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NUP214 Rearrangements in Leukemia Patients: A Case Series From a Single Institution

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Background: The three best-known *NUP214* rearrangements found in leukemia (*SET::NUP214*, *NUP214::ABL1*, and *DEK::NUP214*) are associated with treatment resistance and poor prognosis. Mouse experiments have shown that *NUP214* rearrangements alone are insufficient for leukemogenesis; therefore, the identification of concurrent mutations is important for accurate assessment and tailored patient management. Here, we characterized the demographic characteristics and concurrent mutations in patients harboring *NUP214* rearrangements.

Methods: To identify patients with *NUP214* rearrangements, RNA-sequencing results of diagnostic bone marrow aspirates were retrospectively studied. Concurrent targeted next-generation sequencing results, patient demographics, karyotypes, and flow cytometry information were also reviewed.

Results: In total, 11 patients harboring *NUP214* rearrangements were identified, among whom four had *SET::NUP214*, three had *DEK::NUP214*, and four had *NUP214::ABL1*. All *DEK::NUP214*-positive patients were diagnosed as having AML. In patients carrying *SET::NUP214* and *NUP214::ABL1*, T-lymphoblastic leukemia was the most common diagnosis (50%, 4/8). Concurrent gene mutations were found in all cases. *PFH6* mutations were the most common (45.5%, 5/11), followed by *WT1* (27.3%, 3/11), *NOTCH1* (27.3%, 3/11), *FLT3*-internal tandem duplication (27.3%, 3/11), *NRAS* (18.2%, 2/11), and *EZH2* (18.2%, 2/11) mutations. Two patients represented the second and third reported cases of *NUP214::ABL1*-positive AML.

Conclusions: We examined the characteristics and concurrent test results, including gene mutations, of 11 leukemia patients with *NUP214* rearrangement. We hope that the elucidation of the context in which they occurred will aid future research on tailored monitoring and treatment.

Key Words: Gene rearrangement, High-throughput nucleotide sequencing, Leukemia, *NUP214*, Oncogene fusion

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INTRODUCTION

Nucleoporin 214 (NUP214), a member of the nuclear pore complex (NPC), is the only means by which proteins and protein–nucleic acid complexes can cross the nuclear envelope [1]. NUP214 is located on the cytoplasmic side of the NPC and plays

important roles in nucleocytoplasmic transport and cell cycle regulation [2]. NUP214 is composed of three structural domains: an N-terminal domain (NTD) containing a seven-bladed β-propeller, a coiled-coil domain in the middle, and a C-terminal FG-repeat domain, also known as the intrinsically disordered region (IDR) [3]. The NTD is assumed to interact with the DEAD-box helicase Ddx19 and is involved in mRNA export [4, 5]. The coiled-coil domain is crucial for the incorporation of NUP214 in the NPC via interaction with NUP88 [3]. FG repeats have interspersed charged residues that render them disorderly and secondary structure-less [6]. They line the lumen of the nuclear pore, allowing the selective transport of molecules, and interact with chromosomal maintenance 1 (CRM1), a major nuclear export receptor [7]. The unique roles of each domain are implicated in their fusion proteins, depending on which domains form part of the fusion protein.

Translocations involving *NUP214* are infrequently found in various leukemias, with the specific type generally depending on the partner gene. The three best-known *NUP214* rearrangements found in patients with leukemia are *SET::NUP214*, *NUP214::ABL1*, and *DEK::NUP214*. *SET::NUP214* is frequently observed in T-cell acute lymphoblastic leukemia (T-ALL) but rarely in AML or acute undifferentiated leukemia (AUL) [8-11]. *NUP214::ABL1* is detected in up to 6% of T-ALL patients, and only one case in AML has been reported to date [12, 13]. *DEK::NUP214* is involved in 1% of AML and myelodysplastic syndromes [14]. In the *SET::NUP214* and *DEK::NUP214* fusion genes, the NTD is lost, whereas part of the coiled-coil motif and the entire IDR are retained. Therefore, the NUP214 portion in the fusion protein may exert its function via interaction with CRM1 or with other NUP214 fusion proteins to form transcriptional condensates that may alter the transcription of downstream genes such as its cousin, NUP98 [15, 16]. In *NUP214::ABL1*, the NTD and coiled-coil domain are retained, whereas the C-terminal FG repeats are lost. Unlike in the other fusion proteins, the NUP214 portion in the NUP214-ABL1 fusion protein is incorporated into the NPC, thereby combining the ABL1 tyrosine kinases to ensure that they can cross-phosphorylate and thus activate each other [17]. Regardless of the *NUP214* partner gene or disease type, a common characteristic of all *NUP214* rearrangements is that they are associated with treatment resistance and poor prognosis [18, 19]. However, numerous studies in mice have shown that *NUP214* rearrangements alone are insufficient for or at least inefficient in leukemogenesis [17, 20-22]. Therefore, the identification of concurrent mutations and sensitive detection of fusion genes are important for accurate assessment and tailored management of patients with leukemia.

Although numerous studies have reported mutation profiles of patients with AML and ALL, few patients harbored *NUP214* rearrangements, indirectly elucidating the concurrent mutations in patients with *NUP214* rearrangement [19, 23, 24]; direct studies on *NUP214* rearrangements in leukemia patients are scarce. Therefore, we assessed the characteristics and concurrent test results, including gene mutations detected via targeted next-generation sequencing (NGS), of leukemia patients with *NUP214* rearrangement.

MATERIALS AND METHODS

Patients and samples

To identify patients with *NUP214* rearrangements, results of RNA-sequencing of diagnostic bone marrow aspirates conducted between January 2017 and July 2023 were retrospectively reviewed. Before November 2022, RNA-sequencing was done using FusionPlex Pan-Heme Kits (ArcherDx, Boulder, CO, USA); from November 2022 onwards, TruSeq Stranded mRNA Kits (Illumina, San Diego, CA, USA) were used. Eleven patients had *NUP214* rearrangements, and their medical information, including concurrent results of targeted NGS using genomic DNA, was summarized. Research involving human samples complied with all relevant national regulations, institutional policies, and the tenets of the Declaration of Helsinki (as revised in 2013). This study was approved by the Institutional Review Board of Severance Hospital, Seoul, Korea (4-2023-0721), and the requirement for informed consent was waived.

RNA-sequencing

Total RNA was extracted from freshly obtained mononuclear cells using the QIAamp RNA Blood Mini Kit (Qiagen, Valencia, CA, USA). RNA quantity and quality were assessed using an Agilent 4200 TapeStation and RNA Screen Tape (Agilent, Santa Clara, CA, USA), and samples with RNA integrity scores >7.0 were used. For sequencing using the FusionPlex Pan-Heme Kit, 500 ng of total RNA was processed for library preparation, including random priming, cDNA synthesis, end-repair, adapter ligation, and PCR amplification. The libraries were quantified using a Qubit Flex Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). mRNA-based libraries were constructed using a TruSeq Stranded mRNA Kit (Illumina), with poly-A mRNA selection and cDNA synthesis. Library quality was verified using the 4200 TapeStation. Both types of libraries were sequenced on a

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NextSeq 550Dx sequencer (Illumina, San Diego, CA, USA) in 300-bp paired-end mode.

Sequencing data were analyzed using Archer Analysis version 6.0.3.2 (ArcherDx, Boulder, CO, USA) for the FusionPlex Pan-Heme Kits and an in-house algorithm for the TruSeq Stranded mRNA Kits. The in-house algorithm involved converting bcl files to fastq format, demultiplexing, and trimming using Trimmomatic. Arriba (GitHub, San Francisco, CA, USA) was used to detect fusions, reads were aligned to the human reference genome (GRCh37) using STAR, and gene annotation was performed using Arriba and a customized pipeline.

Targeted panel sequencing

Genomic DNA was extracted from bone marrow aspirates using a QIAsymphony DNA Mini Kit (Qiagen). A custom capture panel (Dxome, Seoul, Korea) targeting coding exons and intron–exon boundaries of 497 genes related to hematologic disorders (Supplemental Data Table S1) was used. Prepared libraries were hybridized with capture probes and sequenced as paired-end reads (2×150 bp) using the NextSeq 550Dx sequencer. NGS data were analyzed using DxSeq Analyzer (Dxome). Single-nucleotide variants, small insertions and deletions, and copy number variants were identified [25, 26]. All variants were classified into four tiers according to the American College of Medical Genetics and Genomics, American Society of Clinical Oncology, and College of American Pathologists [27].

RESULTS

Patient diagnoses, gene fusion types, and demographics

Between January 2017 and July 2023, 528 patients underwent RNA fusion testing because of suspected acute leukemia. Among them, 366 received a diagnosis of AML, 129 were diagnosed as having B-cell acute lymphoblastic leukemia (B-ALL), and 17 were found to have T-ALL. The diagnoses of the 16 remaining patients included AUL, mixed-phenotype acute leukemia, Burkitt lymphoma, and chronic myelomonocytic leukemia, among others. Within this group, 11 patients (2.08%) harbored *NUP214* rearrangements. Six of the 366 AML patients (1.64%) and four of the 17 T-ALL patients (23.53%) had *NUP214* rearrangements. *NUP214* rearrangements were not observed in B-ALL patients.

Among the 11 patients harboring *NUP214* rearrangements, four had *SET::NUP214*, three had *DEK::NUP214*, and four had *NUP214::ABL1* (Table 1). All four *SET::NUP214* fusions were between exon 7 of *SET* and exon 18 of *NUP214*. Two patients were

Table 1. Patient characteristics and concurrent NGS results

No. Case	Diagnosis	Sex	Age at diagnosis (yrs)	Fusion gene 1 (exon breakpoint)	Fusion gene 2 (exon breakpoint)	Immunophenotype	Karyotype	Tier 1/2 variants from targeted NGS (VAF, %)*	Total follow-up period/relapse/death (months)
1	T-ALL	F	26	*SET* (7)	*NUP214* (18)	CD34, CD3, HLA-DR, CD7, CD13, CD33, CD79a	Uninterpretable result	*WT1* c.1372C>T (40.0), *TP53* c.560-27_560delinsTAGA (23.7), *TP53* c.844C>T (42.7), *EZH2* c.1707_1708insGCGTTTCG (15.0)	9 / 6 / 9
2	AML	M	12	*SET* (7)	*NUP214* (18)	CD117, CD34, CD11c, CD11b, HLA-DR, CD33, CD7	46,XY [20]	*PHF6* c.902_903delinsTTATTGTT (18.0), *PHF6* exon 9 deletion (NA)	37 / - / -
3	AUL	F	31	*SET* (7)	*NUP214* (18)	CD117, CD34, CD38, CD7, CD33	46,XX [20]	*WT1* c.1384del (43.8), *PHF6* c.834+1G>C (40.1), *GNB1* c.229G>A (43.4), *EED* c.676_677insCCAG (41.2)	19 / - / -
4	T-ALL	F	11	*SET* (7)	*NUP214* (18)	CD38, CD7, CD33, CD34, CD5, cCD3	46,XX,del(1)(p36.2) [18]	*NOTCH1* c.6943C>T (47.3), *SUZ12* c.814G>T (51.9), *DNM2* c.1153C>T (46.7), *IL7R* c.711_729delinsAAGATGCGTT (43.6), *PHF6* c.681_682insGAGAC (45.3)	4 / - / -
5	AML	M	69	*DEK* (9)	*NUP214* (18)	CD117, CD34, cMPO, CD38, HLA-DR, CD33, CD13	46,XY,t(6;9)(p22;q34) [20]	*FLT3*-ITD (NA), *NRAS* c.35G>A (14.8)	13 / - / -

Table 1. Continued

No. Case	Diagnosis	Sex	Age at diagnosis (yrs)	Fusion gene 1 (exon breakpoint)	Fusion gene 2 (exon breakpoint)	Immunophenotype	Karyotype	Tier 1/2 variants from targeted NGS (VAF, %)*	Total follow-up period/relapse/death (months)
6	AML	F	76	*DEK* (8)	*NUP214* (18)	CD117, CD34, cMPO, CD7, CD11c, HLA-DR, CD33, CD13	46,XX,t(6;9)(p22;q34)[20]	*FLT3*-ITD (NA)	4 / - / 4
7	AML	F	59	*DEK* (9)	*NUP214* (18)	Not done†	46,XX,t(6;9)(p22;q34)[16]/46,XX [4]	*FLT3*-ITD (NA), *KDM6A* c.3490_3494dup (12.1)	32 / - / -
8	AMKL	M	3	*NUP214* (34)	*ABL1* (2)	CD34, CD7, CD33, CD13, CD117	46,XY,der(13;21)(q10;q10)c, der(18)t(1;18)(q12;q22), +21c [24]	*BRCA2* c.8912del (49.3)‡, *GATA1* c.137_164del (14.7), *EGLN1* c.2T>G (4.9)	74 / 21 / -
9	T-ALL	M	9	*NUP214* (32)	*ABL1* (2)	CD38, CD7, CD8, CD4, CD5, CD1a, cCD3	46,XY [20]	*DNM2* c.1609G>A (49.8), *CTCF* c.1369C>T (49.3), *NOTCH1* c.7327_7328insGG (43.4), *NOTCH1* c.4775T>C (47.5), *PHF6* c.507_508insGT (94.1), *CDKN2A* whole-gene deletion, *CDKN2B* whole-gene deletion, *EZH2* whole-gene deletion, *RB1* whole-gene deletion	9 / - / -
10	T-ALL	F	12	*NUP214* (32)	*ABL1* (2)	CD117, CD34, CD7, CD33, CD4, cCD3	46,XX [22]	*WT1* c.366dup (11.1), *WT1* c.1156_1157insGGCGGACCGGT (63.6), *PHF6* c.720T>G (16.9), *PHF6* c.720T>G (44.1), *FBXW7* c.1513C>T (15.1), *NRAS* c.34G>C (14.4), *NOTCH1* c.4754T>C (14.3), *DNM2* c.1948G>T (37.5)	25 / 22 / -
11	AML	F	72	*NUP214* (29)	*ABL1* (2)	CD117, CD34, CD7, CD33, CD4, cCD3	45–47,XX,-2,-3,der(3)t(3;?)(p21;?),-5,?der(5)t(5;?)(q12;?),-7,der(7)t(7;?)(?p21;?),+8,+?der(16)t(16;?)(q12-13;?),-18,-19,-19,+20,+3–5mar[cp20a/46,XX [1]	*TP53* c.524G>A (45.1), *TP53* c.613T>G (46.8), *IDH2* c.515G>A (24.5), *RAD50* c.3883C>T (7.5), *DDX41* whole-gene deletion, *NPM1* whole-gene deletion, *RAD50* whole-gene deletion	1 / - / -

*Reference transcripts were as follows: NM_024426.4 for *WT1*, NM_000546.5 for *TP53*, NM_004456.4 for *EZH2*, NM_001015877.1 for *PHF6*, NM_001282539.1 for *GNB1*, NM_003797.3 for *EED*, NM_017617.5 for *NOTCH1*, NM_015355.4 for *SUZ12*, NM_001005360.3 for *DNM2*, NM_002185.5 for *IL7R*, NM_002524.5 for *NRAS*, NM_021140.4 for *KDM6A*, NM_000059.3 for *BRCA2*, NM_002049.3 for *GATA1*, NM_022051.2 for *EGLN1*, NM_006565.3 for *CTCF*, NM_033632.3 for *FBXW7*, NM_002168.4 for *IDH2*, NM_005732.4 for *RAD50*.
†Positive expression of CD34 and CD117 in leukemic blasts was confirmed via immunohistochemistry.
‡This was a germline pathogenic mutation.
Abbreviations: NGS, next-generation sequencing; T-ALL, T-cell acute lymphoblastic leukemia; AML, acute myeloid leukemia; AUL, acute undifferentiated leukemia; AMKL, acute megakaryocytic leukemia; VAF, variant allele frequency; ITD, internal tandem duplication; NA, not available.

diagnosed as having T-ALL, one as having AML, and one as having AUL. Their age at diagnosis ranged from 11 to 31 yrs. Among the three *DEK::NUP214* fusions, two were between exon 9 of *DEK* and exon 18 of *NUP214*, and one was between exon 8 of *DEK* and exon 18 of *NUP214*. All three patients were diagnosed as having AML, and their age at diagnosis ranged from 59 to 76 yrs. Among the four patients with *NUP214::ABL1* fusions, two patients diagnosed as having T-ALL had breakpoints at exon 32 of *NUP214*, whereas of the two patients who were diagnosed as having AML, one had a breakpoint at exon 34 of NUP214 and the other at exon 29 of NUP214; all four were fused to exon 2 of *ABL1*. Their age at diagnosis ranged from 3 to 72 yrs.

Follow-up data

The average follow-up period was 20.6 months, during which four of the 11 patients had relapsed and/or died. One patient with *SET::NUP214*-positive T-ALL relapsed after six months and died three months after relapse. One patient with *DEK::NUP214*-positive AML died at four months of follow-up without ever having achieved complete remission. Two patients with *NUP214::ABL1* relapsed at 21 and 22 months of follow-up. One patient with *NUP214::ABL1*-positive AML and another with *DEK::NUP214*-positive AML had persistent residual blasts even after initiation of induction chemotherapy.

Concurrent mutations

SET::NUP214 and *NUP214::ABL1* mutations were cryptic, whereas all *DEK::NUP214* rearrangements were identified as t(6;9)(p22;q34) using conventional karyotyping. Concurrent gene mutations were found in all 11 patients harboring *NUP214* rearrangements. *PHF6* mutations were detected in three out of four *SET::NUP214* cases and two out of four *NUP214::ABL1* cases. All *DEK::NUP214*-positive AML cases had concurrent *Fms*-like tyrosine kinase 3 with internal tandem duplication (*FLT3*-ITD) mutations. Other mutations commonly found in *NUP214*-rearranged cases included *WT1* (3/11), *NOTCH1* (3/11), *NRAS* (2/11), and *EZH2* (2/11) mutations.

DISCUSSION

The overall frequency of *NUP214* rearrangements was 2.08%; notably, the frequency was significantly higher in T-ALL (23.53%) than in AML (1.64%). Previous studies have reported frequencies of 6% for *SET::NUP214* [18] and up to 6% for *NUP214::ABL1* in T-ALL cases [12]. Collectively, these figures amount to 12%, which is approximately half the frequency found in the present study. This discrepancy may indicate a higher frequency of *NUP214* rearrangements among Korean patients with T-ALL than previously reported. A more plausible explanation is that RNA-sequencing is more sensitive in detecting genetic fusions, unlike conventional techniques such as FISH employed in previous studies.

Although *NUP214* rearrangements are relatively rare, we identified a diverse set of cases. Their presentations were mostly in line with those reported in previous studies, with some exceptions. The *SET::NUP214* rearrangement, known to be the most common in T-ALL, has also been found in AUL, AML, B-ALL, and myeloid sarcomas [9, 11, 28]. Our results align with those in previous reports as, out of the four cases identified, two were T-ALL, one was AUL, and one was AML. In a previous study, *PHF6*, an X-linked tumor-suppressor gene in T-ALL, was concurrently mutated in all *SET::NUP214*-positive T-ALL cases [23]. In our study, three out of four *SET::NUP214* and two out of four *NUP214::ABL1* cases had concurrent mutations in *PHF6*. Although the two *NUP214::ABL1* cases were both T-ALL, two of the *SET::NUP214*-positive cases with *PHF6* mutations were AML and AUL. *PHF6* mutations in AML are not unheard of. In fact, they were identified in 2.83% of patients with AML in a previous study and showed male predominance [29]. The identification of *SET::NUP214* may be of substantial value as a high correlation between minimal residual disease (MRD) associated with this fusion transcript and clonal immunoglobulin/T-cell receptor rearrangement has been reported [30].

DEK::NUP214 rearrangements have been implicated in approximately 1% of AML cases, among which the incidence of *FLT3*-ITD was 70%, which is substantially higher than the 25–30% found in other AML cases [31, 32]. Accordingly, all three patients with *DEK::NUP214*-positive AML in this study carried *FLT3*-ITD mutations. The median age at diagnosis in this group was 69 (59–76) yrs, which is significantly higher than that in the other two groups, which comprised pediatric and young-adult patients. A previous study reported a median age of 34.5 yrs (range, 2–79 yrs) in *DEK::NUP214*-driven AML, suggesting that with a larger sample size, the median age would decrease, although it would still be higher than that in the other two *NUP214* fusion groups [14]. *DEK::NUP214* fusion gene products promote cell proliferation via the activation of mammalian target of rapamycin (mTOR). mTOR inhibitors can reverse this proliferative effect, suggesting that they may be a useful treatment modality in patients with *DEK::NUP214* rearrangement [33].

The *NUP214::ABL1*-positive cases included two patients with AML patients. To our knowledge, only one case of *NUP214::*

ABL1-positive AML has been reported [13], making our patients the second and third cases reported to date. Case 8 was referred to our hospital in 2017 after having been diagnosed with myeloid leukemia associated with Down syndrome. After two rounds of cytarabine, idarubicin, and etoposide induction chemotherapy, the patient was placed on a maintenance regimen when the leukemia relapsed in January 2019. The patient underwent four rounds of treatment with fludarabine, cytarabine, idarubicin, and granulocyte colony-stimulating factor and achieved complete remission in April, after which he received an allogenic hematopoietic stem cell transplant in May 2019. The patient has been stable since. Karyotyping revealed constitutional trisomy 21 along with two additional derivative chromosomes; however, the *NUP214::ABL1* fusion was cryptic and was only identified after RNA-sequencing. NGS revealed a pathogenic *BRCA2* germline variant as well as two additional somatic mutations. The mutation profile of this patient was distinct and did not share any characteristics with those of the other patients with *NUP214* rearrangement (Table 1). Case 11 was the first Korean patient with AML harboring *NUP214::ABL1*. She was a 72-yr-old patient admitted to our hospital because of persistent fever and myalgia for two weeks. A complete blood count revealed leukocytosis (49.21×10^3 cells/μL) with increased immature cells. Subsequent bone marrow analysis confirmed AML, with a blast count of 39.6%. Despite a first induction chemotherapy cycle, the blast count remained 38.0% after one month. Similar findings were made in case 5, where substantial residual blasts (32.9%) persisted even after the initiation of induction chemotherapy.

Similar to the well-known *BCR::ABL1* fusion, *NUP214::ABL1* relies on constitutive tyrosine kinase activation via the coming together of the tyrosine kinases, allowing them to cross-phosphorylate each other. The N-terminal coiled-coil domain of BCR accomplishes this by promoting homotetramerization of the BCR::ABL1 fusion protein [34]. However, the coiled-coil domain of NUP214 in NUP214::ABL1 plays a role in incorporating the fusion protein into the NPC by interacting with NUP88, rather than via self-interaction [17]. In other words, the NUP214::ABL1 fusion protein competes with wild-type NUP214 for its position within the NPC, and only when sufficient NUP214::ABL1 fusion protein is expressed relative to wild-type NUP214 will they be effectively activated and exert a pathogenic effect. Therefore, the amplification of *NUP214::ABL1* is key to its pathogenicity, and this is usually achieved via episomal amplification. Such episomes are often observed in leukemic cells to varying degrees by FISH [12]. Unfortunately, FISH data were lacking in this study because of the unavailability of *NUP214*-specific probes in our institution. Nevertheless, using RNA-sequencing, the relative expression levels of wild-type *NUP214* versus *NUP214::ABL1* can be measured in transcripts per million, and this may compensate for the lack of FISH data.

Another limitation of our study was the small sample size, with only 3–4 patients per *NUP214* fusion type. However, the limited number of patients included in our study was anticipated because *NUP214* rearrangements are relatively rarer than other gene rearrangements. Despite their rarity, the accurate detection of *NUP214* rearrangements has considerable clinical significance. Studies have highlighted these fusion genes as precise MRD markers [30] and/or promising potential therapeutic targets [33, 35, 36]. Therefore, identifying and understanding *NUP214* rearrangements may facilitate improved disease monitoring, prognosis assessment, and the development of targeted therapeutic approaches. Given that these fusions typically manifest as secondary genetic events occurring late in the progression and seldom appear as solitary genetic alterations, it is crucial to acknowledge the specific milieu in which they emerge. Our study provided essential contextual information regarding these fusions, and we are hopeful that additional reports and studies on tailored treatments will continue to improve the treatment outcomes of patients.

In conclusion, we determined the characteristics and concurrent test results, including gene mutations detected by targeted NGS, of 11 leukemia patients with *NUP214* rearrangements. Two patients represented the second and third reported cases of *NUP214::ABL1*-positive AML. As these gene rearrangements can serve as potential MRD markers as well as therapeutic targets, we hope that the elucidation of the context in which they occurred will aid future research on tailored monitoring and treatment.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.3343/alm.2023.0301

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None.

AUTHOR CONTRIBUTIONS

Choi YJ conceived the study and wrote the manuscript; Ming YK

conducted the experiments; Lee ST and Choi JR provided insights and infrastructure, and Shin S supervised the study, revised the manuscript, and acquired the funding.

CONFLICTS OF INTEREST

None declared.

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