Adverse karyotype subcategories in acute myeloid leukemia display significant differences in mutation composition and transplant-augmented survival

The 2017 European LeukemiaNet (ELN) genetic risk stratification for acute myeloid leukemia (AML) considers three cytogenetic categories: favorable, intermediate, and adverse karyotype (AK);[1] the latter includes t(6;9)(p23;q34.1), *DEK-NUP214*; t(v;11q23.3), *KMT2A* rearranged; t(9;22) (q34.1;q11.2), *BCR-ABL1*; inv(3)(q21.3q26.2)/t(3;3)(q21.3; q26.2), *GATA2, MECOM(EVI1)*; −5; del(5q); −7; −17; abn(17p); and complex (CK) or monosomal (MK) karyotype.[1] CK was defined as three or more unrelated abnormalities that do not include t(8;21), inv(16)/t(16;16), t(9;11), t(v;11q23,3), t(6;9), or inv(3)/t(3;3)/t(9;22)(q34.1;q11.2). MK was defined by the presence of an autosomal monosomy, in association with at least one additional autosomal monosomy or structural chromosome abnormality, other than those defining core binding factor (CBF) AML or acute promye-

locytic leukemia.[1] The prognostic heterogeneity among ELN-2017 AK subgroups was reiterated in a recent study that suggested classifying inv(3)(q21.3q26.2)/t(3;3)(q21.3; q26.2), *TP53* and/or 17p abnormalities, and CK, as "very adverse-risk".[2]

The primary objective of the current study was to seek out differences in outcome prediction and mutation composition among ELN-2017 AK subcategories for AML. Our study population was recruited from Mayo Clinic databases following approval from Institutional Review Board and documentation of newly diagnosed AML treated with intensive induction chemotherapy. The latter typically consisted of "7+3" regimens, with a minority of patients receiving, in addition, midostaurin or other drugs, depending on the mutation type; patients induced with less inten-

Table 1. Presenting clinical and laboratory characteristics of 189 acute myeloid leukemia (AML) patients with European LeukemiaNet adverse karyotype; a separate cohort of 325 AML patients with normal karyotype is included as a reference; rare adverse variants included inv(3)/t(3;3);i(17) and 17(p) abnormalities. *P* value provided is for comparisons among adverse karyotype categories. CR/Cri: complete remission with incomplete hematologic recovery.

Variables	Mono-somal N=103	Complex N=36	Rare adverse variants N=5	-7 N=9	-5/5q- N=7	t(v;11q23.3) N=13	t(6;9) N=8	t(9;22) N=8	*P* value	Normal karyotype N=325
Median age (range), years	61 (24-79)	63 (23-78)	62 (44-74)	65 (27-67)	60 (40-81)	57 (22-69)	40 (20-58)	50 (26-73)	0.0002	60 (18-82)
Males, N (%)	55 (53)	23 (64)	3 (60)	6 (67)	5 (71)	8 (61)	2 (25)	5 (62)	0.6	186 (57)
AML subtype, N (%) Primary Secondary Therapy-related	40 (39) 53 (51) 10 (10)	14 (39) 16 (44) 6 (17)	1 (20) 4 (80) 0	4 (44) 4 (44) 1 (12)	3 (43) 3 (43) 1 (14)	13 (100) 0 0	7 (87) 1 (13) 0	4 (50) 4 (50) 0	0.001	248 (76) 55 (17) 22 (7)
Median leukocytes x10⁹/L (range)	3.2 (0.2-169)	7.5 (0.7-113)	6.7 (1.7-59.6)	7.8 (1.2-137)	1.8 (0.5-44)	9.1 (0.9-76.4)	11.4 (2-40)	68.9 (1-144)	0.01	14.6 (0.3-350)
Median platelets x10⁹/L (range)	39 (3-431)	50 (7-223)	77 (54-112)	22 (5-943)	70 (10-178)	66 (25-298)	46 (28-85)	87 (36-432)	0.3	64 (3-471)
FLT3-ITD; mutated/tested, N (%)	0/34 (0)	0/15 (0)	0/1 (0)	0/4 (0)	0/3 (0)	0/5 (0)	7/7 (100)	0/1 (0)	<0.0001	73/220 (33)
TP53, mutated/tested (%)	8/13 (62)	3/6 (50)	0/0 (0)	0/2 (0)	0/0 (0)	0/2 (0)	0/3 (0)	0/0 (0)	0.04	1/78 (1)
CR/CRi, N (%)	55 (53)	22 (61)	3 (60)	6 (67)	5 (71)	11 (85)	8 (100)	6 (75)	0.04	285 (88)
Transplanted, N (%)	30 (29)	8 (22)	1 (20)	4 (44)	3 (43)	8 (61)	6 (75)	2 (25)	0.04	104 (32)
Deaths, N (%)	96 (93)	34 (94)	5 (100)	7 (78)	6 (86)	7 (54)	2 (25)	4 (50)	<0.0001	190 (58)

sive regimens, such as hypomethylating agents with or without venetoclax, were excluded. Consolidation chemotherapy often utilized high-dose cytarabine. Treatment period spanned from January 2004 through August 2020 and follow-up information was updated as of April 2022. Conventional criteria were used to diagnose AML, assign cytogenetic risk category, and classify treatment response.[1,3] Survival analyses were performed without censoring for allogeneic hematopoietic stem cell transplant (AHSCT). Conventional methods were used for cytogenetic and molecular studies, including next-generation sequencing (NGS).[4] Statistical analysis was performed using JMP Pro 16.0.0 software package, SAS Institute, Cary, NC. We recruited 189 intensively treated AML patients (median age 61 years; 57% males) with ELN-2017 AK, from a database of 758 intensively treated AML cases. Our operational hierarchy of AK group designation was i) MK excluding CBF abnormalities (n=103); ii) CK, not including MK, t(8;21), inv(16)/t(16;16), t(9;11), t(v;11q23.3), t(6;9), inv(3)/t(3;3) or t(9;22) (n=36); iii) non-monosomal karyotype with specific adverse variants (n=50). The latter included t(9;22) (n=8); t(6;9) (n=8); rare adverse variants (RAV), including two cases of inv(3), one of t(3;3), one of isochromosome 17, and one of 17(p) abnormalities (n=5); t(v;11q23.3), excluding t(9;11) (n=13); -5/5q- (n=7); and monosomy 7 (n=9). With regards to cases with t(9;22), in order to minimize ambiguity of disease definition,[5] we included all cases regardless of antecedent history of chronic myeloid leukemia (CML). Of note, 96 (93%) of the 103 patients with MK harbored ≥3 abnormalities. The number of informative patients for *FLT3*-ITD mutation was 70 (7 mutated), *NPM1* 58 (3 mutated), *CEBPA* 44 (3 mutated), *TP53* 26 (11 mutated), *DNMT3A* 25 (3 mutated), *RUNX1* 25 (1 mutated), *ASXL1* 25 (2 mutated), *IDH1* 25 (none mutated), and *IDH2* 25 (1 mutated).

Comparison of our operational seven AK subcategories showed the following differences (Table 1): i) age distribution; <60 years 100% for t(6;9), 75% for t(9;22), 69% for t(v;11q23.3), and 20-43% for others (P=0.002); ii) AML subtype distribution; primary AML 100% for t(v;11q23.3), 88% for t(6;9), and 20-50% for others; iii) *FLT3*-ITD mutations; 100% for t(6;9) and 0% for all others (P<0.001); iv) *TP53* mutations; 62% for MK, 50% for CK, and 0% for all others (P=0.04); v) achievement of complete remission with incomplete hematologic recovery (CR/CRi): 100% for t(6;9), 85% for t(v;11q23.3), 53% for MK, and 60-75% for others (P=0.04); vi) documentation of AHSCT; 75% for t(6;9), 62% for t(v;11q23.3), and 20-44% for others (P=0.04). AHSCT in CR1 was documented in 75% for t(6;9), 54% in t(v;11q23.3), and 22-43% in others (P=0.02). Additional information on phenotype and mutation comparisons is outlined in Table 1, which also includes a comparator arm of 325 patients with normal karyotype (NK).

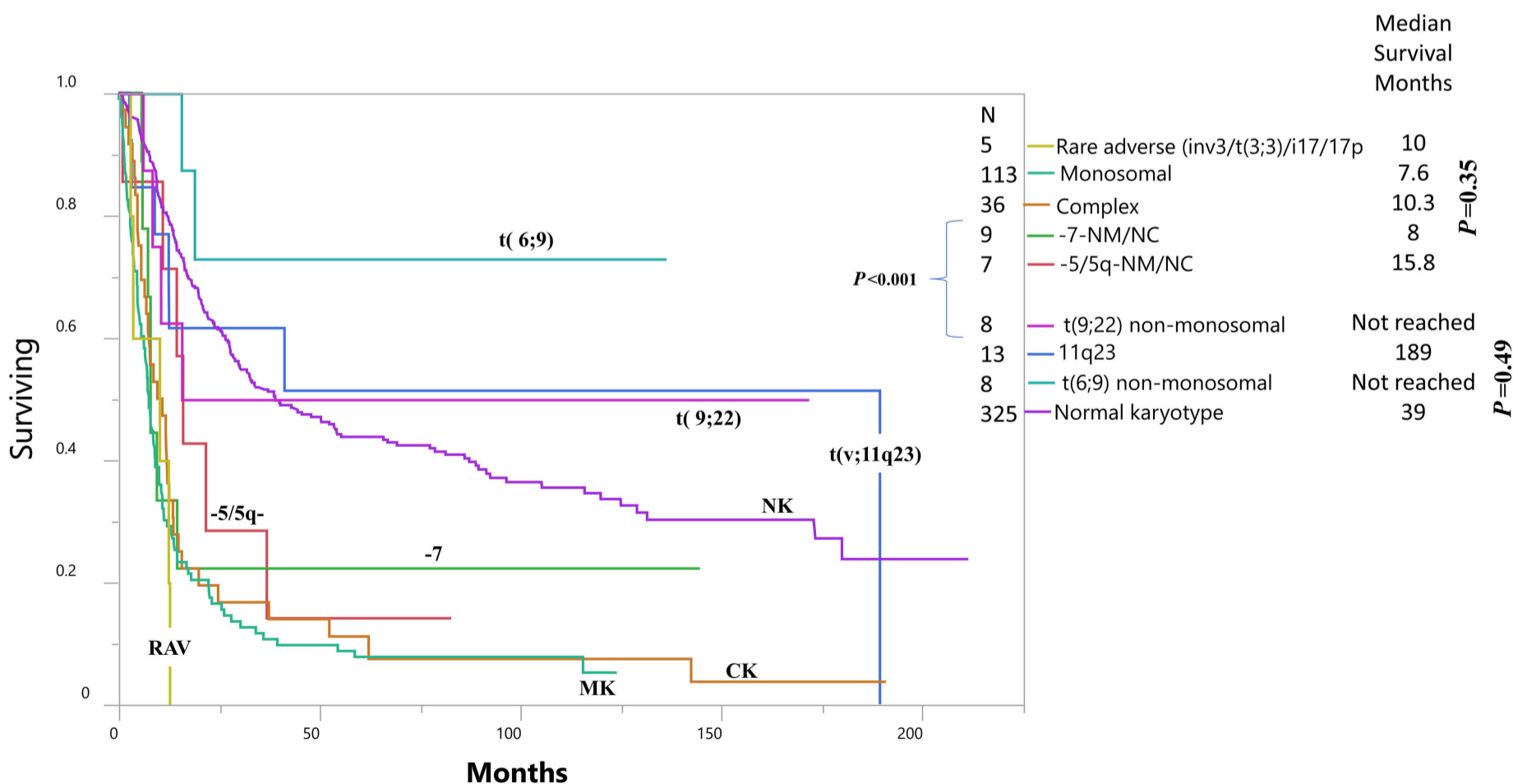After a median follow-up of 9.7 months (range, 0.2-191),

Figure 1. Overall survival of 189 intensively treated Mayo Clinic patients with newly diagnosed acute myeloid leukemia (not including promyelocytic), stratified by European LeukemiaNet (ELN) adverse karyotype subcategories. RAV: rare adverse variants; MK: monosomal karyotype; CK: complex karyotype; NK: normal karyotype (survival analysis did not censor for allogeneic hematopoietic stem cell transplant).

161 (85%) deaths, 57 relapses (49% of those achieving CR/CRi), and 62 (33%) AHSCT, including 56 in CR1, were documented. The 30-day mortality overall was 7% (13/189) with no significant difference between the AK subcategories (*P*=0.2). The 60-day mortality overall was 12% (22/189); the corresponding percentages were 19% for MK, 14% for -5/del(5q), 6% for CK, and otherwise 0% for all the remaining AK categories (*P*=0.02). In age-adjusted multivariable analysis, using NK-AML as a reference, survival was adversely affected by MK (hazard ratio n [HR] 3.8, 95% confidence interval [CI]: 2.9-4.8; *P*<0.001), CK (HR 3.1, 95% CI: 2.1-4.4; *P*<0.001), and RAV (HR 5.6, 95% CI: 2.3-13.7; *P*<0.001); in addition, borderline significance for adverse outcome was also noted for monosomy 7 (*P*=0.06) and -5/5q- (*P*=0.09). On the other hand, there was no difference in survival between NK-AML and t(6;9) (*P*=0.43), t(9;22) (*P*=0.95) or t(v;11q23.3) (*P*=0.67). There was also no difference in survival between MK *versus* RAV (*P*=0.39), MK *versus* CK (*P*=0.31), CK *versus* RAV (*P*=0.21), or -5/5q *versus* monosomy 7 (*P*=0.97).

Comparative survival data are depicted in Figure 1 and identify AML with t(6;9), t(9;22) or t(v;11q23.3) as having superior survival, compared to AML with MK, CK, RAV, -5/5q- or monosomy 7. In multivariable analysis, additional prognostic contribution, to that of AK cytogenetic risk stratification, was not evident for any AML subtype (*P*=0.12), *NPM1* (0.32), or *TP53* (*P*=0.71) mutations, but was evident for *FLT3*-ITD mutation (*P*=0.04); the latter was sustained when analysis was adjusted for age (*P*=0.01). Similarly, the presence of *TP53* mutation, in patients with MK or CK, did not appear to further influence survival (Figure 2A). The relatively favorable survival data in patients with t(6;9), t(9;22) or t(v;11q23.3) was attributed to their younger age distribution and significantly higher utilization of AHSCT (Figure 2B); it should be noted that 14 of 21 patients with these translocations received AHSCT, including 13 in CR1. On the other hand, although AHSCT also improved survival in patients with MK/CK (Figure 2C), it was
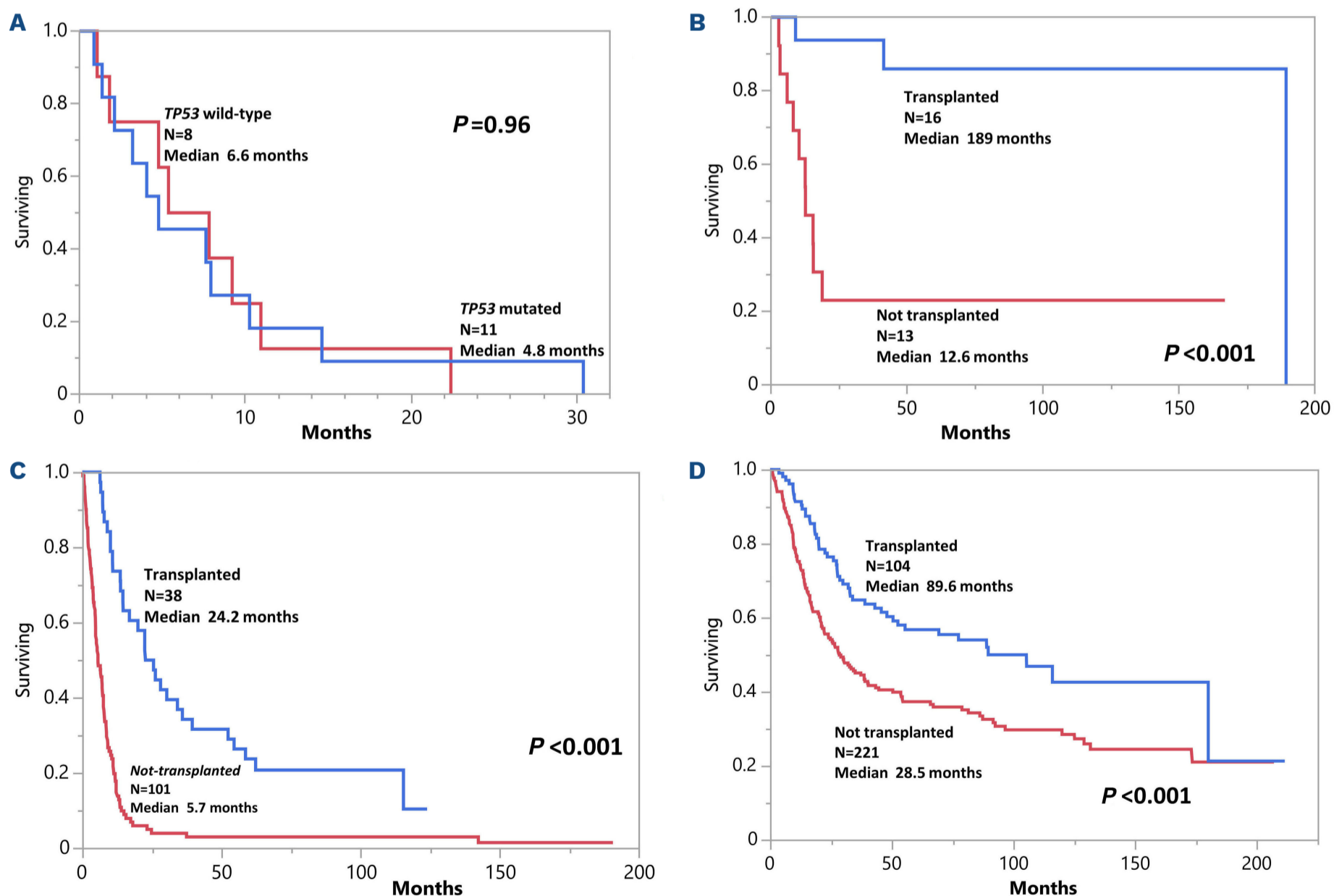

Figure 2. Overall survival data in 189 intensively treated acute myeloid leukemia patients with adverse karyotype. (A) survival data in a subset of 19 patients expressing monosomal or complex karyotype and stratified by *TP53* mutation status; (B) survival data in a subset of 29 patients expressing t(6;9), t(9;22) or t(v;11q23) and stratified by documentation of allogeneic hematopoietic stem cell transplant (AHSCT); (C) survival data in a subset of 139 patients expressing monosomal or complex karyotype and stratified by documentation of AHSCT; (D) survival data in a separate cohort of 325 intensively treated patients with acute myeloid leukemia and expressing normal karyotype and stratified by documentation of AHSCT.

not as effective as it was in the setting of t(6;9), t(9;22) or t(v;11q23.3) (Figure 2B) or NK (Figure 2D). Among 62 AK patients who received AHSCT, 26 (42%) experienced post-transplant relapse, including 18 (60%) of 30 with MK, two (25%) of eight with CK, three (38%) of eight with t(v;11q23.3), none of six with t(6;9), one (25%) of four with monosomy 7, one (33%) of three with -5/5q-, none of two with t(9;22), and one (100%) of one with RAV (*P*=0.02). The current study highlights significant differences in phenotype and mutation composition among ELN-2017 AK subcategories and confirms the value of AHSCT in securing long-term survival in patients with t(6;9)(p23;q34.1) or t(v;11q23.3), excluding t(9;11)(p21.3;q23.3) abnormalities. The AHSCT-enabled survival data in patients with t(6;9) or t(v;11q23.3) were superior to those seen in patients with MK/CK and did not appear to be inferior to that expected in the setting of NK; of note, only three of our eight patients with AML-t(6;9) received FLT3 inhibitor as part of their induction. Similar observations regarding the value of AHSCT in overcoming the otherwise poor prognosis in t(6;9)-AML were also previously reported.[6,7] In a recent study, younger patients with t(9;11)(p22;q23)/*KMT2A-MLLT3* (considered ELN intermediate-risk) had better outcomes and different mutational composition, compared to patients with other 11q23/*KMT2A* rearrangements, suggesting prognostic influence from the specific *KMT2A* fusion partner.[8] In the current study, the *KMT2A* rearrangements included t(11;19)(q23;p13.1)/*KMT2A-ELL* (n=5), t(6;11)(q27;q23)/*AF6/KMT2A* (n=3), del(11)(q21q23-25) (n=2), and others (n=3), with AHSCT-enabled survival data that appeared to be similar to that seen in NK-AML (see above), as has been noted in another recent study;[9] of note, disruption of *KMT2A* in the two cases with del(11)(q21q23-25) was not confirmed but their exclusion from survival analysis did not affect the overall results. We were pleasantly surprised by the favorable survival data in our eight AML patients with t(9;22)(q34.1;q11.2), which included four with antecedent CML. We are cognizant of the 2016 World Health Organization criteria for t(9;22)-AML, which requires absence of evidence for an underlying CML or AML with recurrent genetic aberrations.[10] However, ongoing challenges in clearly distinguishing *de novo* from blast phase CML remain[5,11] and, accordingly, for the purposes of the current study, we included all cases of t(9;22)-AML, with or without antecedent CML. Regardless, our observations suggest that long-term survival is a possibility in t(9;22)-AML, in the context of contemporary treatment strategies. In contrast to previous reports,[12] our observations suggest the prognostic contribution of *TP53* mutations in AML might be fully accounted for by its close association with MK/CK.

Whether or not further accounting for *TP53* allelic state modifies our observations is yet to be determined.[13,14] Unlike the case with t(6;9)(p23;q34.1) or t(v;11q23.3), AHSCT alone might not always secure durable remission in AML patients with MK/CK, although it currently remains the preferred treatment option. Finally, we recognize the relatively small number of informative cases for specific mutation categories, which makes it difficult to draw definitive conclusions on the outcome and advised treatment approach for these groups.

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No conflicts of interest to disclose.

Contributions
AT, RK, and KHB participated in writing the paper, data analysis and interpretation, and abstraction of patient data. All authors participated in patient care, abstraction of patient data, laboratory result interpretation or data analysis. All authors reviewed and approved the manuscript.

Data-sharing statement
Data is available on request.

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