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Targeted sequencing aids in identifying clonality in chronic myelomonocytic leukemia

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ABSTRACT

Chronic myelomonocytic leukemia (CMML) typically shows monocytosis in the peripheral blood (PB), which must be differentiated from reactive monocytosis. To determine the clonality of CMML, we performed molecular and cytogenetic analysis in Korean patients. To investigate whether monocytes in the PB harbored clonal mutational changes, we performed single-cell sequencing after selecting monocytes, neutrophils, and lymphocytes by morphology-aided laser microdissection. Targeted sequencing was performed in 35 patients with CMML with 41 bone marrow samples. Single-cell analysis was performed in two cases. Most (94.3%) patients harbored at least one variant, in genes considered as potential therapeutic targets, while cytogenetic aberrations occurred in only 28.6% of cases. *ASXL1* (54.3%), *SRSF2* (37.1%), *NRAS* (31.4%), and *TET2* (25.7%) were frequently mutated, with lower frequencies of *TET2* mutation and higher frequencies of *NRAS*, *DNMT3A* (17.1%), and *NPM1* (11.4%) mutations compared to in previous studies of Caucasians. Patients with *SETBP1* mutation and those with more than two variants showed poorer survival than those without mutation ($P < 0.001$ and $P = 0.007$, respectively). Most (70.8%) variants were detected at diagnosis and follow-up with no significant differences in variant allele frequency, warranting sequencing during follow-up if diagnostic samples were unavailable. Single-cell analysis revealed clonal monocytes with mutations, and the same mutations were also identified in lymphocytes and neutrophils. Targeted sequencing aided in clonality detection in most patients with CMML and single-cell sequencing facilitated identification of clonal monocytes and the co-existence of mutations in non-myeloid cells, suggesting that certain mutations are acquired by pluripotent stem cells.

1. Introduction

Chronic myelomonocytic leukemia (CMML) is characterized by persistent peripheral blood (PB) monocytosis and dysplasia of one or more myeloid cell lineages in the PB or bone marrow (BM) with fewer than 20% of blasts. CMML presents with overlapping features of myeloproliferative neoplasm (MPN) and myelodysplastic syndrome (MDS) [1–3]. Monocytosis can occur under in various conditions including inflammation, infection, and malignancies, and must be ruled out to diagnose CMML [4,5]. Specific cytogenetic or molecular aberrations have not been identified for CMML and genomic aberrations present in CMML are also commonly in other myeloid neoplasms [4,6]. Several studies have shown that patients with CMML exhibit an increase in CD14+/CD16− classical monocytes, which can help in differentiating CMML from other monocytosis [7,8]. However, clinical information, pathology evaluation, and molecular characteristics should be comprehensively analyzed for diagnosis. The presence of genomic abnormalities can aid in the diagnosis and prognosis prediction of CMML by identifying clonal aberrations [3], and thus has been incorporated in many recently proposed prognostic models [9,10].

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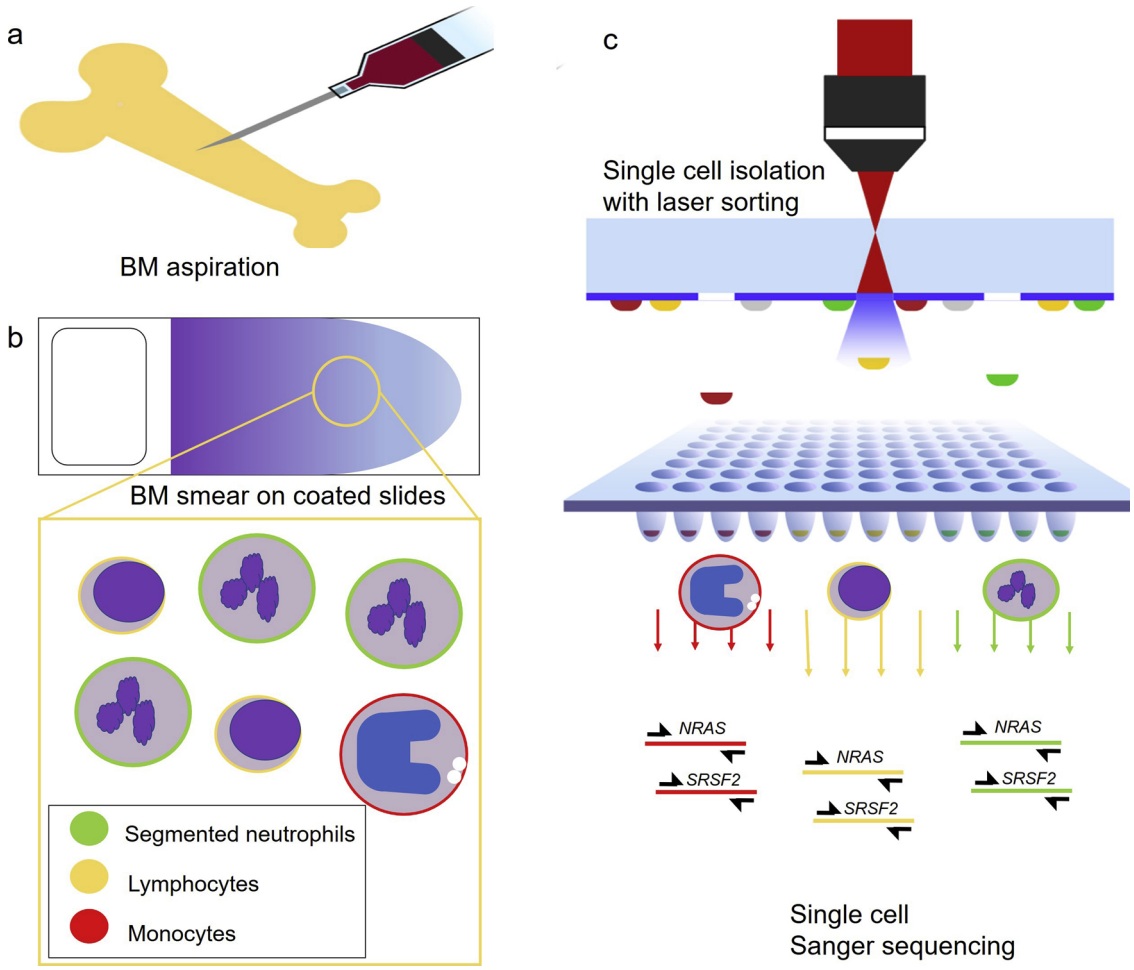

Fig. 1. Morphology aided single cell microdissection. Bone marrow aspirate smeared on coated slides were used for single cell isolation and laser sorting.

In Korea, CMML is a relatively rare disease with a crude incidence rate of 0.14 in 100,000 individuals per year, and thus is diagnosed in fewer than 100 patients per year according to the 2015 Korean Central Cancer Registry [11]. This rate is approximately 1/10 of that in Western countries [12]. Because genomic analysis of CMML has not been reported in Korean patients, we characterized molecular and cytogenetic abnormalities in patients with CMML. We also evaluated whether monocytes in the PB harbor clonal mutational changes, which can help in differentiating clonal and reactive monocytes. Single cells, monocytes, neutrophils, and lymphocytes were isolated by morphology-aided laser microdissection [13] and sequenced to identify the molecular changes found in each patient to confirm monocyte clonality.

2. Materials and methods

2.1. Study populations

A total of 41 samples from 35 patients with CMML recruited from January 2004 to December 2014 were included in the present study. This study was approved by the institutional review board of our institution (H-1311-091-535). Informed consent was acquired from the patients. Diagnosis was based on the 2016 World Health Organization (WHO) criteria [3]. Diagnostic BM aspirate samples were used for targeted sequencing except for five cases, for which follow-up BM samples were used. Additionally, six serial samples were analyzed in five patients.

2.2. Conventional cytogenetics and interphase fluorescence in situ hybridization (FISH) analysis

Cytogenetic analyses using standard Giemsa-banding techniques were performed on heparinized BM samples as part of the diagnostic work-up. Karyotyping results were described according to the International System for Human Cytogenetic Nomenclature (ISCN) [14].

Common chromosomal abnormalities found in MDS, acute myeloid leukemia (AML), and MPN were investigated using commercial FISH probes with BM aspirate as previously reported [15]. Each patient was tested with different FISH probes at the request of the clinicians. The probes used are listed in the supplementary methods. The results were recorded according to ISCN [14].

2.3. Targeted gene sequencing

Targeted gene sequencing was performed using BM aspirate samples to detect 87 genes (Supplementary Table 1) found to be mutated in myeloid neoplasm (n = 49) [4,16] or in other hematologic malignancies (n = 38) [17,18]. Red blood cells in the BM aspirate samples were lysed with ammonium chloride. The QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) was used to extract DNA and the quality control was conducted with the Agilent 2200 TapeStation System (Agilent Technologies, Santa Clara, CA, USA). Fragmentation of genomic DNA and library production were performed by Celemics, Inc. (Seoul, Korea). Sequencing was performed on the Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA) in paired-end 150-baes pair

(bp) rapid run sequencing mode. FASTQ files obtained from the HiSeq 2500 system were processed and aligned to human reference genome 19 using the Burrows-Wheeler Aligner [20] with default parameters. Duplicate reads were removed using Picard MarkDuplicates (http://broadinstitute.github.io/picard/). Low-quality data were eliminated with the rescue of candidate mutations registered in the COSMIC v60 database (http://www.sanger.ac.uk/genetics/CGP/cosmic). Among the filtered single-nucleotide variations with a low depth, those known to represent hotspot mutations based on previous MDS studies [4,16] were rescued. Mapping errors were confirmed by visual inspection with the Integrative Genomics Viewer [19].

2.4. Single-cell sequencing to confirm mutations in cells of different lineages

In patients for whom BM aspirates had been smeared on discharging layer-coated glass slides, single cells of different lineages (segmented neutrophils, lymphocytes, and/or monocytes) were isolated by high-throughput laser isolation [13,20] (Fig. 1). Giemsa-stained BM slides were scanned with an automated microscope (Inverted Microscope Eclipse Ti-E, Nikon Instruments, Tokyo, Japan), and images were obtained at 20x magnification. Stitching was performed with a built-in algorithm (NIS-Elements AR Auto Research, Nikon Instruments) to obtain larger images. Different types of cells, segmented neutrophils, lymphocytes, and/or monocytes were identified by a hematopathologist and their locations were marked for single-cell isolation. At least 10 of each cell type were isolated from each patient. Single-cell isolation was conducted by evaporating the discharging layer underneath the targeted cell using an infrared laser, which generates pressure to transfer the cell downward [20]. The transferred cells were lysed and amplified by PCR to confirm the existence of mutations already found in the bulk BM samples in neutrophils, lymphocytes, and/or monocytes. Primers and PCR conditions are shown in the supplementary methods.

2.5. Statistical analysis

The genes were divided into the following subcategories: DNA methylation, chromatin modification, splicing machinery, receptor/kinases, cell signaling, DNA repair/cell cycle, cohesion complex, and RAS pathway (Supplementary Table 1). Categorical variables were compared by the χ^2 test or Fisher's exact test. Correlations between mutations and categorical clinical parameters were assessed using the Kendall tau-b (T_b) correlation. The *P*-values of multiple comparisons were corrected using the false discovery rate method [21] with GraphPad Prism version 8.0.0 for Windows (GraphPad, Inc., San Diego, CA, USA). Clinical parameters with and without certain mutations were compared by the Mann-Whitney method for genes showing mutations in more than 10.0% of patients.

The period from the date of diagnosis to the date of death from any cause was used to calculate overall survival (OS), and the date of relapse or death from leukemia was used to calculate leukemia-free survival (LFS). OS and LFS were compared by the Kaplan-Meier method (log-rank test). The R statistical program (http://www.r-project.org), and SPSS version 22.0 (SPSS, Inc., Chicago, IL, USA) were used for statistical analyses. *P*-values < 0.05 were considered statistically significant.

3. Results

3.1. Patient characteristics

In total, 35 patients with CMML were included in the study. The median age of patients with CMML was 71.0 years (range: 18 − 85 years) and male patients were predominant (68.6%). The median hemoglobin level was 8.9 g/dL (5.0–15.5); median white blood cell count was 19.4 (4.6 − 141) × 10^9/L; median absolute neutrophil count was 9.8 (1.0 − 77.3) × 10^9/L; median absolute monocyte count was 6.4 (1.1–53.2) × 10^9/L; and median platelet count was 98 (5 − 744) × 10^9/L. Leukemic transformation was present in 10 (28.6%) of patients with CMML, and 14.3% of patients had undergone allogeneic hematopoietic transplantation. Fifteen patients (42.9%) were classified as CMML-0, 7 patients (20.0%) as CMML-1, and 13 (37.1%) as CMML-2 based on the WHO 2016 classification. Based on the French-American British classification, which separates myeloproliferative type (MP)-CMML and myelodysplastic type (MD)-CMML by the white blood cell count (13 × 10^9/L), 7 patients (20.0%) were categorized as having MD-CMML, while the remaining 28 patients (80.0%) were categorized as MP-CMML.

3.2. Cytogenetic results by karyotyping and interphase fluorescence in situ hybridization

An abnormal karyotype was observed in 28.6% (n = 10) of patients with CMML, while interphase FISH was positive in 20.0% (n = 7) of patients. A complex karyotype was observed in 2 (5.7%) of patients with CMML, and −7/del(7q) was present in 4 (11.4%) patients as a recurrent cytogenetic abnormality. The list of cytogenetic aberrations detected by conventional Giemsa-banding and FISH analyses of CMML are shown in Supplementary Table 2.

3.3. Gene mutations

A total of 131 candidate variants were found in 39 genes, including 93 single-nucleotide variations (71.0%) and 38 indels (29.0%) in patients with CMML (Supplementary Table 3). Among the 35 patients, 33 patients with CMML (94.3%) harbored at least one variant, with a median of 4 variants (1 − 10). Frequently mutated genes in CMML were *ASXL1* (54.3%), *SRSF2* (37.1%), *NRAS* (31.4%), *TET2* (25.7%), *DNMT3A* (17.1%), and *RUNX1* (17.1%). The following genes showed variations in more than one patient: *JAK2* (14.3%), *KRAS* (14.3%), *NPM1* (11.4%), *SETBP1* (11.4%), *FLT3* tyrosine kinase domain (TKD) (8.6%), *IDH2* (8.6%), *BCOR* (5.7%), *ETV6* (5.7%), *NF1* (5.7%), and *ZRSR2* (5.7%) (Fig. 2). RAS pathway-related genes were most frequently mutated; the most common variant was in *ASXL1*, c.1934dupG (p.Gly646Trpfs*12), which was found in 42.1% of *ASXL1*-mutated patients.

The correlations between gene mutations present in more than 5% of patients with CMML were evaluated. After correction for multiple comparisons, significant positive correlations were found for *ASXL1* vs. *NRAS* ($T_b = 0.621$, $P < 0.001$, $q = 0.02$) and *DNMT3A* vs. *NPM1* mutation ($T_b = 0.790$, $P < 0.001$, $q < 0.001$) (Fig. 3). The prevalence of *ASXL1* ($P = 0.002$) and *NPM1* mutations ($P = 0.022$) differed according to the 2016 WHO criteria, with more *NPM1* mutations present in CMML-2 and more *ASXL1* mutations in CMML-0.

The frequency of variants identified in genes, which may be potential therapeutic targets, listed in the Drug Gene Interaction Database (http://dgidb.org) [22] was assessed. Among the 39 genes containing variants, 23 genes were reported to have drug-gene interactions in the literature, clinical trials, or practice; these genes were *ASXL1*, *ATM*, *BCOR*, *CDKN2A*, *CSF3R*, *DNMT3A*, *ETV6*, *EZH2*, *FLT3*, *IDH1*, *IDH2*, *JAK2*, *KIT*, *KRAS*, *NF1*, *NOTCH1*, *NPM1*, *NRAS*, *POLG*, *RUNX1*, *STAG2*, *TET2*, and *WT1*. All patients with CMML (n = 35) who harbored at least one variant exhibited a variant in one of the genes listed above.

3.4. Single-cell analysis

Single-cell sequencing was performed for two CMML cases with available BM aspirate smears on discharging layer-coated glass slides for high-throughput laser isolation and sequencing. The first case (P317) exhibited mutations in *ASXL1*, *KRAS*, *NRAS*, *RUNX1*, and *SRSF2* in the BM aspirate bulk sample. We confirmed the *NRAS* and *SRSF2* mutations by single-cell sequencing. Segmented neutrophils, lymphocytes, and monocytes were separately isolated and sequenced (Fig. 4).

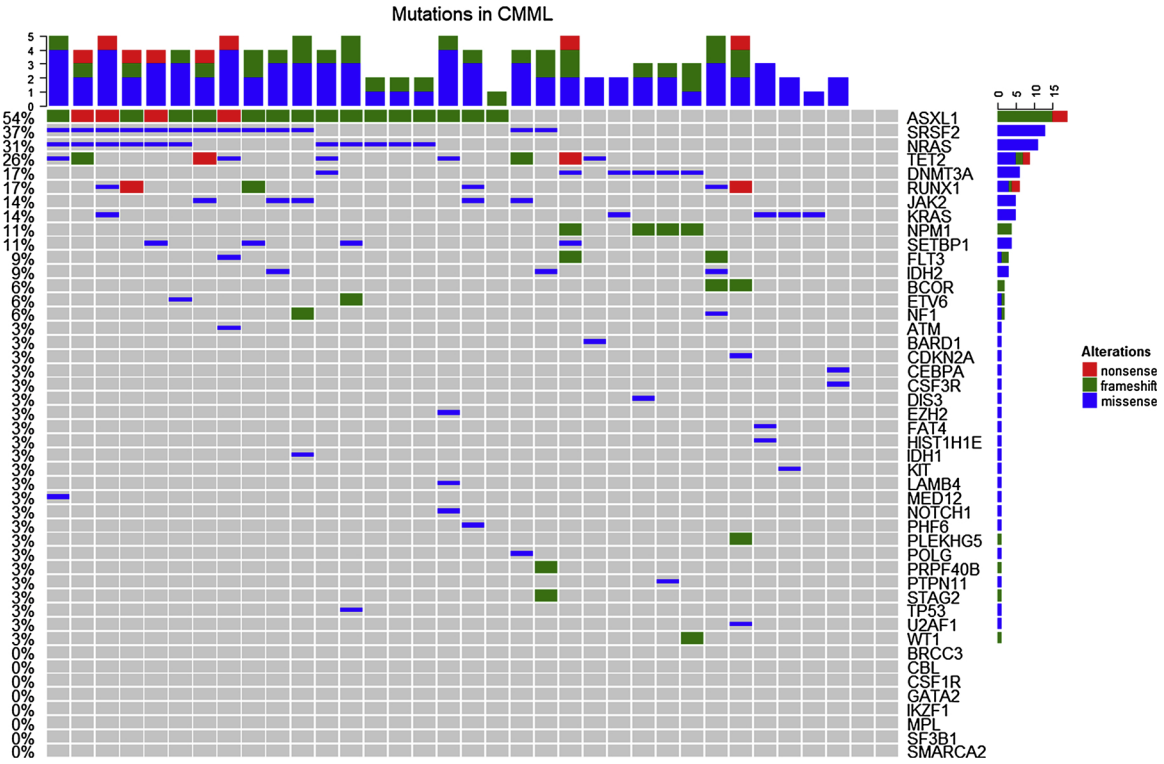

Fig. 2. Distribution of mutations in chronic myelomonocytic leukemia.

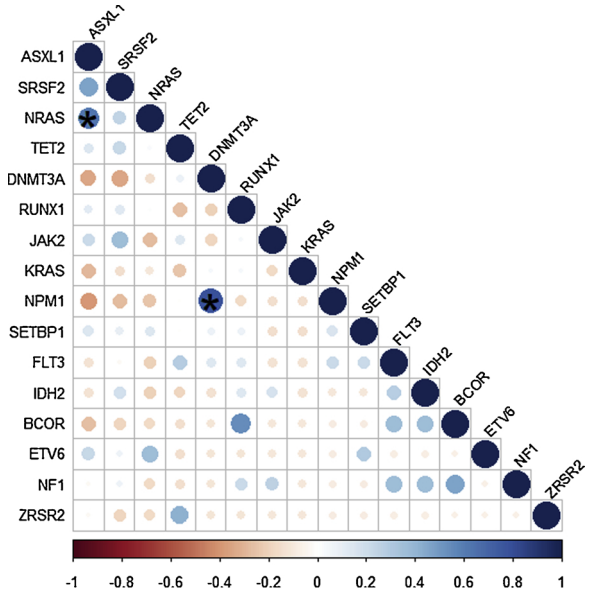

Fig. 3. The correlations between gene mutations present in more than 5% of patients with CMML. Significant correlations after multiple comparisons are shown with a star(*).

Among the single cells selected, *SRSF2* mutation was commonly identified in 66.7% of the amplified segmented neutrophils, lymphocytes, and monocytes. *NRAS* mutation was found in 50.0% of the amplified segmented neutrophils and lymphocytes and 57.1% of monocytes in single-cell analysis. In the second patient (P288), mutations were present in *ASXL1*, *ETV6*, *NRAS*, *SETBP1*, and *TP53* in the BM aspirate sample. Segmented neutrophils and lymphocytes were isolated and the *NRAS* mutation were confirmed by single-cell sequencing. However, monocytes could not be selected because of the poor slide quality in this patient.

3.5. Survival analysis

Among the 35 patients with CMML, 16 patients (45.7%) were administered hypomethylating agents (HMA); 2 patients (7.4%) were administered hydroxyurea; and 3 patients (11.1%) were treated with induction chemotherapy. Among the 16 patients treated with HMA, 11 patients (68.8%) were administered azacitidine and 5 patients (21.2%) with decitabine. A median of 4 cycles of therapy was administered (2–26 cycles). A complete response was observed in 3 patients (18.8%), and a marrow CR was observed in 3 patients (18.8%), with an overall response rate of 37.5%. Stable disease was observed in 5 patients (35.8%), and 3 patients (21.4%) showed progressive disease. The median follow-up period was 16.8 months (0.1–101.2 months). The median OS of the patients with CMML was 21.5 months (95% CI: 16.5–26.4 months), and the median survival of patients administered HMA was 21.5 months (95% CI: 14.2–28.8 months), while that of patients not treated with HMA therapy was 19.1 months (10.3–28.0 months), showing no significant difference in OS ($P = 0.197$). OS in the high-risk group according to the Mayo Molecular Model [23] or Groupe Francophone des Myelodysplasies prognostic model [10], treated with or without HMA was compared. However, there were no significant differences in OS among patients in the high-risk group according to Mayo Molecular model or GFM model treated with or without HMA ($P = 0.280$, 0.608, respectively).

Median leukemia progression-free survival (PFS) was not significantly different in patients treated with HMA (22.7 months) and those not treated with HMA therapy (28.6 months) ($P = 0.318$). There was no significant difference in OS between MD-CMML and MP-CMML ($P = 0.284$), classified based on the French–American–British criteria or between different WHO 2016 classifications of CMML-0, CMML-1, and CMML-2 ($P = 0.527$).

OS was compared in patients with or without specific gene mutations. The only significant difference in OS was observed for *SETBP1* mutation, in which patients with *SETBP1* mutation showed a worse survival than those without mutation ($P < 0.001$). There were no significant differences in OS according to the gene mutation status for

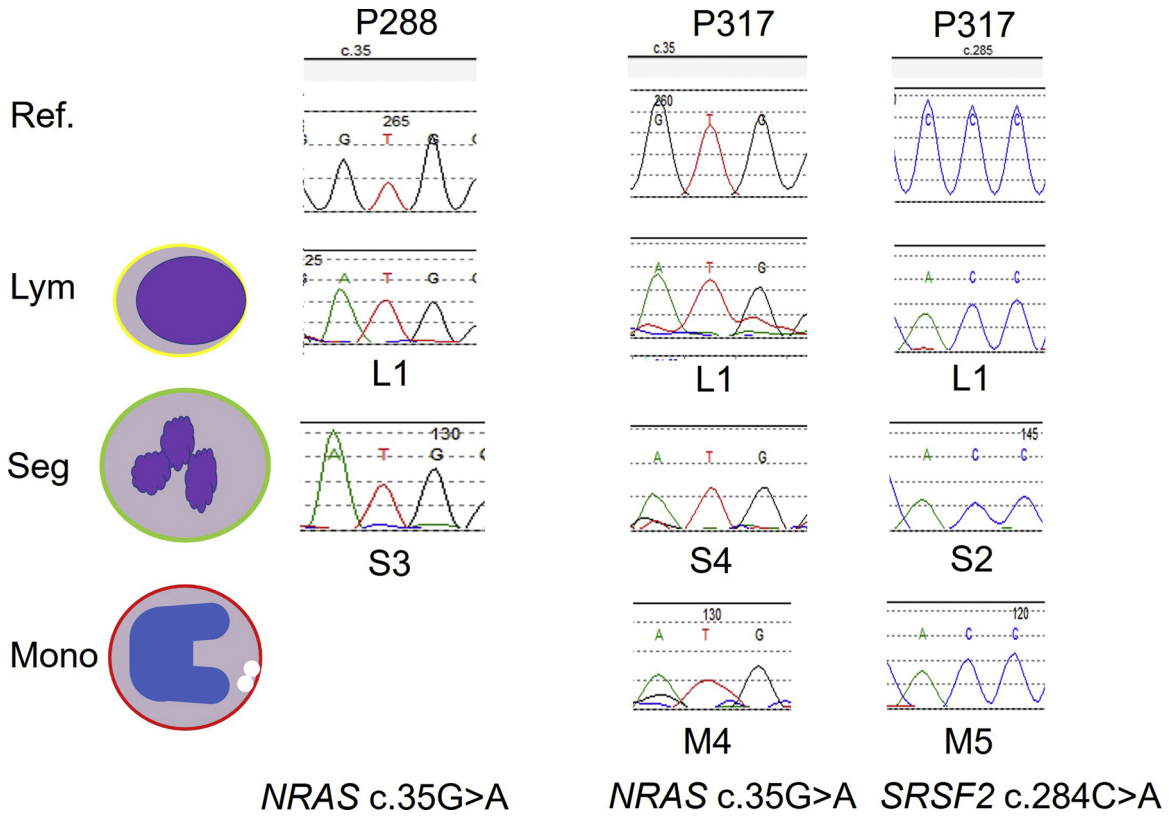

Fig. 4. Results of single cell sequencing in segmented neutrophils, monocytes and lymphocytes by morphology-aided laser microdissection.

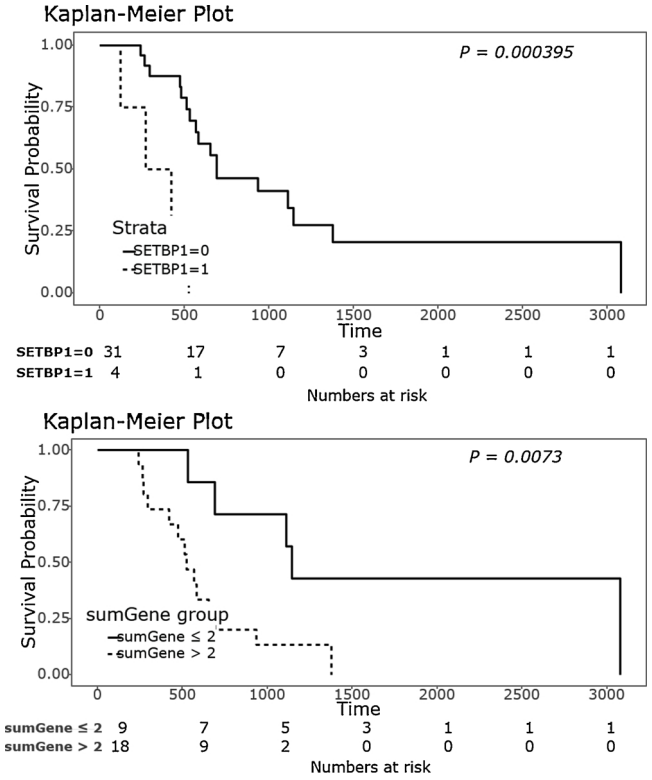

Fig. 5. Kaplan meier survival curve.

ASXL1 ($P = 0.267$), *SRSF2* ($P = 0.108$), *NRAS* ($P = 0.122$), *TET2* ($P = 0.914$), *DNMT3A* ($P = 0.895$), *RUNX1* ($P = 0.362$), *KRAS* ($P = 0.240$), *JAK2* ($P = 0.250$), *NPM1* ($P = 0.305$). There was no significant OS difference between $TET2^{mutant}/ASXL1^{wildtype}$ vs. others ($P = 0.799$). However, the number of genes mutated had effect on survival; patients with more than 2 genes mutated had worse survival than those with ≤2 mutations ($P = 0.007$) (Fig. 5). We also examined whether the OS of patients who presented epigenetic regulator gene mutations (*ASXL1*, *DNMT3A*, and *TET2*) had survival difference from those treated with HMA or not. There were no significant differences in OS between those who received HMA and $DNMT3A^{wild\ type}$ *vs.* $DNMT3A^{mutant}$, $TET2^{wild\ type}$ *vs.* $TET2^{mutant}$, $ASXL1^{wild\ type}$ *vs.* $ASXL1^{mutant}$ ($P = 0.384$, 0.863, 0.666, respectively). The overall response rate (complete remission, partial remission, and marrow complete remission) of HMA, according to the MDS International Working Group 2006 criteria [24], did not differ between those with or without *DNMT3A, TET2, or ASXL1* mutation ($P = 1.0$, 1.0, 0.315, respectively).

3.6. Sequential analysis with serial samples

Serial BM aspirate samples were available for five patients. Twenty-four variants were identified at diagnosis and/or follow-up. Seventeen variants (70.8%) were commonly found at diagnosis and follow-up, with no significant differences observed in the variant allele frequency (VAF) between the diagnostic and follow-up samples for commonly mutated genes ($P = 0.505$).

One patient (P050) who had undergone hypomethylating therapy showed no significant interval change in the BM results and had the same *KRAS* mutation in diagnostic and follow-up samples. Another patient (P285) who had not been administered cytotoxic reagent and showed progression, had retained the *ASXL1*, *DNMT3A*, *NRAS*, and *TET2* mutations, as well as gain-of-mutations in *BCOR* and *RUNX1* at follow-up. The third patient with CMML (P301) showed progression to AML despite hypomethylating therapy and the same number of mutations prior to and after leukemic transformation with *ASXL1*, *EZH2*, *LAMB4*, *TET2*, and *ZRSR2* mutation but had lost the *NOTCH1* mutation and gained a *CEBPA* mutation after AML transformation. The fourth patients with CMML (P307), who had *ASXL1*, *NRAS*, *SRSF2*, *TET2*, and

MED12 mutations and was treated by induction chemotherapy, but showed loss of the *MED12* mutation after progression to AML. The fifth patient with CMML (P090) had no mutation at diagnosis but showed *MPL* and *KRAS* mutations with transformation to AML

4. Discussion

Somatic mutations were identified in most of the Korean patients (94.3%) with CMML and morphology-based single-cell analysis was performed to identify clonal monocytes with mutations.

In CMML, *ASXL1*, *SRSF2*, *DNMT3A*, *TET2*, *NRAS*, *RUNX1*, *SETBP1*, and *KRAS* were commonly mutated. The rate of cytogenetic aberrations (28.6%) was similar to those found in previous studies [10,25]. In agreement with the meta-analysis results of the prognostic significance of *SETBP1* in CMML [26], patients with *SETBP1* mutation had poorer survival than those without mutation. Moreover, the number of mutations in CMML had prognostic significance as in MDS [16], in which patients with greater than two mutations showed worse survival than those with ≤ 2 mutations.

However, the mutational profiles slightly differed from found in previous studies with lower frequencies of *TET2* and higher frequencies of *DNMT3A*, *NPM1*, and *NRAS* mutations [9,10,27] in Korean patients with CMML (Table 1). *TET2* were only mutated in 25.7% of patients, whereas previous studies identified such mutations in 40–60% of patients with CMML. The differences in mutation profiles may be due to the small number of patients included in this study, but a relatively lower percentage of *TET2* mutations (35.6%) was reported in Chinese patients [28], suggesting differences in somatic mutations among ethnicities, as in other hematologic diseases such as acute lymphoblastic leukemia [29] and chronic lymphocytic leukemia [30]. In contrast, *DNMT3A* was more frequently mutated in our cohort than in previous studies (5, 27). Although *DNMT3A* is the most frequently mutated gene associated with age-related clonal hematopoiesis [31,32], the median age of patients with CMML in the present study was similar to that of patients in other studies [27,33]; thus, age may not be related to the higher frequency of mutations in the Korean population. Patients with *DNMT3A* mutation were associated with inferior OS and LFS in a previous study, but OS or LFS difference was not found in this study, possibly owing to the small number of patients [34]. The frequency of *NRAS* mutations identified in the present study was higher than those reported previously, which may be attributed to the higher proportion of MP-CMML (80.0%), which shows more *RAS* mutations and a higher proliferative potential [35]. We found no significant differences in the mutation frequency between MP and MD-CMML for *KRAS*, while *NRAS* mutations were found only in MP-CMML, supporting the findings of previous studies. *NPM1* mutations were detected in 14.8% of the Korean patients with CMML, indicating a higher prevalence of mutations that are relatively rare in CMML according to previous reports [36]. Previous studies have suggested a higher probability of AML progression in patients with *NPM1* mutations, whereas others have suggested that *NPM1* mutation favors a diagnosis of AML rather than MDS or MDS/MPN because of similarity in molecular and clinical characteristics, which need further validation [36–39]. In this study, 50% of the patients with *NPM1* mutation underwent acute leukemic transformation in 7.4 − 9.6 months. However, there was no significant difference of leukemic transformation between those with or without *NPM1* mutation ($P = 0.361$). Moreover, there was no significant difference in OS or PFS between patients with or without *NPM1* mutations in the present study ($P = 0.122$, 0.573, respectively), either because of the small number of patients or a different clinical implication in our group. One patient with *NPM1* mutation had concomitant *FLT3* TKD mutation, where others may favor a diagnosis of AML, but there are previous studies reporting cases with concomitant *NPM1* and *FLT3* mutations in MDS or MDS/MPN [40]. Larger studies are needed to confirm the prognostic effect and common features of patients with *NPM1* mutations in CMML.

Because the diagnosis of CMML is primarily based on the morphology of PB and BM, we assessed the genetic changes by single-cell analysis. Single cells were selected based on their morphology by laser isolation, and we identified the same mutations in single cells of segmented neutrophils, monocytes, and lymphocytes as conducted for the whole BM sample in some cases. Although CMML is known as a myeloid neoplasm depending on the gene involved, certain mutations may be present at the hematopoietic stem cell progenitor stage, and lymphocytes can therefore show the same mutations as observed in myeloid cells [41]. With our preliminary study, we have confirmed this finding in our cases for two genes, *NRAS* and *SRSF2*, which are commonly detected in myeloid and lymphoid cells. This suggests that some mutations are acquired at the pluripotent stem cell level. However. owing to the limited number of available samples, this finding could not be assessed in other patients, which is a limitation of our study and hence needs further investigation. The presence of mutations in monocytes can facilitate the differential diagnosis of reactive and clonal monocytosis through morphology-based single-cell isolation and sequencing.

All patients with CMML who harbored mutations had at least one variant in genes that had drug-gene interaction [22], thus they can be potential therapeutic targets. However, only few drugs have been approved by the FDA and even less for the treatment of CMML [5], thus our mutation data may have future therapeutic implications, as the indications of drugs may change when additional clinical data are acquired.

Survival analysis did not show similar results as recently published prognostic studies, with regard to *ASXL1*, *RUNX1,* and *NRAS* mutation, which may be because of the small number of patients included in this study [42]. However, we showed consistent findings for the poor prognosis of *SETBP1* mutation in CMML and a worse survival when a larger number of mutations (> 2 vs. ≤ 2) was present in CMML. Additionally, the mutational status of epigenetic regulatory genes did not affect the OS, PFS, and response rate of HMA therapy, which was proposed in a previous CMML study. However, other studies have shown that $TET2^{mutant}/ASXL1^{wildtype}$ was associated with better OS and a higher CR, thus the genomic features and its effect on HMA activity are yet to be validated in a larger number of patients [42–44]. This is in contrast to MDS, for which several studies have shown that individuals with mutations in epigenetic regulation-related genes show better responses to HMA [45,46].

Targeted gene sequencing of serial samples enabled the

Table 1
Prevalence of mutations in CMML in other studies.

Gene	A. France Itzykson et al. [10] (n = 312)	B. Spain, Elena et al. [9] (n = 214)	C.US, Patnaik et al. [23] (n = 175)	D. This study (n = 35)	*P*-value (A vs. D)
ASXL1	40.1	37.0	46.9	54.3	0.150
SRSF2	45.9	38.8	53.1	37.1	0.423
NRAS	11.0	11.7	12.0	31.4	0.002
TET2	57.6	44.4	45.7	25.7	< 0.001
DNMT3A	2.2	3.7	5.1	17.1	< 0.001
RUNX1	14.8	7.9	14.3	17.1	0.899
KRAS	7.6	8.9	NA	14.3	0.194
JAK2	8.0	7.0	4.0	14.3	0.207
NPM1	1.2	NA	2.9	11.4	0.003
SETBP1	NA	8.9	18.9	11.4	NA
IDH2	5.7	5.6	4.5	8.6	0.457
NF1	NA	3.3	NA	5.7	NA
FLT3	3.0	NA	0.6	8.6	0.110
EZH2	4.6	7.0	1.1	5.7	0.663
CBL	10.2	8.4	14.3	0.0	0.058
ZRSR2	7.9	4.2	5.1	5.7	0.882
SF3B1	5.9	5.6	5.7	0.0	0.235
U2AF1	5.0	4.2	8.0	2.9	0.859
ETNK1	NA	3.3	NA	NA	NA

NA, not available.

identification of recurrent mutations with no significant differences in VAFs, despite therapeutic interventions. Two of the five patients included in the analysis of serial samples were administered HMA therapy. The presence of mutations with similar VAFs despite HMA therapy has been reported previously, suggesting an epigenetic effect of the drug rather than a cytotoxic effect, thus allowing the mutant clone to persist [43,47]. The presence of mutations throughout treatment suggests that using sequential samples to assess mutations when diagnostic samples are unavailable and for response monitoring based on VAFs after HMA therapy in CMML is unsuitable, as VAFs showed no significant differences prior to and after therapy.

We examined the demographic features and genetic profiles of Korean patients with CMML patients, revealing clonality by targeted sequencing and/or cytogenetics in most patients and showing some differences in genetic profiles compared to in Caucasians. Confirmation of the identified mutations by single-cell sequencing in a case revealed mutations in monocytes, demonstrating the clonality of monocytes, which may be useful for developing rapid differential diagnosis methods for CMML.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.leukres.2019.106190.

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