, i(17q), any other single or double independent clones; Poor = -7, inv(3)/t(3q)/del(3q), double including − 7/del(7q), complex (3 abnormalities); Very poor = complex (> 3 abnormalities) | |
| WHO, World Health Organization; MDS, myelodysplastic syndrome; RS, ring sideroblasts; SLD, single lineage dysplasia; U, unclassifiable; MLD, multilineage dysplasia; EB, excess blasts; CMML, chronic myelomonocytic leukemia; N/A, not available; ANC, absolute neutrophil count; BM, bone marrow; IPSS-R, Revised International Prognostic Scoring System | |

# Treatment data and clinical outcomes

The treatment for the patients included allogeneic HCT in 234 (88.0%) patients, hypomethylating therapy in 166 (62.4%), cytotoxic chemotherapy in 19 (3.8%), immunosuppressive therapy in 8 (3.0%), and androgens in 11 (4.1%) patients (Table 2).

Table 2
Treatment data.

| Characteristic | No. of patients (%) |
|---|---|
| Allogeneic HCT | 234 (88.0) |
| Disease status at HCT | |
| MDS | 190 (81.2) |
| AML evolution | 44 (18.8) |
| Donor type for HCT | |
| HLA matched sibling | 86 (36.8) |
| HLA matched unrelated | 71 (30.3) |
| HLA mismatched unrelated | 12 (5.1) |
| HLA mismatched familial | 65 (27.8) |
| Conditioning therapy intensity for HCT | |
| Myeloablative | 81 (34.6) |
| Reduced-intensity | 153 (65.4) |
| Hypomethylating therapy | 166 (62.4) |
| Azacitidine | 95 (57.2) |
| Decitabine | 71 (42.8) |
| Response to hypomethylating therapy | |
| Complete response (CR) | 18 (10.9) |
| Partial response | 1 (0.6) |
| Marrow CR with HI | 12 (7.3) |
| Marrow CR without HI | 25 (15.2) |
| Stable disease with HI | 28 (17.0) |
| Stable disease without HI | 58 (35.2) |
| Progressive disease | 19 (11.5) |
| Not assessable | 4 (2.4) |
| Cytotoxic chemotherapy | 19 (3.8) |
| Immunosuppressive therapy | 8 (3.0) |

HCT, hematopoietic cell transplantation; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; HI, hematologic improvement

| Characteristic | No. of patients (%) |
|---|---|
| Androgen | 11 (4.1) |

HCT, hematopoietic cell transplantation; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; HI, hematologic improvement

The median follow-up duration of surviving patients was 6.9 years (range 1.03–27.21); 124 patients died, and 63 experienced AML evolution. The probabilities of OS and AFS at 5 years were 59.7% and 76.2%, respectively.

# General features of somatic mutations and copy number alterations

The median depth of coverage for targeted deep sequencing was 2215.5 x (range, 878–5118) across the target genome (Additional file 1: Table S1). Overall, 215 (80.8%) patients were found to carry at least one somatic mutation (Additional file 2: Table S2), while 67 (25.2%) had at least one CNA (Additional file 3: Table S3). Altogether, 227 (85.3%) had either somatic mutation or CNA, 160 (60.1%) had somatic mutations without CNA, and 12 (4.5%) had only CNA without somatic mutation (Fig. 1). The average number of mutated genes per patient was 1.9 (range, 0–6), and the average number of genes with CNAs per patient was 0.4 (range, 0–10). Eight genes were found to be mutated in more than 10% of the patients, i.e., *U2AF1* (n = 58 [21.8%]), *TET2* (n = 47 [17.7%]), *ASXL1* (n = 36 [13.5%]), *TP53* (n = 35 [13.2%]), *SETBP1* (n = 34 [12.8%]), *NF1* (n = 29 [10.9%]), *SF3B1* (n = 28 [10.5%]), and *RUNX1* (n = 28 [10.5%]). Five genes with CNAs were detected in 10 or more patients, i.e., *EZH2* (loss in 7q, n = 18 [6.8%]), *KRAS* (gain/loss in 12p, n = 14 [5.3%]), *ASXL1* (gain/loss in 20q, n = 12 [4.5%]), *LAMB4* (loss in 7q, n = 10 [3.8%]), and *RUNX1* (gain/loss in 21q, n = 10 [3.8%]). Somatic mutations were found in all 28 genes, whereas targeted CNAs were found in 18 (64.3%) of the 28 genes (Table 3). Interestingly, all five patients with *TP53* deletion exhibited *TP53* mutation as well, indicating a bi-allelic alteration.

Table 3
Frequency of genetic aberrations: somatic mutations and copy number alterations (CNAs).

| Gene | No. of patients with somatic mutations (%) | No. of patients with CNAs [Gain/Loss] (%) | No. of patients with somatic mutations or CNAs (%) |
|---|---|---|---|
| *NRAS* | 13 (4.9) | 2 [2/0] (0.8) | 15 (5.6) |
| *DNMT3A* | 24 (9.0) | - | 24 (9.0) |
| *SF3B1* | 28 (10.5) | - | 28 (10.5) |
| *IDH1* | 8 (3.0) | - | 8 (3.0) |
| *TET2* | 47 (17.7) | 8 [4/4] (3.0) | 54 (20.3) |
| *NPM1* | 3 (1.1) | 2 [0/2] (0.8) | 5 (1.9) |
| *LAMB4* | 17 (6.4) | 10 [0/10] (3.8) | 26 (9.8) |
| *EZH2* | 13 (4.9) | 18 [0/18] (6.8) | 30 (11.3) |
| *JAK2* | 9 (3.4) | 5 [2/3] (1.9) | 14 (5.3) |
| *CBL* | 11 (4.1) | 6 [6/0] (2.3) | 17 (6.4) |
| *ETV6* | 22 (8.3) | 2 [0/2] (0.8) | 24 (9.0) |
| *KRAS* | 13 (4.9) | 14 [6/8] (5.3) | 27 (10.2) |
| *FLT3* | 3 (1.1) | - | 3 (1.1) |
| *IDH2* | 3 (1.1) | 9 [8/1] (3.4) | 12 (4.5) |
| *PRPF8* | 9 (3.4) | 3 [0/3] (1.1) | 12 (4.5) |
| *TP53* | 35 (13.2) | 5 [0/5] (1.9) | 35 (13.2) |
| *NF1* | 29 (10.9) | - | 29 (10.9) |
| *SRSF2* | 6 (2.3) | - | 6 (2.3) |
| *SETBP1* | 34 (12.8) | 1 [1/0] (0.4) | 35 (13.2) |
| *DNMT1* | 4 (1.5) | 3 [3/0] (1.1) | 7 (2.6) |
| *ASXL1* | 36 (13.5) | 12 [6/6] (4.5) | 47 (17.7) |
| *RUNX1* | 28 (10.5) | 10 [6/4] (3.8) | 36 (13.6) |
| *U2AF1* | 58 (21.8) | 7 [4/3] (2.6) | 64 (24.1) |
| *ZRSR2* | 7 (2.6) | 1 [0/1] (0.4) | 8 (3.0) |
| *ATRX* | 7 (2.6) | - | 7 (2.6) |
| *STAG2* | 15 (5.6) | - | 15 (5.6) |

| Gene | No. of patients with somatic mutations (%) | No. of patients with CNAs [Gain/Loss] (%) | No. of patients with somatic mutations or CNAs (%) |
|---|---|---|---|
| *MMP8* | 4 (1.5) | - | 4 (1.5) |
| *ARID2* | 7 (2.6) | - | 7 (2.6) |

# Prognostic factor analysis

The number of genes with somatic mutations per patient did not significantly affect the outcomes of the patients (data not shown). In contrast, a higher number of genes with CNAs per patient showed a significant association with inferior OS ($P < 0.001$) and AFS ($P = 0.007$) (Fig. 2).

We performed a univariate analysis for the prognostic values of clinical variables and genetic aberrations (Additional file 1: Table S4 and Additional file 1: Table S5). Among the clinical variables, sex (OS), age (OS), and IPSS-R (OS and AFS) were significant factors for clinical outcomes. Considering only the somatic mutations, *NRAS* (OS and AFS), *DNMT3A* (AFS), *TET2* (OS), *LAMB4* (AFS), *PRPF8* (OS), *TP53* (OS), *DNMT1* (OS), and *MMP8* (OS) mutations were significant prognostic factors. Taking into account both somatic mutations and CNAs, the genetic aberrations of *NRAS* (OS and AFS), *DNMT3A* (AFS), *NPM1* (OS), *LAMB4* (OS and AFS), *EZH2* (OS), *CBL* (OS and AFS), *TP53* (OS), and *MMP8* (OS) were found to be significant prognostic factors. In addition, the combination of complex karyotype and *TP53* aberrations showed a significant association with AFS (Additional file 1: Table S5).

Upon taking into consideration only the clinical variables and somatic mutations, our multivariate analysis demonstrated that the independent prognostic factors for OS were sex, IPSS-R, *NRAS* mutation, and *TP53* mutation, and those for AFS were sex, IPSS-R, *NRAS* mutation, *DNMT3A* mutation, and complex karyotype/ *TP53* mutation. When clinical variables and somatic mutations as well as CNAs were taken into account, genetic alterations of *TET2*, *LAMB4*, *U2AF1*, and *CBL* were seen to have an additional significant impact on OS or AFS (Table 4).

Table 4
Multivariate analysis for overall and acute myeloid leukemia-free survivals.

| *Overall survival* | | | | | | |
|---|---|---|---|---|---|---|
| | **Somatic mutations only** | | | **Somatic mutations + CNAs** | | |
| Variable | HR | 95% CI | *P*-value | HR | 95% CI | *P*-value |
| Sex | | | | | | |
| Female | 1 | | | 1 | | |
| Male | 1.722 | 1.145–2.589 | 0.009 | 1.558 | 1.030–2.358 | 0.036 |
| Age, year | | | | | | |
| < 50 | 1 | | | 1 | | |
| ≥ 50 | 1.715 | 1.178–2.498 | 0.005 | 1.649 | 1.114–2.442 | 0.012 |
| IPSS-R | | | | | | |
| Lower-risk | 1 | | | 1 | | |
| Higher-risk | 3.763 | 1.973–7.178 | < 0.001 | 1.647 | 1.115–2.434 | 0.012 |
| *NRAS* | | | | | | |
| Wild type | 1 | | | 1 | | |
| Mutant type | 2.408 | 1.505–3.852 | < 0.001 | 1.900 | 0.986–3.663 | 0.055 |
| *TP53* | | | | | | |
| Wild type | 1 | | | 1 | | |
| Mutant type | 1.722 | 1.145–2.589 | 0.009 | 2.211 | 1.346–3.633 | 0.002 |
| *TET2* | | | | | | |
| Wild type | | | | 1 | | |
| Mutant type | | | | 1.544 | 1.008–2.365 | 0.046 |
| *LAMB4* | | | | | | |
| Wild type | | | | 1 | | |
| Mutant type | | | | 1.758 | 0.999–3.094 | 0.051 |

CNAs, copy number alterations; HR, hazards ratio; CI, confidence interval; IPSS-R, Revised International Prognostic Scoring System (Lower-risk if score ≤ 3.5, Higher-risk if score > 3.5); AML, acute myeloid leukemia; CK, complex karyotype (three or more chromosomal abnormalities); WT, wild type; MT, mutant type

| *Overall survival* | | | | | | |
|---|---|---|---|---|---|---|
| *U2AF1* | | | | | | |
| Wild type | | | | 1 | | |
| Mutant type | | | | 1.536 | 1.002–2.354 | 0.049 |
| *CBL* | | | | | | |
| Wild type | | | | 1 | | |
| Mutant type | | | | 2.110 | 1.059–4.203 | 0.034 |
| AML-free survival | | | | | | |
| | **Somatic mutations only** | | | **Somatic mutations + CNAs** | | |
| Variable | HR | 95% CI | *P*-value | HR | 95% CI | *P*-value |
| Sex | | | | | | |
| Female | 1 | | | 1 | | |
| Male | 1.858 | 1.032–3.346 | 0.039 | 1.856 | 1.029–3.350 | 0.040 |
| IPSS-R | | | | | | |
| Lower-risk | 1 | | | 1 | | |
| Higher-risk | 2.056 | 1.133–3.729 | 0.018 | 2.347 | 1.338–4.118 | 0.003 |
| *NRAS* | | | | | | |
| Wild type | 1 | | | 1 | | |
| Mutant type | 3.891 | 1.685–8.987 | 0.001 | 4.452 | 2.115–9.372 | < 0.001 |
| *DNMT3A* | | | | | | |
| Wild type | 1 | | | 1 | | |
| Mutant type | 3.714 | 1.889–7.303 | < 0.001 | 3.614 | 1.893–6.897 | < 0.001 |
| CK/ *TP53* | | | 0.094 | | | |
| CK-/WT | 1 | | | | | |
| CK-/MT | 0.277 | 0.038–2.018 | 0.205 | | | |
| CK+/WT | 1.333 | 0.510–3.479 | 0.558 | | | |
| CK+/MT | 2.538 | 1.062–6.065 | 0.036 | | | |

CNAs, copy number alterations; HR, hazards ratio; CI, confidence interval; IPSS-R, Revised International Prognostic Scoring System (Lower-risk if score ≤ 3.5, Higher-risk if score > 3.5); AML, acute myeloid leukemia; CK, complex karyotype (three or more chromosomal abnormalities); WT, wild type; MT, mutant type

| *Overall survival* | | | |
|---|---|---|---|
| *LAMB4* | | | |
| Wild type | 1 | | |
| Mutant type | 2.797 | 1.349–5.801 | 0.006 |
| *CBL* | | | |
| Wild type | 1 | | |
| Mutant type | 2.438 | 1.017–5.845 | 0.046 |

CNAs, copy number alterations; HR, hazards ratio; CI, confidence interval; IPSS-R, Revised International Prognostic Scoring System (Lower-risk if score ≤ 3.5, Higher-risk if score > 3.5); AML, acute myeloid leukemia; CK, complex karyotype (three or more chromosomal abnormalities); WT, wild type; MT, mutant type

# Discussion

CNAs are the most abundant form of genetic alteration in cancer; however, due to the difficulties associated with their identification, only a limited number of CNAs can be used in a clinical setting. Targeted cancer-related gene panel NGS has become a routine clinical tool in recent times and can be used for the detection of CNAs, in addition to the detection of somatic mutations. One typical example is the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) study, which enables precision oncology in patients with solid tumors [19]. Prospective clinical sequencing of 10,000 patients with advanced solid cancer using the MSK-IMPACT cancer panel revealed the presence of highly frequent genes with CNAs, such as *PIK3CA*, *EGFR*, and *KIT*, in addition to frequently mutated genes such as *TP53* and *KRAS* [20]. It has also been reported that the concurrent detection of CNAs and mutations using targeted cancer-related gene panel is possible in myeloid malignancy [21]. The authors analyzed the mutations and CNAs in 270 patients with myeloid malignancy (MDS, MDS/MPN, MPN, and AML) using a targeted cancer-related gene panel and identified the presence of targeted CNAs in 68 patients [21]. However, the clinical and prognostic implications of CNAs from the target gene panel analysis in MDS have not been well-studied. In this study, our focus was to investigate whether the detection of CNAs defined from the targeted NGS data provided an additional prognostic value over somatic mutations, and also to explore whether a synergistic effect is observed on combining the somatic mutation and CNA data. Indeed, on taking into account both somatic mutations and CNAs, more genetic aberrations were found to be significantly associated with the prognosis of MDS, and the significance level became more reliable than it was on considering somatic mutations alone.

The identification of prognostic genetic factors and their integration into the clinical data is an essential process for the prediction of survival and disease progression of MDS. Karyotype has been the gold standard of MDS genetic data, and is a crucial component of the IPSS and the revised IPSS [22, 23]. Conventional karyotype analysis can detect chromosome abnormalities in only half of the *de novo* MDS patients. Thus, several studies have attempted to utilize molecular genetic data such as somatic

mutations and CNAs to improve the precision of MDS prognostication and to aid the identification of therapeutic targets. In this study, we simultaneously detected somatic mutations and CNAs using the same targeted NGS data, which led to the identification of further genetic aberrations in 17 genes, compared to those detected upon using somatic mutations alone (Table III). Somatic mutations of two genes, *TP53* and *NRAS*, had significant prognostic impacts on the survival of our MDS patients, as previously reported [1, 6, 7, 24, 25]. All five patients with *TP53* CNAs also harboured *TP53* mutations, and only two patients had *NRAS* CNAs without *NRAS* mutations. In the case of these two genes, the detection of CNAs did not prove to be of additional clinical significance. In comparison, genetic aberrations of four genes, including *TET2*, *LAMB4*, *U2AF1*, and *CBL*, showed a significant correlation with survival only after a combined analysis of both somatic mutations and CNAs, but not by the analysis of somatic mutations alone. We found eight *TET2* CNAs (loss and gain in four each). The adverse prognostic impacts of copy number loss [11] or somatic mutations [24, 26] of *TET2* have been previously reported, but none of reports have demonstrated the clinical significance of copy number gain of *TET2*. When we analysed the prognostic impacts of each combination of copy number loss or copy number gain with somatic mutations, each of these was associated with poor OS ($P = 0.039$ for loss and $P = 0.037$ for gain, data not shown). However, this kind of analysis does not seem to be appropriate given the small size. *CBL* mutations are known to contribute to myeloid leukemogenesis through a gain-of-function phenotype of *CBL* mutant [27]. The mutations recurrently occur in various myeloid neoplasms, including MDS and secondary AML [28]. In this study, copy number gain of *CBL* was found in four patients without *CBL* mutations, and the analysis of combined CNAs and somatic mutations of *CBL* showed a significant adverse impact of *CBL* aberrations on OS and AFS. *U2AF1* mutations result in altered pre-mRNA splicing and may contribute to MDS pathogenesis [29]. Some studies have shown inferior survival in MDS patients with *U2AF1* mutations [30, 31]. In this study, seven patients harboured *U2AF1* CNAs (loss in 3 and gain in 4), and *U2AF1* aberrations were associated with inferior OS. *LAMB4* mutations are recurrently found in MDS patients, but little is known about their prognostic implications and contribution to MDS pathogenesis [2]. In this study, 17 patients harbored *LAMB4* mutations, and detection of CNAs resulted in 9 more patients being identified with *LAMB4* aberrations. The results suggest that concurrent detection of targeted CNAs and mutations is feasible [21] and may improve the prognostic implications of genetic aberrations in several MDS-related genes through detection of more aberrations.

There are several issues which should be considered while interpreting our results. Firstly, we suggest that the prognostic implications of CNAs identified in this study on survival should be confined to MDS patients receiving active treatment, as most of the patients in this study received chemotherapy and/or allogeneic hematopoietic stem cell transplantation. The timing of collection of bone marrow samples should also be taken into consideration, with some of them being acquired after hypomethylating treatment, though none after transplantation. This should be done in order to account for the clonal dynamics of genetic aberrations during the course of MDS [32–34]. Our targeted cancer-related gene panel was designed to target 28 genes based on PCR amplification, hence, technically it is possible to detect only the gene level CNAs. An NGS panel with more cancer-related genes and backbone targets may identify the large structural alterations and chromosomal rearrangement.

# Conclusions

In this study, we identified the independent prognostic factors for OS (sex, IPSS-R, and mutations in *NRAS* and *TP53*) and AML-free survival (sex, IPSS-R and mutations in *NRAS*, *DNMT3A*, and complex karyotype/ *TP53* mutations). When we consider clinical variables along with somatic mutations and CNAs, genetic alterations in *TET2*, *LAMB4*, *U2AF1*, and *CBL* showed additional significant impact on the survivals. Our study suggests that the concurrent detection of somatic mutations and targeted CNAs using the same panel-based NGS data may provide clinically useful information for the prognosis of MDS patients.

# Abbreviations

NGS: next-generation sequencing

CNAs: copy number alterations

MDS: myelodysplastic syndrome

IPSS-R: revised International Prognostic Scoring System

AML: acute myeloid leukemia

HMT: hypomethylating therapy

HCT: allogeneic hematopoietic cell transplantation

SNVs: single-nucleotide variants

Indels: short insertions/deletions

CMML: chronic myelomonocytic leukemia

OS: overall survival

AFS: AML-free survival

# Declarations

### Ethics approval and consent to participate

This study was approved by the institutional review board at Seoul St. Mary's Hospital and Asan Medical Center. Each one of the patients gave written informed consent for molecular analysis.

### Consent for publication

Not applicable.

### Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

### Competing interests

The authors declare no competing interests relevant to this article.


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### Authors' contributions

Lee JH, Chung, YJ, Kim YJ, and Jung SH designed the research study, performed the study, analyzed the data and wrote the first draft of the manuscript; Hur EH, Choi EJ, Lee KH, Park S, and Lee SH provided clinical data and critically revised the manuscript; Park HC, Kim HJ, and Kwon YR performed laboratory studies and contributed to the analysis of the data. All authors participated in reviewing the manuscript drafts and approved the final version of the manuscript.


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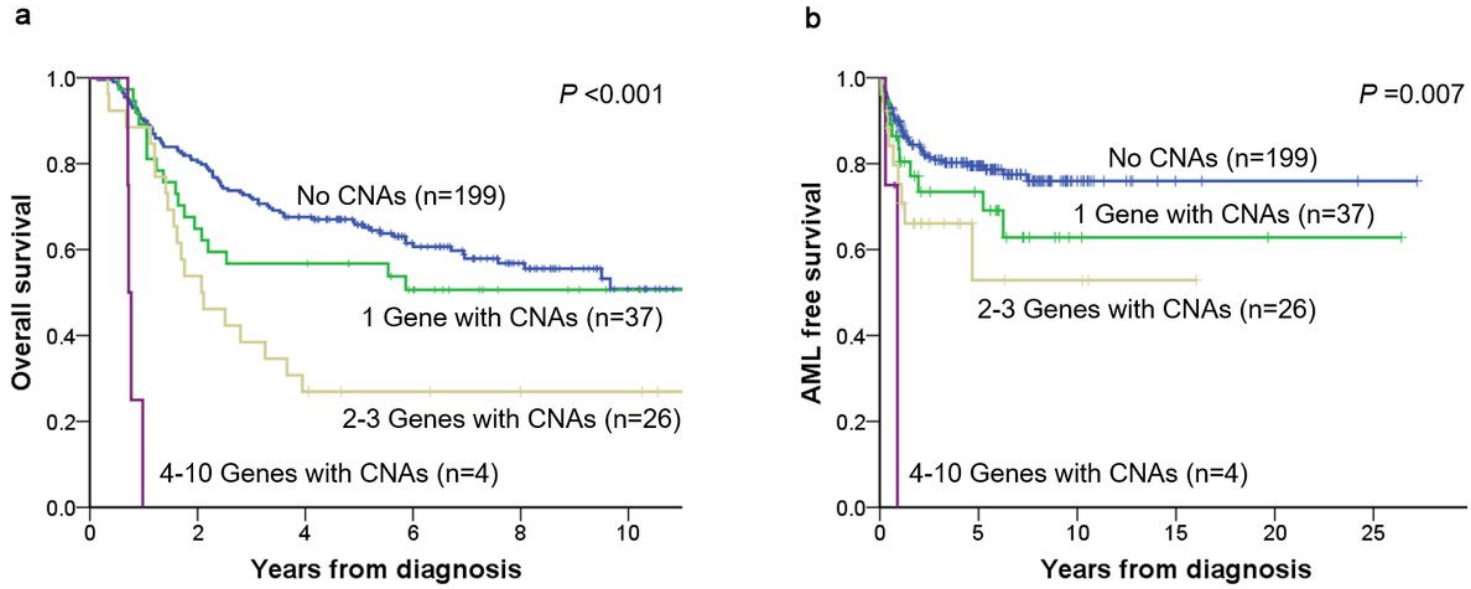
# Figures


## Figure 1

Features of genetic aberrations (somatic mutations + copy number alterations).




## Figure 2

Survival curves based on the number of genes with copy number alterations (CNAs) per patient. (A) Overall survival; (B) Acute myeloid leukemia-free survival.

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