CME/SAM

Acute Myeloid Leukemia With t(9;11)(p21-22;q23)

Common Properties of Dysregulated *Ras* Pathway Signaling and Genomic Progression Characterize De Novo and Therapy-Related Cases

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Key Words: Leukemogenesis; Mixed lineage leukemia (MLL); *Ras* Signaling; Treatment effects; microRNA; let-7

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Upon completion of this activity you will be able to:
- describe the salient clinicopathologic and molecular genetic features of acute myeloid leukemia with t(9;11)(p21-22;q23), including factors that may contribute to leukemogenesis and/or leukemic progression.
- discuss similarities and differences between de novo and therapy related disease with t(9;11)(p21-22;q23).

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Questions appear on p 815. Exam is located at www.ascp.org/ajcpcme.

Abstract

*We compared pathogenetic features of 32 de novo and 29 therapy-related (t) t(9;11)(p21-22;q23)/*MLLT3-MLL *acute myeloid leukemia (AML) cases to identify progression factors and to assess whether distinction between these manifestations is warranted.* MLLT3-MLL *rearrangement was commonly the sole karyotypic abnormality at diagnosis, with many secondary chromosomal changes emerging at relapse in both subgroups.* Ras *point mutations were common in both groups (overall, 18/50 [36%]) and associated with monocytic phenotype and aneuploid progression. Expression patterns of 675 microRNAs profiled in 7 cases were also similar, with let-7 species linked to* Ras *down-modulation expressed at low levels. Outcome for both groups was poor (relapsed or refractory in 49/61 [80%] cases); however, patients with t-AML were generally older and female, with worse outcome (*P = .03*), likely secondary to t-AML mostly arising in patients with breast cancer following topoisomerase inhibitor–containing chemotherapy. Ras activation seems to complement the* MLLT3-MLL *oncogene in transformation with features of de novo and t-AML with* MLLT3-MLL *being similar.*

The mixed lineage leukemia (*MLL*) gene located at chromosome 11q23 encodes a transcription factor with pleiotropic effects on hematopoietic gene expression and chromatin remodeling.[1] *MLL* fusion with 1 of more than 50 different known chromosomal partners produces acute leukemias with a nearly uniform poor outcome.[2,3] The t(9;11)(p21-22;q23) chromosomal translocation and its variants, hereafter described as t(9;11)-AML, fuses the *MLL* gene with *MLLT3* (also known as *AF9* or *LTG9*) and is the most common *MLL* translocation producing acute myeloid leukemia (AML). t(9;11)-bearing-AML can occur as a primary neoplasm (de novo) or secondarily as a result of previous therapy (t-AML), most commonly with topoisomerase II inhibitors.[4,5] Previous studies on the tumorigenesis of the *MLLT3-MLL* fusion indicate that it mostly produces AML with a monocytic immunophenotype and has an aggressive course with frequent relapse and short survival.[3,4]

The 2008 classification of the World Health Organization (WHO) divides AML into various prognostic categories based on clinical, morphologic, immunophenotypic, and molecular genetic alterations.[6] De novo AML with t(9;11) is classified under the WHO heading of "AML with recurrent genetic abnormalities," whereas t-AML with t(9;11) is placed separately in the category of "therapy-related myeloid neoplasms."[5,7] In this study, we assessed whether this separation of de novo and therapy-related cases was warranted by comparing clinical, morphologic, immunophenotypic, genetic, and epigenetic features and found similar patterns in both

groups. By using karyotype, genomic profiling, and mutation analysis, we also identified genetic factors contributing to leukemogenesis and disease progression in t(9;11)-AML.

Materials and Methods

Patients and Workup

This institutional review board–approved study included 61 patients with acute leukemias harboring t(9;11)(p21-22;q22) seen at our institution between 1995 and 2007. Diagnoses were based on current WHO classification criteria using a combination of clinical and morphologic features and cytochemical, flow cytometric (FC), and immunohistochemical methods **Table 1**.[6]

Separation of our patient cohort into de novo vs t-AML was based on the definitions provided by the current WHO classification.[6] All patients with t-AML were previously treated for a prior malignancy with chemotherapy and/or radiation **Table 2**. Presence of myeloid, erythroid, and/or megakaryocytic dysplasia was based on morphologic criteria outlined in the current WHO classification. Cytochemical stains for myeloperoxidase (a marker of differentiated myeloid blasts) and nonspecific esterase/butyrate esterase (NSE; a marker of differentiated monoblasts and monocytes) were used to establish blast lineage.

A standard FC panel was performed on diagnostic bone marrow (BM) aspirate samples, including the markers CD3, CD4, CD7, CD8, CD10, CD13, CD14, CD15, CD19, CD20, CD34, CD38, CD64, and CD117, as well as HLA-DR and

Table 1
Clinicopathologic Features of 61 Patients With AML Harboring the *MLLT3-MLL* Translocation

	No. of Cases*	*P* for Correlates of Poor OS	No. of t-AML Cases*	No. of De Novo Cases*	*P* for De Novo vs t-AML
Total	61	—	29	32	NS
Sex					
Male	22	NS	6	16	
Female	39		23	16	.04
Median (range) age (y)	50 (1-81)	.0005 (for >60 y)	57 (12-81)	42 (1-76)	.01
Median (range) OS (mo)	11.3 (0.2-98.1)	—	4.7 (0.2-79.9)	15.3 (0.2-98.1)	.03
Clinical course†					
Relapsed/refractory‡	49	—	26	23	NS
Alive	12	—	3	9	NS
Died of disease§	49	—	26	23	NS
Durable remission	8	—	2	6	NS
Median (range) initial WBC ($\times 10^9$/L)‖	5.4 (0.4-287)	.029 (for >15 $\times 10^9$/L)	4.2 (0.4-260)	9.8 (0.8-287)	NS
Median (range) initial platelet count ($\times 10^9$/L)‖	61 (7-356)	NS	56 (7-240)	76 (9-356)	NS
Percentage (range) initial peripheral blood blasts	40 (0-94)	NS	48 (0-91)	38 (0-94)	NS
No. (%) FAB subtype					
M0	3 (5)	—	2 (7)	1 (3)	NS
M1	9 (15)	—	2 (7)	7 (22)	NS
M2	8 (13)	—	7 (24)	1 (3)	NS
M4	16 (26)	—	9 (31)	7 (22)	NS
M5	25 (41)	—	9 (31)	16 (50)	NS
MPO (>20%)	23	NS	12	11	NS
NSE (>20%)	34	.025	16	18	NS
No. (%) with karyotype at diagnosis (with progression)					
MLLT3-MLL only	40 (23)	NS	17 (13)	23 (10)	NS
Other changes	21 (38)	NS	11 (22)	10 (16)	NS
Mutational analysis					
No./total tested (%) with *Ras* mutation	18/50 (36)	NS	9/22 (41)	9/28 (32)	NS
KRAS, NRAS, both	10, 5, 3		6, 1, 2	4, 4, 1	
No./total tested with *FLT3* ITD	3/48	.08	2	1	NS
No./total tested with *FLT3 TK* mutation	1/48	NS	0	1	NS

AML, acute myeloid leukemia; *FLT3 TK,* codon 835/836 kinase domain mutation; ITD, internal tandem duplication; MPO, myeloperoxidase; NS, not statistically significant; NSE, nonspecific esterase; OS, overall survival.

* Unless otherwise indicated.

† Initial therapies included cytarabine-containing regimens (27 idarubicin/cytarabine; 8 fludarabine/cytarabine; 3 dexamethasone, cytarabine, 6-thioguanine, etoposide, and daunorubicin; 3 topotecan and cytarabine; 2 with liposomal daunorubicin/topotecan/thalidomide/cytarabine; and 1 low-dose cytarabine), 7 with demethylating agents (3 azacytadine,4 decitabine), 3 with gemtuzumab ozogamicin (Mylotarg) with or without etoposide, 1 with daunorubicin/etoposide, 4 with investigational agents (2 VNP-40101M, 1 imatinib and LDAC), 1 with anti-CD33 immunotherapy (HUM-195/rGel), and 1 with hydroxyurea only. Allogeneic stem cell transplantation was done in first remission in 11 patients.

‡ Salvage therapies at relapse included cytarabine alone (1), azacitidine (1), dacarbazine-containing regimens (3), cenersen sodium (1), topotecan/cytarabine (2), liposomal daunorubicin (1), etoposide/cytarabine/clofarabine (1), mitomycin/etoposide (1), gemtuzumab ozogamicin with or without etoposide (5), azathioprine (4), etoposide-containing regimens (1), HUM-195/rGEL (1), idarubicin/clofarabine (2), anti-BCL2 therapy (2), allogeneic stem cell transplantation (6), and unknown (1).

§ Deaths due to disease progression (n = 44), chemotherapy complications (n = 3), or transplant-related complications (n = 2).

‖ Values for WBC and platelet counts are given in Système International values; conversions to conventional values are as follows: WBC count (/μL), divide by 0.001; platelet count ($\times 10^3$/μL), divide by 1.0.

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terminal deoxynucleotidyl transferase. Monocytic morphologic features and NSE positivity were correlated with CD14, CD56, and CD64 expression. For statistical analysis, marker positivity was defined as expression in at least 10% of the blast population, with higher expression being defined as expression in greater than 75% of the blast population.

Cytogenetic, Genomic, and Mutation Analysis

Conventional G-banded karyotype analysis was performed on short-term cultures of BM aspirate. Fluorescence in situ hybridization for the *MLL* gene rearrangement was performed in a subset of 20 cases on metaphase spreads using a commercially available *MLL* break-apart probe (Abbott Molecular/Vysis, Downers Grove, IL). In a subset of samples, array-based comparative genomic hybridization (CGH) was performed on genomic DNA extracted from diagnostic BM samples containing at least 30% blasts. CGH was done using a 44,000-feature, customized, 60-mer oligonucleotide array (4 × 44 format, Agilent, Santa Clara, CA) using a mixed normal female DNA control (Promega, Madison, WI) as the reference sample, as previously described.[8] Following laser scanning and feature extraction of the hybridized arrays, aberration calls were made using Nexus Copy Number 3.1 (Biodiscovery, El Segundo, CA) and compared by visual inspection using the Nexus viewer. Aberrations spanning fewer than 4 contiguous probes were not regarded as definitive.

FLT3 codon 835/836 and internal tandem duplication mutational status was performed by polymerase chain reaction (PCR)-based amplification of genomic DNA and capillary electrophoresis as previously described.[9] Codons 12, 13, and 61 of *KRAS* and *NRAS* were sequenced following PCR amplification by using the Sanger or a pyrosequencing-based method as previously described.[10,11]

MicroRNA Expression Profiling

RNA from diagnostic BM aspirate samples, available in 7 cases, was reverse transcribed to complementary DNA by using Megaplex RT primers and the TaqMan Micro RNA reverse transcription kit according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). Complementary DNA was loaded onto a TaqMan Human MicroRNA Array, v.2.0 (Applied Biosystems), centrifuged, and sealed. Each sample was run on 2 microfluidic cards (pool A and pool B) that assessed expression levels of 675 unique human microRNAs (miRNAs) via separate real-time PCR reactions. miRNA levels were quantified by using the PCR 9700 Thermocycler ABI Prism 7900HT and the sequence detection system (Applied Biosystems) according to the manufacturer's instructions.

Statistical Analysis

Clinical features (age, sex, relapse status, time to relapse, persistent disease, transplant status, and presence of prior malignancy), hematologic features (initial blood leukocyte, blast, and platelet counts; hemoglobin levels; and initial percentage of BM blasts), morphologic features (presence of dysplasia, cytochemical or flow cytometric evidence of monocytic differentiation, French-American-British subtype, and flow cytometric immunophenotype), cytogenetic findings, and molecular genetic features were recorded as categorical variables to assess their impact on overall survival (OS) and progression-free survival (PFS) by the Kaplan-Meier method in combination with the log-rank test using Statistica 6 software (StatSoft, Tulsa, OK). Associations between clinical and pathologic features were determined through Kruskal-Wallis and Fisher exact tests. The Cox proportional hazards regression method was used for multivariate analyses. All reported *P* values were 2-sided and obtained by using the Statistica and/or SPSS software package (SPSS 15.0 for Windows, SPSS, Chicago, IL).

We analyzed miRNA expression data by using the R statistical package. Of 675 miRNAs, 297 were removed from the analysis because they were expressed at a very low level for at least 5 of 7 samples. For the remaining 378 miRNAs, the expression level of each miRNA was calculated by the Δ cycle threshold (Ct) method. The ΔCt value was obtained by normalizing against the median Ct value of all test miRNAs for each sample. Differentially regulated miRNAs were identified as follows: First, the mean ΔCt value was calculated

Table 2
Relation of Prior Malignancies to 29 Cases of Treatment-Related t(9;11) Acute Myeloid Leukemia

Primary Tumor	No. (%) of Cases	Regimens With Topoisomerase Inhibitors	Agents Used	Median Time to Leukemia, mo (Range)
Breast carcinoma	18 (62)	17*	Doxorubicin (14); epirubicin (3)	25 (10-147)
Sarcoma	4 (14)	4	Doxorubicin (3); irinotecan (1)	21 (12-38)
Large B-cell lymphoma	3 (10)	2*	Doxorubicin (2)	26 (24-26)
Hodgkin lymphoma	2 (7)	2	Doxorubicin (2)	24
Testicular carcinoma	1 (3)	1	Etoposide	72
Prostate carcinoma	1 (3)	0	—	24

* Treatment regimen unknown in the remaining case.

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for each miRNA. Then the median (M) and median absolute deviation (MAD) of all mean ΔCt values was computed. The miRNAs of the mean ΔCt values that were larger than M + 2 × MAD were defined as being highly expressed, and mean ΔCt values that were less than M – 2 × MAD were defined as having low expression.

Student *t* tests were performed to compare the expression difference for each miRNA between various subgroups (eg, t-AML vs de novo and *Ras*-mutated vs not mutated). The Benjamini-Hochberg method was used to maintain a false discovery rate at the 0.1 level.

Results

Summary of Demographic Features and Outcome

The 61 patients (22 males and 39 females) with t(9;11)-AML had a median age of 50 years (range, 1-81 years). There were 29 secondary cases occurring following therapy for other malignancies (t-AML) and 32 cases arising de novo (Table 1). Among t-AML cases, the most common preceding malignancy was breast carcinoma (21/35 [60%]) and 30 (86%) of 35 were treated with topoisomerase II inhibitors (mostly doxorubicin and epirubicin), including 25 with a regimen also containing alkylating agents (Table 2).

Following development of AML, most patients were treated with multiagent chemotherapy, most commonly anthracycline and cytarabine in 44 patients, followed by stem cell transplantation at first remission in 12 and after relapse in an additional 7 patients (see Table 1 footnote). The median OS was 11.3 months (range, 0.2-98.1 months), with 16 patients having persistent disease following therapy and 33 with relapses at a median time from initial diagnosis of 9.5 months (range, 0.5-72.5 months). At last follow-up, 49 (80%) of 61 patients had died of disease or transplant complications. Older age was associated with decreased PFS and OS (P = .0005; Table 1). There was not a strong correlation between outcome and initial hematologic features except for an adverse OS for patients with initial WBC counts of more than 15,000/μL (15×10^9/L; P = .029; Table 1).

Correlation of Immunophenotypic Findings With Outcome

Most t(9;11)-AML cases showed at least partial monocytic differentiation in the blasts, including French-American-British type M4 in 16 (26%) and M5 in 25 (41%) of 61 cases (Table 1). Biphenotypic lymphoid/myeloid immunophenotypes were not observed. The most commonly expressed markers included CD13, CD15, CD33, CD38, CD64, and HLA-DR, whereas CD14, CD117, and myeloperoxidase showed more variable expression **Table 3**. Of the 61 cases, 41 (67%) expressed at least 1 marker of monocytic differentiation, whereas an immature blast phenotype was rare, with only 15 (25%) of 60 cases expressing CD34. Terminal deoxynucleotidyl transferase and CD56 expression were rare. In univariate analysis, cases with partial or complete monocytic differentiation were associated with shorter OS, when assessed by surface CD14 expression by FC (P = .017) **Figure 1**, CD64 by FC (P = .07), or NSE cytochemical staining (P = .025).

Cytogenetic and Molecular Features

At diagnosis, t(9;11) (or related derivative chromosomes due to extra or complex *MLLT3-MLL* translocations) was the sole cytogenetic abnormality in 40 (66%) of 61 cases, with

Table 3
Marker Expression by Flow Cytometry in Blasts From t(9;11)-AML Cases at Diagnosis*

Marker	Total (n = 61)	Therapy-Related AML (n = 29)	De Novo AML (n = 32)
CD33	58/59 (98)	27/28 (96)	31/31 (100)
CD38	57/58 (98)	27/28 (96)	30/30 (100)
CD15	14/16 (88)	6/7 (86)	8/9 (89)
CD64	53/60 (88)	22/28 (79)	31/32 (97)
CD13	50/60 (83)	25/28 (89)	25/32 (78)
HLA-DR	49/60 (82)	22/28 (79)	27/32 (84)
CD117	41/60 (68)	19/28 (68)	22/32 (69)
MPO	25/39 (64)	11/16 (69)	14/23 (61)
CD14	11/41 (27)	8/18 (44)	3/23 (13)
CD34	15/60 (25)	9/28 (32)	6/32 (19)
CD56	6/41 (15)	3/18 (17)	3/23 (13)
TdT	1/57 (2)	0/27 (0)	1/30 (3)

AML, acute myeloid leukemia; MPO, myeloperoxidase; TdT, terminal deoxynucleotidyl transferase.
* Data are given as number positive/total tested (percentage).

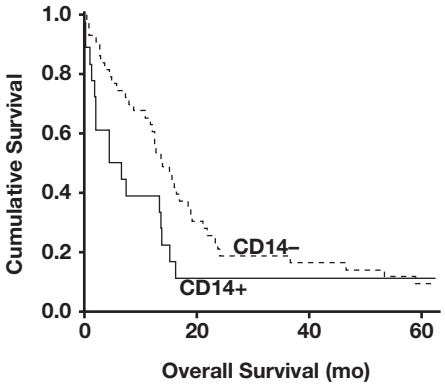

Figure 1 Kaplan-Meier survival curves. Monocytic differentiation in leukemic blasts, as assessed by flow cytometric expression of CD14, is associated with decreased overall survival in t(9;11) acute myeloid leukemia (P = .017).

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the most common additional cytogenetic changes at diagnosis being +8 in 7 (33%), +6 in 2 (10%), and +21 in 2 (10%) of 21 cases. Array CGH analysis performed in 15 cases with sole t(9;11) at diagnosis demonstrated no other detectable genomic aberration in 11 cases. In 1 case, +8 not detected by karyotype was shown, and 3 showed several small loci of extra chromosomal material, with gains of chromosome 1p13 spanning the *NOTCH2* gene and chromosome 4q28 spanning *PLK4* seen in 2 cases each.

With disease progression and/or relapse, repeated karyotypes demonstrated 25 (74%) of 34 cases with other chromosomal changes unrelated to the *MLLT3-MLL* translocation **Figure 2**. These additional chromosomal changes were heterogeneous, including exclusively chromosome copy number aberrations (most commonly +8 in 3 and +21 in 2 cases), structural chromosomal changes, or complex karyotypes with different types of aberrations. The presence of additional karyotypic changes at diagnosis besides *MLLT3-MLL* translocation (detected by CGH or karyotype) was not significantly associated with an adverse outcome.

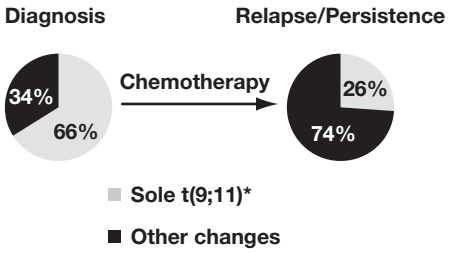

Figure 2 High rates of cytogenetic progression in t(9;11) acute myeloid leukemia (AML). At diagnosis, 66% (40/61) of patients demonstrated t(9;11) [*or derivative chromosome related to t(9;11)] as the sole cytogenetic abnormality. Of 34 patients with sequential karyotypes, 74% (25/34) showed a more complex karyotype at the time of leukemic relapse or persistence.

Table 4
Correlations Between Genetic and Immunophenotypic Features in t(9;11) Acute Myeloid Leukemia

Findings/Association	*P*
Mutational status	
NRAS or *KRAS* mutation	
Monocytic differentiation (>20% NSE+)	.05
CD14 expression in blasts by FC	.01
Any *Ras* or *FLT3* mutation	
Aneuploid changes on karyotype	.01
Shorter OS	.07
Cytogenetic findings	
Aneuploid changes on karyotype	
Decreased PFS	.04
Increased HLA-DR expression by FC	.02
Increased MPO expression by FC	.03
FLT3 or *NRAS/KRAS* mutation	.01

FC flow cytometry; MPO, myeloperoxidase; NSE, nonspecific esterase; OS, overall survival; PFS, progression-free survival.

Mutation of *KRAS* or *NRAS* was found at diagnosis in 18 (36%) of 50 cases and correlated with monocytic blast phenotype (CD14+, $P = .01$; NSE+, $P = .05$) **Table 4**. *FLT3* mutations were identified in 4 (8%) of 48 cases overall, including 3 with t-AML, and were mutually exclusive with *Ras* mutations. The few cases with *FLT3* mutation had an aggressive clinical course consisting of persistent disease or relapse, but this observation was not statistically significant. The blasts in *Ras*-mutated cases rarely expressed CD34, and +8 was more common in cases with *FLT3* or *Ras* mutations compared with cases without either change (7/18 vs 1/4 vs 2/24, respectively); +21 was also associated with *Ras* mutation (3/5 cases) but not with *FLT3* mutation (1/4) or cases without either (1/24). Overall, *Ras* or *FLT3* mutations were much more commonly associated with cases showing single chromosome aneuploidy (mostly +8 and +21; $P = .01$) and had a trend toward decreased OS ($P = .07$), supporting a complementary role with *MLLT3-MLL* of *Ras* pathway activation in oncogenesis.

Given this apparent association between growth factor mutations and aggressive course **Figure 3**, we assessed whether a *Ras*/*FLT3*-driven miRNA expression pattern could be identified in 7 cases with adequate RNA and high blast counts. By profiling 675 miRNAs using a TaqMan-based quantitative PCR method, we noted low expression of members of the let-7 miRNA family, including hsa-let-7f, hsa-let-7g, and hsa-let-7i (all P values $< .005$), which are known to modulate the *Ras* pathway.[12] Higher expression of miRNA-92a was seen in *Ras*-mutated (n = 3) as compared to not mutated (n = 4) cases ($P = .04$). There were no statistically significant differentially expressed miRNAs between de novo AML (n = 2) and t-AML (n = 5). We also evaluated the contribution of aneuploidy to miRNA expression; 6 (30%) of 20 of the most highly expressed miRNAs were located on chromosome 3 (not commonly involved in copy number alterations), whereas miRNAs located on the frequently aneuploid chromosomes 6, 8, and 21 were not overexpressed except in 1 case each **Table 5**.

Similarities and Differences Between De Novo and Therapy-Related Cases

In univariate analysis, patients with t-AML were more likely to be women ($P = .04$), older ($P = .01$), and to have an inferior outcome (median survival, 4.7 vs 14.7 months for de novo disease; $P = .03$) **Figure 4**. There were no other statistically significant differences between de novo and t-AML with regard to clinical features; immunophenotype; morphologic, cytogenetic, and molecular genetic features; or miRNA expression. These included a similar incidence

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Table 5
Highly Expressed miRNAs in t(9;11) Acute Myeloid Leukemia

hsa-miRNA	Normalized Mean Expression	Standard Deviation	*P*	Genomic Location (Chromosome)
30a	107.83	1.90	.004	6
15b	64.92	1.34	.006	3
374b	28.91	1.88	.01	X
195	25.70	1.61	.005	17
28-3p	25.02	1.85	.009	3
451	24.06	1.58	.002	17
25	23.99	1.52	.007	7
425*	23.22	2.14	.007	3
30d	17.62	2.08	.0002	8
20a*	17.14	1.72	.005676	13
565	13.03	1.63	.003	3
103	11.25	1.47	.0001	5
106b*	10.29	2.47	.008	7
532-5p	10.08	2.02	.009	X
28-5p	9.84	1.73	.004	3
532-3p	8.66	1.72	.003	X
15b*	8.49	2.09509	.0004	3
145	8.359569	1.98	.009	5
328	8.31	2.27	.008	16
34a*	8.31	2.14	.0004	1

miRNA, microRNA.

of *Ras* mutations in t-AML and de novo cases (9/22 vs 9/28, respectively) and a similar distribution of *NRAS* vs *KRAS* mutation sites. In a Cox proportional hazards regression model incorporating significant or borderline significant factors predicting for OS in univariate analysis (from Table 1 and Table 4: age > 60 years, female sex, t-AML, initial WBC count > 15,000/μL [15 × 10^9/L], markers of monocytic differentiation, *FLT3*/*Ras* mutation, and stem cell transplant status), only no transplant (relative risk [RR], 13.6; *P* = .0002), older age (RR, 2.6; *P* = .008), high WBC count (RR, 2.1; *P* = .03), and NSE positivity in blasts (RR, 1.5; *P* = .04) operated as adverse predictors after adjustment of other covariates, with sex and t-AML status eliminated.

Discussion

We confirm an aggressive clinical course in nearly all cases of t(9;11)-AML, with frequent and rapid relapses following initial response to anthracycline/cytarabine-based chemotherapy. Although the current WHO classification separates t-AML and de novo AML into separate categories, we demonstrate that the ranges of morphologic, immunophenotypic, and genetic features are similar between these 2 groups in t(9;11)-AML. Shared properties included frequent monocytic differentiation in blasts (67% M4/M5), common absence of CD34 expression in blasts (~75%), frequent mutation in *NRAS* or *KRAS* (36%), and low incidence of *FLT3* mutation (8%) compared with other AML subtypes.

The major differences noted were that treatment-related cases were more likely to be in older patients and occur in women and had a slightly worse outcome, which was not significant in a multivariate model that incorporated age and blast phenotype. Indeed, all of these differences in t-AML could be attributable to the finding that the majority of these cases arose in patients with advanced-stage breast cancer treated with topoisomerase inhibitors, which is a disease setting preferentially associated with older women.[13,14] In a review of other t-AML cases diagnosed at our institution during the same period, 62.0% of patients with t(9;11) t-AML had previously treated breast cancer compared with only 18.8% of patients with t-AMLs with any other cytogenetic

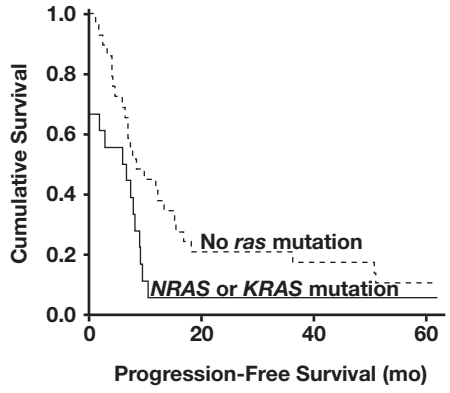

Figure 3 Kaplan-Meier survival curves for t(9;11) acute myeloid leukemia (AML) with point mutation *Ras* genes. Mutation in *NRAS* or *KRAS* is associated with decreased progression-free survival (*P* =.042; Kruskal-Wallis method).

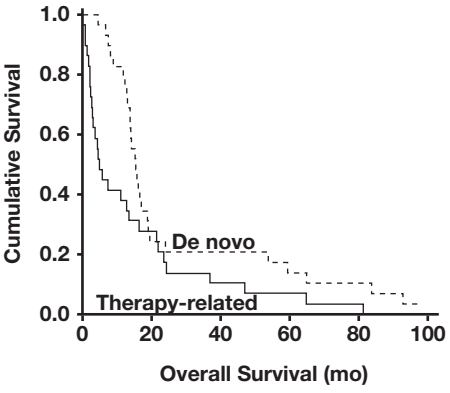

Figure 4 Kaplan-Meier survival curves. Therapy-related t(9;11) acute myeloid leukemia has a worse outcome than de novo cases (*P* =.03).

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change ($P < .0001$). This strongly suggests that topoisomerase inhibitors used at the dose and duration common in breast cancer regimens are particularly likely to drive *MLL* translocations and that most if not all differences between secondary and de novo t(9;11)-AML are related to the prior therapy setting. We thus find little evidence for intrinsic leukemia differences between de novo and t-AML cases that would warrant separate recognition.

The reasons for such poor outcomes in t(9;11)-AML appear primarily related to a high rate of rapid relapses following successful induction chemotherapy. These relapses, which often occurred in 1 to 4 months following successful induction therapy, frequently contained additional karyotypic changes in contrast with the sole *MLLT3-MLL* translocation commonly present at initial diagnosis. Indeed, array CGH performed on a subset of the diagnostic samples confirmed the absence of other preexisting genomic aberrations, making the rapid clonal progression even more remarkable. These results suggest that there may be a high intrinsic sensitivity of t(9;11)-bearing blasts to chromosome missegregation following chemotherapy exposure. A direct effect of the MLL protein on overriding the checkpoint block signals that arise following exposure to DNA-damaging cytotoxic chemotherapy is one possible mechanism for this effect.[15]

In mouse models, the *MLLT3-MLL* gene fusion has been shown to disrupt proliferation and survival of some myeloid progenitors, thereby indirectly driving monocyte outgrowth,[16] but the fusion appears insufficient in these models to directly induce leukemia.[17] Complementing genetic events in AML often include growth factor mutations that can be subtype-specific, such as the association of *KIT* mutations with inv(16)- and t(8;21)-bearing AML. Our study implicates Ras pathway dysregulation as a common secondary transforming factor in *MLLT3-MLL*-AML, either through *Ras* point mutations[18-20] or possibly by down-regulation of let-7 miRNA species. The effects of Ras activation on tumor phenotype are complex, with effects on suppressing apoptosis and arrest of hematopoietic differentiation.[7,18-22] In that regard, *Ras* mutations have been shown to synergize with *MLLT3-MLL* in vitro by suppressing apoptosis and blocking differentiation.[23] Let-7 miRNA alterations have also been commonly reported in other AML subtypes with simple karyotypes,[12,24-26] and it remains to be determined whether these effects are mediated through or influenced by aberrant Ras signaling.[27]

Although *Ras* mutations are common in all AML subtypes with monocytic differentiation, their prognostic significance has been difficult to demonstrate.[28] We show herein that *Ras* mutation was associated with a modest adverse effect on PFS in t(9;11)-AML (Figure 3), suggesting that blockade of this pathway may improve outcomes. Farnesyltransferase inhibitors can inhibit Ras signaling (at least in vitro) and have shown some single-agent efficacy in myeloproliferative neoplasms harboring *Ras* mutations, such as chronic myelomonocytic leukemia,[29] and possibly in combination therapy in AML.[30] If the frequent Ras pathway dysregulation is confirmed in subsequent studies, specific targeting of the t(9;11)-AML subtype with adjuvant farnesyltransferase inhibitors may be warranted. Given the rapid relapses and high rate of genomic progression following chemotherapy, adjuvant strategies to reduce the level of residual disease are likely to be critical to improving outcome in t(9;11)-AML.

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