



Myelodysplastic syndrome with t(6;9)(p22;q34.1)/DEK-NUP214 better classified as acute myeloid leukemia? A multicenter study of 107 cases

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Abstract

t(6;9)(p22;q34.1)/DEK-NUP214 is a recurrent genetic abnormality that occurs in 1–2% of patients with acute myeloid leukemia (AML), and rarely in myelodysplastic syndrome (MDS). It has been suggested by others that all myeloid neoplasms with t(6;9)/DEK-NUP214 may be considered as AML, even when blast count is <20%. In this study, we compared the clinicopathologic features of 107 patients with myeloid neoplasms harboring t(6;9)/DEK-NUP214: 33 MDS and 74 AML. Compared with patients with AML, patients with MDS were older ($p = 0.10$), had a lower white blood cell count ($p = 0.0017$), a lower blast count in the peripheral blood ($p < 0.0001$) and bone marrow ($p < 0.0001$), a higher platelet count ($p = 0.022$), and a lower frequency of FLT3-ITD mutation ($p = 0.01$). In addition, basophilia was not a common feature in the patients of this cohort. Although there was no difference in overall survival between MDS and AML patients ($p = 0.18$) in the entire cohort, the survival curves did show a trend toward favorable survival in MDS patients. Multivariate analyses showed that initial diagnosis of MDS vs. AML and allogeneic hematopoietic stem cell transplantation were prognostic factors for survival of patients with t(6;9)/DEK-NUP214 ($p = 0.008$ and $p < 0.0001$, respectively). Our data suggest that MDS with t(6;9)/DEK-NUP214 is prognostically not equivalent to AML with t(6;9)/DEK-NUP214. These data also show that stem cell transplantation greatly improves the survival of MDS and AML patients with myeloid neoplasms associated with t(6;9)/DEK-NUP214.

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Introduction

t(6;9)(p22;q34.1)/*DEK-NUP214* is a recurrent genetic abnormality that occurs in 1–2% of patients with acute myeloid leukemia (AML) [1, 2]. AML associated with t(6;9)/*DEK-NUP214* has been recognized as a distinct entity in the World Health Organization (WHO) classification since 2008 [3]. This translocation in AML was first identified by Rowley and Potter in 1976 [4], and was shown subsequently to involve *DEK* on chromosome 6p22.3 and *NUP214* (formerly known as *CAN*) on chromosome 9q34.1, leading to formation of a *DEK-NUP214* fusion gene. Consistent with its association with AML, increased expression of the fusion protein has been reported to be restricted to cells of myeloid lineage [5, 6].

AML associated with t(6;9)/*DEK-NUP214* has morphological features that overlap with AML with maturation or acute myelomonocytic leukemia [1, 7]. Frequently, these neoplasms show evidence of myelodysplasia and in some cases, basophilia, Auer rods, and ring sideroblasts [1, 7–10]. These neoplasms also commonly harbor an internal tandem duplication (ITD) of *FLT3* [1, 7, 9–12]. Others have reported that adults and children with AML associated with t(6;9)/*DEK-NUP214* have a poor prognosis [1, 2, 8, 9, 11, 13], and data suggest that allogeneic hematopoietic stem cell transplantation (HSCT) may improve survival [1, 12].

Rarely, the t(6;9)/*DEK-NUP214* has been reported in cases of myelodysplastic syndrome (MDS) [8, 10, 14–18]. These patients have a high risk of progression to AML [8, 10, 14, 18–20]. In a study of 62 pediatric patients including 8 patients with MDS and 54 with AML associated with t(6;9)/*DEK-NUP214*, Sandahl *et al.* found similar clinical and morphological characteristics between t(6;9)/*DEK-NUP214*-positive MDS and AML, suggesting that MDS with t(6;9)/*DEK-NUP214* may be better considered as AML regardless of the blast percentage, analogous to myeloid neoplasms associated with t(8;21)(q22;q22.1)/*RUNX1-RUNX1T1*, and inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB-MYH11* [9].

MDS associated with t(6;9)/*DEK-NUP214* is rare and only a few small series have been reported. No large studies have compared patients with MDS with t(6;9)/*DEK-NUP214* vs. AML with t(6;9)/*DEK-NUP214*. In this multicenter study of 107 patients, we compared the clinicopathologic features of patients with MDS vs. AML harboring t(6;9)/*DEK-NUP214*. Our aim was to address the clinical and prognostic impact of t(6;9)/*DEK-NUP214* in MDS, and more specifically, address this question: should MDS associated with t(6;9)/*DEK-NUP214* be classified as AML, even with a blast percentage of <20%?

Materials and methods

Patient selection and data collection

Cases of myeloid neoplasms diagnosed at The University of Texas MD Anderson Cancer Center since 1997 were reviewed for the presence of t(6;9)/*DEK-NUP214* detected by conventional chromosomal analysis, fluorescence in situ hybridization (FISH), and/or molecular testing, yielding 16 MDS and 50 AML cases. An additional 41 cases (17 MDS and 24 AML) were contributed from nine collaborating institutions where the contributors prioritized identification of MDS cases. Clinical information and follow-up data were obtained from the electronic medical records, including age, gender, history of cytotoxic therapy before the emergence of t(6;9)/*DEK-NUP214*, initial pathological diagnosis, immunophenotyping, interval from initial diagnosis of MDS to progression to AML, therapy, clinical response, status of last follow-up, and overall survival (OS). The types of treatment included standard 7 + 3 chemotherapy (cytarabine and anthracycline), usually with additional chemotherapies including etoposide, hypomethylating agents (HMA) including azacitidine and decitabine, targeted therapies including *FLT3* inhibitors, and HSCT. Laboratory findings at initial diagnosis of MDS or AML were recorded, including complete blood count and differential counts. This study was approved by the Institutional Review Boards of all participating institutions.

Morphologic assessment

Wright–Giemsa-stained peripheral blood (PB) and bone marrow (BM) aspirate smears and hematoxylin-eosin-stained BM clot and core biopsy sections were reviewed. These specimens were assessed for morphologic dysplasia of all lineages. The assessment of dysplasia followed criteria described previously [7]. A total of 200 leukocytes in PB and 500 cells in BM were counted to determine cell differentiation including blast and basophil percentages. Evidence of progression to AML in patients with MDS was also evaluated, defined as the occurrence of ≥20% blasts in BM or PB after the initial diagnosis of MDS.

Chromosomal, FISH analyses, and acute leukemia translocation panel

Conventional chromosomal analysis was performed on G-banded metaphases prepared from unstimulated 24- and 48-h BM aspirate cultures using standard GTG banding. Twenty metaphases were analyzed, and the results were reported using the 2016 International System for Human Cytogenetics Nomenclature. A complex karyotype was defined as ≥3 chromosomal abnormalities. FISH analysis

for *DEK-NUP214* was performed on cultured cells with *DEK-NUP214* dual-color dual fusion probes (Abbott Molecular, Des Plaines, IL, USA) according to the manufacturer's instructions. Two hundred interphase nuclei were analyzed. The presence of one red, one green, and two yellow (fusion) signals was considered as the typical signal pattern for *DEK-NUP214* fusion. Nanofluidics-based qualitative multiparametric reverse-transcriptase PCR was performed for the detection of a panel of fusion transcripts associated with acute leukemia including t(6;9)/*DEK-NUP214* on MDS or AML patients at MDACC, with an analytical sensitivity of 0.1–0.01% [21].

Flow cytometric analysis

Flow cytometry immunophenotypic analysis was performed on fresh BM aspirate specimens collected in tubes containing ethylenediaminetetraacetic acid as part of routine clinical service in the clinical flow cytometry laboratories of each institution. Samples were examined with antibody panels designed to assess acute leukemia, and the antibodies used mainly to evaluate myeloblasts were as follows: CD2, surface and cytoplasmic CD3, CD4, CD5, CD7, CD9, CD10, CD13, CD14, CD15, CD19, CD33, CD34, CD36, CD38, CD41, CD56, CD64, CD117, CD123, HLA-DR, MPO, and TdT. At MDACC, the acquisition of events was performed on FACSCalibur instruments (BD Biosciences, San Jose, CA, USA), in which standardization was maintained using CS&T Beads with emphasis on comparable cross platform performance. Data were analyzed using FCS Express (De Novo Software, Glendale, CA).

Mutation analysis

Different panels designed to detect genes commonly mutated in myeloid neoplasms were performed on fresh-frozen BM DNA samples over different time intervals and at different institutions using targeted next-generation sequencing (NGS) methods (Supplementary Materials and Methods: NGS panels). A 28- or 81-gene panel at MDACC was performed using the same methods as described previously [22, 23]. A 30- or 49-gene panel was used for cases contributed by Memorial Sloan Kettering Cancer Center (New York, NY, USA), and the ARUP Myeloid Malignancies Mutation Panel was used by University of Nebraska Medical Center (Omaha, NE, USA). Certain genes were tested separately with or without NGS panels. *FLT3*-ITD, *FLT3* D835, and *NPM1* (exon 12, codons 956–971) mutations were assessed by PCR followed by capillary electrophoresis on a genetic analyzer (Prism ABI 3130, Applied Biosystems, Foster City, CA, USA) as described previously [24]. *NRAS* (codons 12, 13, 61), *KRAS* (codons 12, 13, 61),

IDH1 (exon 4, codons 87–138), *IDH2* (exon 4), *KIT* (exons 8 and 17), *DNMT3A* (exon 23), *CEBPA*, and *JAK2* (codon 617) mutations were assessed by PCR followed by Sanger sequencing with a sensitivity of 10–20% as described previously [25, 26].

Statistical analysis

Clinical and laboratory variables were compared between patients with MDS vs. AML using chi-square or Fisher's exact tests for categorical variables and unpaired t-test or Mann–Whitney U tests for numeric variables. The interval from MDS to AML was calculated as the time from the initial diagnosis of MDS to the diagnosis of AML progression. OS was calculated from the initial diagnosis of MDS or AML to the date of expiration or the last known alive date. Survival distributions were analyzed with the Kaplan–Meier method, with differences compared by the log-rank test. To compare OS of patients with AML associated with t(6;9)/*DEK-NUP214* in our cohort to a more general AML population, a public database from the TCGA AML study was used and only patients equal to or younger than 55 years were included in the survival analysis to achieve a patient group of similar median age to our study cohort [27].

To perform multivariate analysis, a univariate cox model was run for all variables at initial detection of t(6;9)/*DEK-NUP214*, including initial diagnosis (MDS vs. AML), age, white blood cell count, hemoglobin, platelet count, erythroid dysplasia, granulocytic dysplasia, megakaryocytic dysplasia, multilineage dysplasia, complex karyotype, isolated t(6;9), *FLT3*-ITD mutation, 7 + 3 chemotherapy, HMA, *FLT3* inhibitor treatment, and HSCT status. Variables significant at some level ($p \leq 0.20$) were included in the initial multivariate model. Then using stepwise regression procedure, the final main-effect model with a significant level ($p < 0.05$) was performed after backward selection to eliminate non-significant variables at some level ($p \geq 0.10$) and forward selection to add variables with significant level $p \leq 0.10$. Statistical analyses were performed using GraphPad Prism 8 (La Jolla, CA, USA) and R statistical software (version 4.0.2, R core team).

Results

Baseline clinical characteristics

The study cohort included 107 patients with myeloid neoplasms harboring t(6;9)/*DEK-NUP214*: 33 patients with MDS and 74 with AML. There were 55 men and 52 women with a median age of 43 years (range, 10–82) at initial diagnosis. Patients with MDS were older than those with

Table 1 Clinicopathologic features of patients with MDS vs. AML with t(6;9)(p22;q34).

	Overall (N = 107)	MDS (N = 33)	AML (N = 74)	p value
Age, median (range, years)	43 [10–82]	51 [22–74]	38 [10–82]	0.10
Gender (male, %)	55/107 (51%)	18/33 (55%)	37/74 (50%)	0.68
Peripheral blood, median (range)				
WBC ($\times 10^9/L$)	9.5 (0.4–222.2)	3.2 (0.9–61.2)	15.3 (0.4–222.2)	0.0017
Hgb (g/dL)	8.4 (4.4–13.9)	8.6 (4.6–13.0)	8.3 (4.4–13.9)	0.22
Platelets ($\times 10^9/L$)	59 (12–373)	85 (18–242)	52 (12–373)	0.022
Blasts (%)	6% (0–97%)	0% (0–12%)	12% (0–97%)	<0.0001
Basophils (%)	0% (0–3%)	0% (0–2%)	0% (0–3%)	0.61
Bone marrow				
Blasts, median (range, %)	45% (2–90%)	11% (2–19%)	56% (20–90%)	<0.0001
Basophils, median (range, %)	0% (0–4%)	0% (0–2%)	0% (0–4%)	0.1
Dyserythropoiesis, N (%)	75/99 (75%)	26/30 (87%)	49/69 (70%)	0.083
Dysgranulopoiesis, N (%)	63/99 (64%)	21/30 (70%)	42/69 (61%)	0.5
Dysmegakaryopoiesis, N (%)	55/99 (56%)	25/30 (83%)	30/69 (43%)	0.0003
Multilineage dysplasia, N (%)	69/99 (70%)	27/30 (90%)	42/69 (61%)	0.004
Karyotype				
Complex, N (%)	9/92 (10%)	2/26 (8%)	7/66 (11%)	>0.99
Sole t(6;9), N (%)	67/92 (73%)	22/26 (85%)	45/66 (68%)	0.13
FLT3-ITD, N (%)	61/79 (77%)	10/19 (53%)	51/60 (85%)	0.01
Transplant, N (%)	69/107 (64%)	20/33 (61%)	49/74 (66%)	0.66
F/U time, median (range, mos)	24 (1–160)	32 [6–94]	20 (1–160)	0.06
Patient outcome, N (death, %)	60/107 (56%)	17/33 (52%)	43/74 (58%)	0.54

MDS myelodysplastic syndrome, AML acute myeloid leukemia, N number, WBC white blood cell, Hgb hemoglobin, mos months, F/U follow-up.

AML (median, 51 vs. 38 years) but this difference was not significant ($p = 0.10$). Similarly, neither patients with MDS nor patients with AML showed a gender predilection. The clinical, morphologic, and genetic characteristics of the cohort are summarized in Table 1.

In all 101 patients with known time of t(6;9)/DEK-NUP214 emergence, the translocation was detected at time of initial diagnosis of MDS or AML. In the remaining six patients, the time of emergence of t(6;9)/DEK-NUP214 was unknown. In these patients, conventional chromosomal analysis, FISH, and molecular testing for DEK-NUP214 were not performed at initial diagnosis and t(6;9)/DEK-NUP214 was detected at follow-up. Four of these patients were diagnosed initially with MDS, which progressed to AML during follow-up; t(6;9)/DEK-NUP214 was identified 6–13 months after AML progression. However, an earlier karyotype was not available and the possibility of t(6;9)/DEK-NUP214 occurring as a secondary event cannot be excluded.

Laboratory findings

Patients with MDS associated with t(6;9)/DEK-NUP214 presented with a lower white blood cell count (median: 3.2 vs. 15.3 $\times 10^9/L$, $p = 0.0017$) and a higher platelet count

(median: 85 vs. 52 $\times 10^9/L$, $p = 0.022$) than patients with AML (Table 1). There was no significant difference in the hemoglobin level ($p = 0.22$) and basophil percentages in the PB ($p = 0.61$) or BM ($p = 0.1$) between patients with MDS and AML. Of the 96 patients with the PB or BM differential counts available, 7 (7%) had basophils over 2% in the PB and/or BM. In the PB, 66/86 (77%) patients had no basophils in the differential count, including 48/61 (79%) with AML and 18/25 (72%) MDS. In the BM, 68/94 (72%) patients had no basophils, including 46/68 (68%) with AML and 22/26 (85%) MDS. As expected, patients with MDS presented with a lower blast count in the PB (median, 0% vs. 12%, $p < 0.0001$) and BM (median, 11% vs. 56%, $p < 0.0001$) than those with AML.

Morphologic findings

The classification of the 33 MDS cases using the WHO system was as follows: 15 MDS with excess blasts-2 (MDS-EB2), 10 MDS-EB1, 4 MDS with multilineage dysplasia, 1 atypical chronic myeloid leukemia, BCR-ABL1-negative, and 3 MDS of unknown sub-classification. By morphology only, the 74 AML cases were classified as follows: 40 (54%) AML with maturation, 26 (35%) acute myelomonocytic leukemia, 3 (4%) acute monocytic leukemia, 2 (3%)

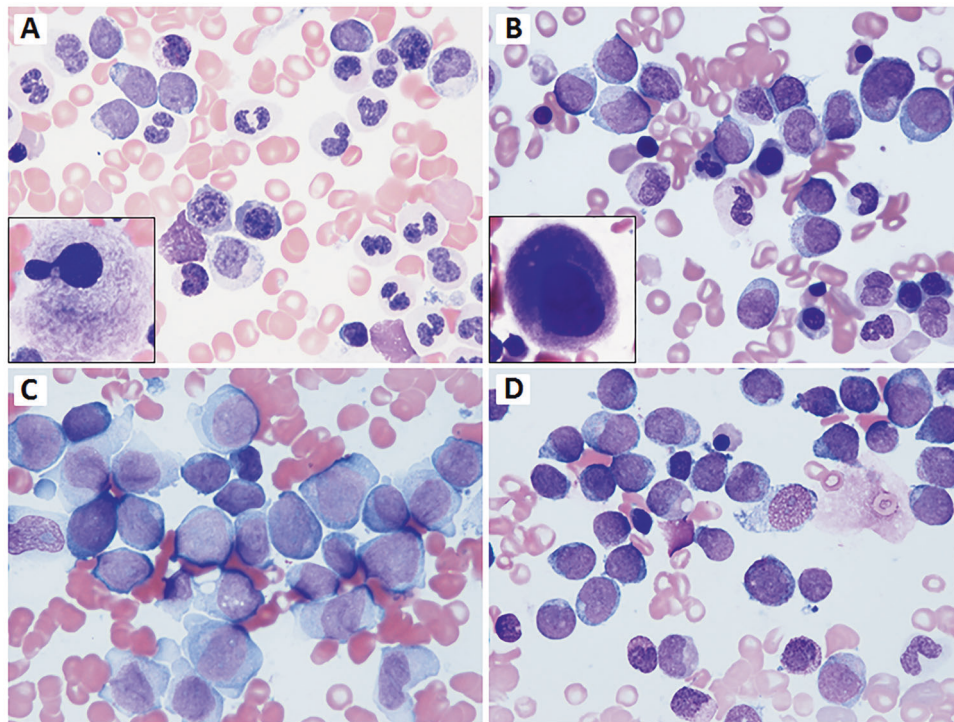


Fig. 1 Morphology of bone marrow and peripheral blood in MDS and AML with t(6;9). **A** Bone marrow from a patient with MDS-EB2 with t(6;9) and mutations of *NRAS* and *KRAS* showed dysplastic neutrophils and megakaryocytes (insert), and increased basophils and blasts. Original magnification: $\times 1000$. **B** Bone marrow from a patient with AML with t(6;9) and *FLT3*-ITD mutation showed trilineage dysplasia and increased myeloblasts. The patient had a history of MDS-EB2 with t(6;9). Insert: dysplastic megakaryocytes. Original

magnification: $\times 1000$. **C** Bone marrow from a patient with AML with t(6;9) and *FLT3*-ITD mutation showed numerous large monoblasts. The patient developed AML progression after HSCT for MDS-EB1 with t(6;9). Original magnification: $\times 1000$. **D** Bone marrow from a patient with de novo AML with t(6;9) showed numerous medium-sized myeloblasts in a background of increased basophils and dysgranulopoiesis. Original magnification: $\times 1000$.

AML without maturation, and 3 (4%) without morphologic classification available.

A variable degree of multilineage dysplasia was seen in 69 of 99 (70%) cases with BM materials available for morphologic evaluation (Fig. 1), including dyserythropoiesis in 75 (75%), dysgranulopoiesis in 63 (64%), and dysmegakaryopoiesis in 55 (56%). MDS with t(6;9)/*DEK-NUP214* showed a higher frequency of multilineage dysplasia than AML with t(6;9)/*DEK-NUP214* (27/30, 90% vs. 42/69, 61%, $p = 0.004$). This difference seems attributable to a higher frequency of dysplasia in the megakaryocytic lineage in MDS with t(6;9)/*DEK-NUP214* (25/30, 83% vs. 30/69, 43%, $p = 0.0003$). Sixty cases were evaluated specifically for Auer rods; in 11 (18%) cases Auer rods were present in blasts.

Immunophenotypic findings

The blasts had a non-specific myeloid immunophenotype, with consistent expression of CD13 (64/64, 100%), CD33 (64/65, 99%), CD34 (60/65, 92%), CD38 (50/50, 100%), CD117 (63/64, 98%), CD123 (21/22, 96%), HLA-DR

(61/61, 100%), and MPO (36/39, 92%). Some cases expressed CD9 (5/8, 63%), CD15 (13/33, 39%), and/or monocyte-associated antigens including CD4 (9/28, 32%), CD14 (8/46, 17%), CD36 (6/13, 46%), and CD64 (39/57, 68%). Most AML cases were negative for lymphoid antigens. Cytoplasmic CD3 ($n = 47$) and CD5 ($n = 36$) were negative in all cases assessed. CD2 was positive (3/45 cases positive, 7%), CD7 (12/56, 21%), and CD19 (6/51, 12%). No AML cases in our cohort met the criteria for a mixed-phenotype acute leukemia. The blasts in most AML cases were negative for CD10 (1/39 cases positive, 3%), CD41 (3/30, 10%), and CD56 (2/44, 5%).

Cytogenetic findings

Complete karyotype results at time of initial diagnosis were available in 92 patients, including 66 with AML and 26 with MDS (Fig. 2 and Table 1). Among these patients, 67 (73%), including 45 AML and 22 MDS, had t(6;9) or its variant translocation as a sole cytogenetic abnormality, including 1 MDS with t(6;9;15)(p23;q34;q25). The remaining 25 patients (21 AML and 4 MDS) had at least

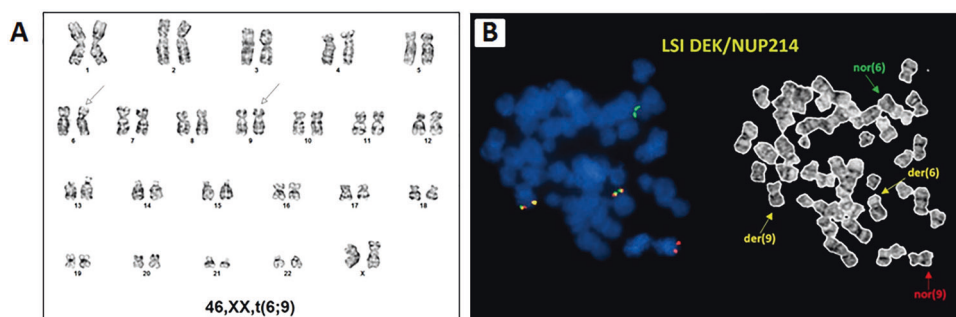


Fig. 2 Karyotype and FISH analyses for $t(6;9)/DEK-NUP214$. **A** $t(6;9)(p22;q34.1)$ by conventional cytogenetics pointed by the arrows. **B** $DEK-NUP214$ rearrangement by FISH analysis. Green signal

marked *DEK* gene on chromosome 6 and orange signal labels *NUP214* gene on chromosome 9 (der derivative, nor normal).

one additional cytogenetic abnormality including 9 with a complex karyotype (7 AML and 2 MDS). The most common additional chromosomal aberrations included trisomy 8 ($n = 9$) and trisomy 13 ($n = 5$), all in patients with AML. In four cases of MDS with additional abnormalities, $del[15q]$ and a complex karyotype were observed in two patients each. No significant differences were observed in the frequencies of a complex karyotype or sole $t(6;9)$ between the MDS vs. AML groups ($p > 0.99$ and 0.13 , respectively).

Twenty-four cases with $t(6;9)$, including 13 AML and 11 MDS, had FISH performed to confirm the $DEK-NUP214$. All cases with positive $DEK-NUP214$ by FISH had their $t(6;9)$ detected by karyotype. In addition, 22 cases, including 13 AML and 9 MDS, were assessed by RT-PCR using an acute leukemia translocation panel. Twenty-one patients were positive for $DEK-NUP214$ fusion transcripts. The only MDS patient negative for the fusion transcript was confirmed to carry $DEK-NUP214$ by FISH.

Of the nine patients without complete karyotype information at the time of initial presentation, two had AML with $t(6;9)/DEK-NUP214$ detected by FISH, and seven had $t(6;9)$ but a complete karyotype was not available (four AML and three MDS). Two MDS patients had a normal karyotype with cryptic $DEK-NUP214$ fusion detected by the acute leukemia translocation panel ($n = 1$) or FISH ($n = 1$) testing.

Molecular findings by targeting sequencing

Molecular profiles (except *FLT3* described separately) at initial diagnosis were available in 58 patients: 44 with AML and 14 with MDS (Supplementary Table 1).

In cases of AML with $t(6;9)/DEK-NUP214$, *RAS* genes were mutated in 12 of 33 (36%) cases assessed including 11 *NRAS* and 1 *HRAS*. Other gene mutations in AML with $t(6;9)/DEK-NUP214$ included *TET2* (3/16, 19%), *IDH2* (3/20, 15%), *PTPN11* (2/13, 15%), *EGFR* (1/8, 13%), *SETBP2* (1/8, 13%), *WT1* (2/16, 13%), *PRPF40B* (1/9, 11%), *U2AF1* (1/9, 11%), *DNMT3A* (1/17, 6%), *IDH1* (1/18, 6%),

and *NPM1* (1/29, 3%). No mutations were detected in *ASXL1* ($n = 9$), *BCORL1* ($n = 6$), *CEBPA* ($n = 28$), *JAK2* ($n = 24$), *KIT* ($n = 26$), *KMT2A* ($n = 5$), *NOTCH1* ($n = 11$), *PHF6* ($n = 11$), *PTEN* ($n = 14$), and *ZRSR2* ($n = 6$).

In cases of MDS with $t(6;9)/DEK-NUP214$, *RAS* was mutated in five of ten (50%) patients assessed (four *NRAS* and one *KRAS*). Other genes mutated in MDS with $t(6;9)/DEK-NUP214$ included *DNMT3A* (2/7, 29%), *WT1* (2/8, 25%), *PTPN11* (1/4, 25%), *ZRSR2* (1/4, 25%), *PHF6* (1/5, 20%), *TET2* (1/7, 14%), and *IDH1* (1/8, 13%). No mutations were found in *ASXL1* ($n = 7$), *BCORL1* ($n = 4$), *CEBPA* ($n = 4$), *KIT* ($n = 8$), *KMT2A* ($n = 2$), *IDH2* ($n = 8$), *NOTCH1* ($n = 5$), and *NPM1* ($n = 8$).

FLT3-ITD mutation was detected in 61 of 79 (77%) cases assessed (Table 1). The frequency of *FLT3*-ITD mutation in MDS was lower than that in AML (10/19, 53% vs. 51/60, 85%, $p = 0.01$). Twelve cases of AML and one MDS had a *FLT3*-ITD ratio available, and four cases including the MDS case showed multiple peaks, whereas the remaining AML cases had a median ratio of 0.042 (range, 0.012–0.782). The median interval for patients with MDS associated with *FLT3*-ITD to develop AML was 16.1 months (range, 0.7–27.4). The three MDS patients without *FLT3*-ITD developed AML after 2.3, 9.5, and 20.7 months. Kaplan–Meier analysis for time interval from MDS diagnosis to AML progression was performed in the MDS group with *FLT3*-ITD mutation vs. without *FLT3*-ITD mutation; there was no difference in AML progression between these two groups ($p = 0.2$). In addition, five patients were found to have *FLT3* D835 mutations (three with MDS and two with AML).

Clinical outcomes

The median follow-up time was 24 months for the entire cohort (range, 1–160 months). After a median interval of 13 months (range, 1–43 months), 20 of 33 (61%) patients with MDS developed AML. Most of patients in this study received the 7 + 3 regimen during their disease

Fig. 3 Overall survival in AML patients with t(6;9) and MDS patients with t(6;9).

A No difference in OS between patients with MDS vs. patients with AML were noted (median: 39 vs. 24 months, $p = 0.18$).

B Patients who received HSCT had a longer OS than patients who did not (median: 62 vs. 17 months, $p < 0.0001$).

C Without HSCT, MDS patients with t(6;9) showed a better survival than AML patients with t(6;9) (median: 26 vs. 13 months, $p = 0.0035$).

D With HSCT, MDS patients with t(6;9) showed a similar survival than AML patients with t(6;9) (median: not reached vs. 48 months, $p = 0.14$).

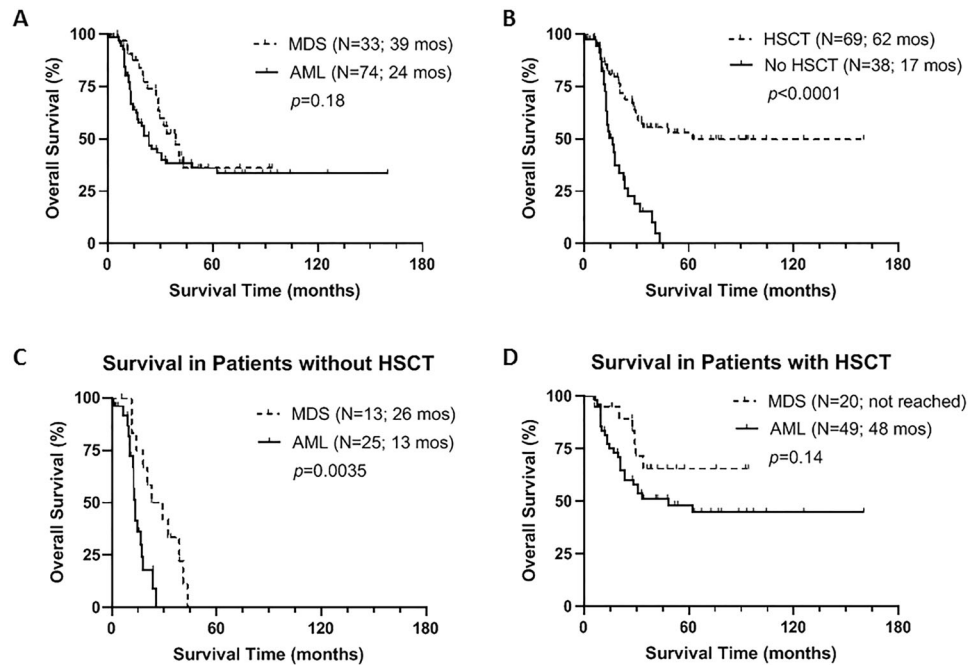


Table 2 Multivariate Cox-regression models for OS in patients with t(6;9)(p22;q34).

Patient group	Prognostic factors	Hazard ratio (95% CI)	p value
All patients ($n = 60$)	MDS vs. AML	0.45 (0.24–0.81)	0.008
	Transplantation	0.20 (0.11–0.36)	<0.0001
Patients without transplantation ($n = 29$)	MDS vs. AML	0.24 (0.09–0.64)	0.005
	Age (>60 years)	3.13 (1.37–7.14)	0.007

OS overall survival, CI confidence interval, MDS myelodysplastic syndrome, AML acute myeloid leukemia.

course, including 63 of 72 (88%) with AML and 24 of 33 (73%) with MDS ($p = 0.09$). In the 24 MDS patients treated with the 7 + 3 regimen, 5 patients were treated during their MDS disease phase, 14 patients during their AML disease phase, and 5 patients did not develop AML during their follow-up period. As expected, more patients with MDS received hypomethylating agent treatment than patients with AML (16/33, 49% vs. 12/72, 17%, $p = 0.0016$). Sixty-nine (64%) patients received HSCT during their clinical course with very similar rates between patients with MDS (20/33, 61%) and patients with AML (49/74, 66%) ($p = 0.66$).

At time of last follow-up, 43 of 74 (58%) AML and 17 of 33 (52%) MDS patients died. There was no difference in OS between patients with MDS and patients with AML (median, 39 vs. 24 months, $p = 0.18$; Fig. 3A). When further stratified by the status of transplantation, patients who received HSCT had a longer OS than those without HSCT (median, 62 vs. 17 months, $p < 0.0001$; Fig. 3B). Without HSCT, patients with MDS had a better OS than patients with AML (median, 26 vs. 13 months, $p = 0.0035$; Fig. 3C).

With HSCT, MDS and AML patients had a similar survival (median, not reached vs. 48 months, $p = 0.14$; Fig. 3D). In addition, to evaluate the effect of induction therapy on OS of patients with t(6;9)/DEK-NUP214, we compared patients who received 7 + 3 chemotherapy vs. those who did not. There was no statistically significant difference between these groups ($p = 0.4$).

Multivariate analyses using Cox-regression models were used to evaluate prognostic factors for OS in patients with MDS or AML with t(6;9)/DEK-NUP214. In an initial model that included 16 variables (see Statistical analysis in Materials and Methods), only initial diagnosis (MDS vs. AML) and transplantation were significant prognostic factors ($p = 0.008$ and $p < 0.0001$, respectively; Table 2). However, similar to the OS analysis, initial diagnosis of MDS vs. AML had a prognostic impact only in patients who were not treated with transplantation ($p = 0.005$; Table 2).

In addition, we compared OS of AML patients with t(6;9)/DEK-NUP214 in our cohort to a more general AML patients in a TCGA cohort, which utilized the European

LeukemiaNet standardized system for cytogenetic subtypes of AML [27, 28]. The analyses showed that the OS of AML patients with $t(6;9)/DEK-NUP214$ was similar to that of AML patients with intermediate cytogenetic risk (median, 24 vs. 27 months, $p = 0.7$), slightly better than that with unfavorable cytogenetic risk (median, 24 vs. 12 months, $p = 0.04$), and significantly worse than that with favorable cytogenetic risk (median, 24 vs. not reached, $p = 0.02$) in the TCGA database.

Discussion

Myeloid neoplasms with $t(6;9)/DEK-NUP214$ are rare. In over two decades, only 50 cases of AML and 16 cases of MDS were identified from at MDACC where over 18,000 BM specimens are processed annually. Although AML with $t(6;9)/DEK-NUP214$ is described as a distinct entity in the WHO classification, many aspects of this disease remain unknown, especially prognostic factors. MDS with $t(6;9)/DEK-NUP214$ has been studied even less. One cohort was reported in which the clinicopathologic features between $t(6;9)/DEK-NUP214$ -positive MDS and AML were compared; the authors suggested that myeloid neoplasms with $t(6;9)/DEK-NUP214$ may be best categorized as AML, irrespective of the blast percentage [9]. However, this study only included eight MDS patients and focused on a pediatric population. To the best of our knowledge, our study represents the largest cohort to characterize and compare the clinicopathologic features between MDS and AML associated with $t(6;9)/DEK-NUP214$, including cytogenetic and molecular findings as well as clinical outcomes.

In our study, MDS patients tend to be older than AML patients. No gender predilection was present in our cohort. The laboratory findings showed that MDS patients had a lower white blood cell count and a higher platelet count than AML patients; hemoglobin levels were not different. These data indicate that MDS patients had a lesser tumor burden than AML patients, likely attributable to their differences in blast count. Previous studies [1, 7] showed that AML with $t(6;9)/DEK-NUP214$ is morphologically and immunophenotypically similar to AML with maturation or acute myelomonocytic leukemia, which was confirmed in our study. In our cohort, about 60% of AML cases showed evidence of multilineage dysplasia, which may be a morphologic hint for the presence of $t(6;9)/DEK-NUP214$, although the frequency of multilineage dysplasia was lower when compared to MDS cases. Although some studies described basophilia as a pathologic feature in AML with $t(6;9)/DEK-NUP214$, basophilia was not present in neither the PB nor BM in most patients in this cohort, consistent with more recent publications [8–10].

The cytogenetic data in this study show that about three quarters of patients with MDS or AML had $t(6;9)$ as an isolated abnormality; only 10% had a complex karyotype. These results are in line with earlier studies and suggest that $t(6;9)/DEK-NUP214$ is a main driver in pathogenesis. Similar to an earlier study [12], trisomy 13 and trisomy 8 were the common additional abnormalities observed in cases without a complex karyotype in this cohort. Similar to previous studies [9, 12], the results in this study show that patients with $t(6;9)/DEK-NUP214$ frequently harbor a concomitant *FLT3*-ITD, although the rate was lower in MDS than AML. Multivariate analyses showed that *FLT3*-ITD is not a significant adverse prognostic factor for OS, concurring with some other studies [9, 10, 12]. *RAS* mutation, especially *NRAS*, was another frequent abnormality in both MDS and AML patients with $t(6;9)/DEK-NUP214$, as was reported by Visconte et al. [10]. Due to the inevitable limitation of a retrospective study, molecular profiles were not complete in our cohort. Further studies are necessary to elucidate the spectrum of molecular abnormalities in patients with MDS or AML associated with $t(6;9)/DEK-NUP214$.

Over half of the patients in this cohort died with a median follow-up of 24 months, despite being treated with aggressive chemotherapy regimens and HSCT. There was no difference in OS between MDS and AML patients in the entire cohort, but the survival curves did show a trend toward favorable survival in MDS patients at the earlier stage of diseases (Fig. 3A). The multivariate analysis on all patients with $t(6;9)/DEK-NUP214$ confirmed the prognostic significance of the initial diagnosis of MDS vs. AML after adjusting 15 potential confounding factors (Table 2). These results argue against the proposal that MDS associated with $t(6;9)/DEK-NUP214$ should be classified as AML irrespective of blast percentage. Our data confirm previous studies [12, 29–31] showing that HSCT significantly improve the survival of patients with myeloid neoplasms with $t(6;9)/DEK-NUP214$: 62 months of median survival in patients who received HSCT vs. 17 months in patients who did not ($p < 0.0001$). In contrast, induction therapy appears to have no effect on the survival ($p = 0.4$). Given the significant prognostic effect of HSCT in these patients, we performed further analyses by stratification according to transplantation status. Multivariate analyses showed that MDS patients with $t(6;9)/DEK-NUP214$ have a better survival than AML patients with $t(6;9)/DEK-NUP214$ among non-transplanted patients. In transplanted patients, OS was not significantly different, indicating that HSCT greatly improves clinical outcomes and may overcome the adverse effect of the initial diagnosis of AML on the survival.

In earlier studies, patients with AML associated with $t(6;9)/DEK-NUP214$ were thought to have a poor prognosis. In a cohort including 69 AML patients with

t(6;9) from 1987 to 2002 [1], the median survival was 14.4 months, worse than the median survival time of 24 months in our cohort. However, HSCT was performed in only 31% of their patients vs. 66% in our cohort. Another cohort of 178 AML patients with t(6;9) from 1989 to 2016 had a median OS of 27 months with a transplantation rate of 62%, which are comparable to our findings [12]. As our data has shown, transplantation has a significant prognostic effect on these patients, and may explain the differences in survival time between these studies. In addition, our analysis comparing our cohort with the TCGA AML database shows that patients with AML associated with t(6;9)/DEK-NUP214 that receive aggressive treatment strategies have a prognosis similar to AML patients in the intermediate-risk cytogenetic group. These data suggest that the poor prognosis of this disease may be overcome with modern intensive therapy.

In our opinion, MDS with t(6;9)/DEK-NUP214 does share some clinicopathologic characteristics with AML with t(6;9)/DEK-NUP214, including a comparable hemoglobin level, frequent multilineage dysplasia, FLT3-ITD, RAS mutation, and a high frequency of t(6;9)/DEK-NUP214 as an isolated abnormality. However, MDS and AML patients also differ in some aspects, especially the differences on OS. Therefore, we suggest that MDS with t(6;9)/DEK-NUP214 should not be categorized with AML with t(6;9)/DEK-NUP214. However, given that over half of MDS patients with t(6;9)/DEK-NUP214 developed AML, and that transplantation can greatly improve their clinical outcomes, early and aggressive treatment strategies such as HSCT may be beneficial for MDS patients associated with t(6;9)/DEK-NUP214.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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