

## ORIGINAL ARTICLE

# Mutations and Treatment Outcome in Cytogenetically Normal Acute Myeloid Leukemia

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## ABSTRACT

**BACKGROUND**

Mutations occur in several genes in cytogenetically normal acute myeloid leukemia (AML) cells: the nucleophosmin gene (*NPM1*), the *fms*-related tyrosine kinase 3 gene (*FLT3*), the CCAAT/enhancer binding protein  $\alpha$  gene (*CEBPA*), the myeloid–lymphoid or mixed-lineage leukemia gene (*MLL*), and the neuroblastoma RAS viral oncogene homolog (*NRAS*). We evaluated the associations of these mutations with clinical outcomes in patients.

**METHODS**

We compared the mutational status of the *NPM1*, *FLT3*, *CEBPA*, *MLL*, and *NRAS* genes in leukemia cells with the clinical outcome in 872 adults younger than 60 years of age with cytogenetically normal AML. Patients had been entered into one of four trials of therapy for AML. In each study, patients with an HLA-matched related donor were assigned to undergo stem-cell transplantation.

**RESULTS**

A total of 53% of patients had *NPM1* mutations, 31% had *FLT3* internal tandem duplications (ITDs), 11% had *FLT3* tyrosine kinase–domain mutations, 13% had *CEBPA* mutations, 7% had *MLL* partial tandem duplications (PTDs), and 13% had *NRAS* mutations. The overall complete-remission rate was 77%. The genotype of mutant *NPM1* without *FLT3*-ITD, the mutant *CEBPA* genotype, and younger age were each significantly associated with complete remission. Of the 663 patients who received postremission therapy, 150 underwent hematopoietic stem-cell transplantation from an HLA-matched related donor. Significant associations were found between the risk of relapse or the risk of death during complete remission and the leukemia genotype of mutant *NPM1* without *FLT3*-ITD (hazard ratio, 0.44; 95% confidence interval [CI], 0.32 to 0.61), the mutant *CEBPA* genotype (hazard ratio, 0.48; 95% CI, 0.30 to 0.75), and the *MLL*-PTD genotype (hazard ratio, 1.56; 95% CI, 1.00 to 2.43), as well as receipt of a transplant from an HLA-matched related donor (hazard ratio, 0.60; 95% CI, 0.44 to 0.82). The benefit of the transplant was limited to the subgroup of patients with the prognostically adverse genotype *FLT3*-ITD or the genotype consisting of wild-type *NPM1* and *CEBPA* without *FLT3*-ITD.

**CONCLUSIONS**

Genotypes defined by the mutational status of *NPM1*, *FLT3*, *CEBPA*, and *MLL* are associated with the outcome of treatment for patients with cytogenetically normal AML.

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N Engl J Med 2008;358:1909-18.

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**A**CUTE MYELOID LEUKEMIA (AML) IS A GENETICALLY heterogeneous disease in which somatic mutations that disturb cellular growth, proliferation, and differentiation accumulate in hematopoietic progenitor cells. The karyotype at the time of diagnosis provides the most important prognostic information in adults with AML,<sup>1-3</sup> but 40 to 50% of patients do not have clonal chromosomal aberrations.<sup>1-3</sup> All such cases of cytogenetically normal AML are currently categorized in the intermediate-risk group, yet this group is quite heterogeneous.<sup>4,5</sup>

In recent years, acquired gene mutations, as well as deregulation of gene expression, have been identified.<sup>6-8</sup> Somatic mutations in AML include partial tandem duplications (PTDs) of the myeloid-lymphoid or mixed-lineage leukemia gene (*MLL*),<sup>9</sup> internal tandem duplications (ITDs)<sup>10</sup> or mutations of the tyrosine kinase domain (TKD)<sup>11</sup> of the *fms*-related tyrosine kinase 3 gene (*FLT3*), and mutations in the nucleophosmin gene (*NPM1*),<sup>12</sup> the CCAAT/enhancer binding protein  $\alpha$  gene (*CEBPA*),<sup>13</sup> and the neuroblastoma RAS viral oncogene homolog gene (*NRAS*).<sup>14</sup> These alterations appear to fall into two broadly defined complementation groups.<sup>15</sup> One group (class I) comprises mutations that activate signal-transduction pathways and thereby increase the proliferation or survival, or both, of hematopoietic progenitor cells. Mutations that activate the receptor tyrosine kinase *FLT3*<sup>16</sup> or *RAS*<sup>17</sup> family members are considered to be class I mutations. The other complementation group (class II) comprises mutations that affect transcription factors or components of the transcriptional coactivation complex and cause impaired differentiation. On the basis of their known physiological functions, mutations in *CEBPA*,<sup>18</sup> *MLL*,<sup>19</sup> and possibly also *NPM1*<sup>20</sup> fall into this group.

Mutations in these genes have prognostic relevance. *FLT3*-ITD<sup>21-25</sup> and *MLL*-PTD<sup>26,27</sup> have been associated with short relapse-free and overall survival, whereas a more favorable outcome is associated with cytogenetically normal cases of AML with mutations in *CEBPA*<sup>28-30</sup> or *NPM1* (without concomitant *FLT3*-ITD).<sup>31-34</sup>

Postremission therapy with repeat cycles of high-dose cytarabine is an effective treatment for cytogenetically normal AML.<sup>4,5</sup> Hematopoietic stem-cell transplantation involving an HLA-matched related donor can reduce the risk of relapse, but this benefit is mitigated by a treatment-related mortality of 15 to 25%.<sup>4,5</sup> Most on-

going clinical trials involving young adults have adopted strategies for balancing treatment-related toxic effects with the risk of relapse. These strategies are particularly relevant to allogeneic stem-cell transplantation, in that it is usually offered to patients with high-risk cytogenetic abnormalities and not to low-risk patients.

In this study, we aimed to assess the frequencies and interactions of mutations in *NPM1*, *FLT3*, *CEBPA*, *MLL*, and *NRAS*. We also planned to evaluate the association of the mutations with treatment outcomes and to analyze the role of the mutations in guiding postremission therapy in patients with cytogenetically normal AML.

## METHODS

### SELECTION OF PATIENTS

Between July 1993 and November 2004, patients were enrolled in one of four multicenter prospective treatment trials of the German–Austrian Acute Myeloid Leukemia Study Group (see Table 1 in the Supplementary Appendix, available with the full text of this article at [www.nejm.org](http://www.nejm.org)). The eligibility criteria of the four trials were similar. The inclusion criterion for the individual-patient–data analysis described here was the presence of a normal karyotype on chromosome-banding analysis.

### THERAPY

All four trials used double-induction therapy with idarubicin, cytarabine, and etoposide; a first cycle of consolidation therapy based on high-dose cytarabine; and a second cycle of consolidation therapy during which patients with an HLA-matched related donor were assigned to undergo stem-cell transplantation and those without a suitable donor received high-dose cytarabine-based chemotherapy or were randomly assigned either to receive such chemotherapy or to undergo autologous stem-cell transplantation (Fig. 1 and Fig. 2 in the Supplementary Appendix). For both autologous and allogeneic transplantation, a conditioning regimen of hyperfractionated total-body irradiation (12.0 to 14.4 Gy) or oral busulfan (16 mg per kilogram of body weight) followed by intravenous cyclophosphamide (120 to 200 mg per kilogram of body weight) was recommended.

### CYTOGENETIC AND MOLECULAR GENETIC STUDIES

Cytogenetic and molecular genetic studies were performed in two central reference laboratories of the German–Austrian Acute Myeloid Leukemia

Study Group, one at the University of Ulm and the other at Hannover Medical School. Blood or bone marrow specimens from each patient were screened for the recurring gene fusions *PML-RARA*, *CBFB-MYH11*, and *RUNX1-RUNX1T1*, by means of the fluorescence in situ hybridization assay or polymerase-chain-reaction assay. Diagnostic samples were also analyzed for mutations in the *FLT3* gene (i.e., the ITD and TKD mutations at codons D835 and I836) and in the *CEBPA*, *MLL*, *NPM1*, and *NRAS* genes (Table 2 in the Supplementary Appendix).

#### STATISTICAL ANALYSIS

The primary end point was relapse-free survival; secondary end points were complete remission after induction therapy and overall survival. To evaluate relapse-free survival and overall survival, we used relapse or death during complete remission and death, respectively. These end points were concordant with those in the primary treatment trials, and all were based on recommended criteria.<sup>35</sup> A conditional logistic-regression model incorporating stratification according to treatment trial was used to analyze associations between baseline characteristics and the achievement of complete remission. A Cox model with stratification to account for the particular treatment trial was used to identify prognostic variables. In addition to the molecular markers, the presence or absence of hepatosplenomegaly, age, white-cell count, and type of AML were added as explanatory variables in all regression analyses. On the basis of data from previous studies, the marker of mutant *NPM1* without *FLT3*-ITD was compared with all other combinations of these two markers.<sup>31-34</sup> We estimated missing data for covariates for patients with at least one molecular marker analyzed by using 50 multiple imputations in chained equations incorporating predictive mean matching.<sup>36</sup> All statistical analyses were performed with the use of the R package (version 2.0-12) of the R statistical software platform (version 2.4.1).<sup>37</sup> P values of less than 0.05 were considered to indicate statistical significance.

## RESULTS

#### ACCRUAL AND CLINICAL CHARACTERISTICS

A total of 1919 patients who were 16 to 60 years of age and had newly diagnosed AML were enrolled in the four treatment trials. Cytogenetically normal AML was identified in 872 patients (45%),

and data for all patients with this variant were used in the current analysis (Table 1 in the Supplementary Appendix). Table 1 lists the baseline characteristics of the 872 patients. The availability of an HLA-matched donor was recorded for 846 of the 872 patients.

#### MOLECULAR MARKERS

Screening for molecular markers was performed in all available samples of blood or bone marrow, or both, that were taken at the time of diagnosis: *NPM1* was screened in 570 patients, *FLT3*-ITD in 531 patients, *FLT3*-TKD in 617 patients, *CEBPA* in 509 patients, *MLL*-PTD in 640 patients, and *NRAS* in 641 patients. The mutational status of all six markers could be determined in 438 of the 872 patients with cytogenetically normal AML (50%), and 693 of the 872 patients (79%) had at least one marker analyzed.

*NPM1* mutations were found in 301 of 570 patients (53%), *FLT3*-ITD in 164 of 531 patients (31%), *FLT3*-TKD in 68 of 617 patients (11%), *CEBPA* in 67 of 509 patients (13%), *MLL*-PTD in 47 of 640 patients (7%), and *NRAS* in 82 of 641 patients (13%). Frequencies and distributions of the mutations differed slightly between the subgroup of the 438 patients with complete mutation data (Fig. 1). At least one mutation was identified in 369 of the 438 patients (84%). In 312 of the 438 patients, there were mutations in hypothetical class II genes (*NPM1*, *CEBPA*, and *MLL*), with only minimal overlap: only 17 patients (5%) had more than one class II mutation. Class I mutations (*FLT3*-ITD, *FLT3*-TKD, and *NRAS*) were identified in 241 of the 438 patients, again with a minimal number of patients (12 patients, 5%) having more than one class I mutation. *FLT3*-ITD ( $P < 0.001$ ) and *FLT3*-TKD mutations ( $P = 0.03$ ), but not *NRAS* mutations ( $P = 0.46$ ), were significantly associated with *NPM1* mutations. As compared with these associations, *FLT3*-ITD ( $P = 0.03$ ) was less frequently associated, and *FLT3*-TKD ( $P = 0.40$ ) and *NRAS* ( $P = 0.34$ ) mutations were not significantly associated, with *CEBPA*. *MLL*-PTD was not significantly associated with class I mutations.

#### INDUCTION THERAPY

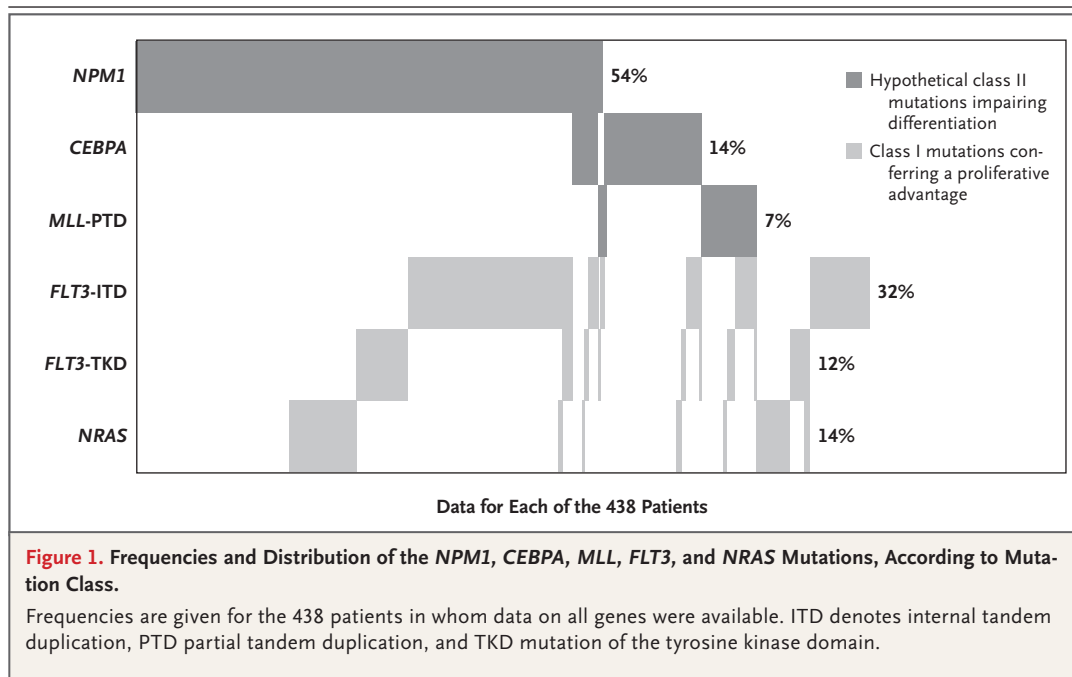
In all trials, response-adapted, double-induction therapy was administered (Fig. 1 in the Supplementary Appendix). Patients in whom there was complete or partial remission after the first course of induction therapy received a second course, whereas patients with refractory disease received

**Table 1. Baseline Characteristics of the 872 Patients with Cytogenetically Normal Acute Myeloid Leukemia (AML).\***

Characteristic	All Patients (N=872)	Patients with $\geq 1$ Molecular Marker Analyzed (N=693)
Sex — no. (%)		
Male	408 (47)	330 (48)
Female	464 (53)	363 (52)
Age — yr		
Median	48	48
Range	16–60	16–60
FAB type — no. (%)		
M0	39 (5)	31 (5)
M1	141 (18)	108 (18)
M2	206 (27)	166 (27)
M4	236 (31)	185 (31)
M5	108 (14)	92 (15)
M6	29 (4)	20 (3)
M7	4 (<1)	4 (1)
Missing data	109 (13)	87 (13)
Lymphadenopathy — no./total no. (%)	177/829 (21)	157/658 (24)
Hepatosplenomegaly — no./total no. (%)	307/831 (37)	263/661 (40)
Gingival hyperplasia — no./total no. (%)	61/829 (7)	52/673 (8)
CNS involvement — no./total no. (%)	7/816 (1)	6/667 (1)
Type of AML — no. (%)		
Primary AML	762 (87)	607 (88)
s-AML	96 (11)	73 (11)
t-AML	13 (1)	12 (2)
Missing data	1 (<1)	1 (<1)
Family donor available — no./total no. (%) †	218/846 (26)	178/670 (27)
White-cell count		
Median — $\times 10^9$ /liter	16.9	20.1
Range — $\times 10^9$ /liter	0.2–372.0	0.2–372.0
Missing data — no. (%)	21 (2)	16 (2)
Platelet count		
Median — $\times 10^9$ /liter	60	60
Range — $\times 10^9$ /liter	4–746	4–746
Missing data — no. (%)	29 (3)	22 (3)
Hemoglobin		
Median — g/liter	91	91
Range — g/liter	25–176	30–176
Missing data — no. (%)	29 (3)	21 (3)
Bone marrow blasts		
Median — %	80	80
Range — %	0–100	0–100
Missing data — no. (%)	77 (9)	64 (9)
Peripheral-blood blasts		
Median — %	41	42
Range — %	0–100	0–100
Missing data — no. (%)	68 (8)	47 (7)

\* CNS denotes central nervous system, FAB French–American–British, s-AML AML that developed after a myelodysplastic syndrome, and t-AML AML that developed after chemotherapy or radiation therapy.

† Family donor data were not available for 26 patients because of death during induction therapy or the first cycle of consolidation therapy.



a salvage regimen. Complete remission was achieved in 668 of the 872 patients (77%), 130 patients (15%) had refractory disease, and 74 patients (8%) had early death or death with hypoplastic bone marrow.

Multivariable analysis of data from the 693 patients with at least one molecular marker analyzed revealed that two genotypes were significantly associated with a complete remission: mutant *CEBPA* (odds ratio, 1.33; 95% confidence interval [CI], 1.01 to 1.74) and mutant *NPM1* without *FLT3-ITD* (odds ratio, 1.48; 95% CI, 1.21 to 1.80). The odds ratio for a complete remission for each 10-year increase in age was 0.91 (95% CI, 0.84 to 0.99).

#### POSTREMISSION THERAPY

A matched donor was available for 182 of the 663 patients (27%) in complete remission (the donor group); allogeneic transplantation was actually performed in 150 patients (82%). Of the 481 patients without a matched donor (the no-donor group), 147 were assigned to receive chemotherapy and 334 were randomly assigned either to receive chemotherapy or to undergo autologous stem-cell transplantation. There was no significant difference in relapse-free or overall survival between those receiving chemotherapy and those undergoing autologous transplantation, on an intention-to-treat basis ( $P=0.78$  and  $P=0.44$ , re-

spectively) or according to the treatment actually received ( $P=0.65$  and  $P=0.88$ , respectively); nor were there significant differences according to the mutational status. Therefore, the no-donor group was considered an appropriately uniform treatment group for comparison with the donor group.

#### SURVIVAL ANALYSES

The median follow-up for survival was 51.6 months. Of the 872 patients, 471 (54%) died; the median overall survival was 30.4 months, and the 4-year rate of overall survival was 43% (95% CI, 39 to 47). Of the 668 patients in whom complete remission was achieved, 80 died in complete remission and 294 had a relapse; the median relapse-free survival was 22.2 months, and the 4-year rate of relapse-free survival was 42% (95% CI, 38 to 46). To address a potential source of bias, we compared the 693 patients who had at least one molecular marker analyzed and the 179 patients without any marker analyzed. The 693 patients with at least one marker analyzed had significantly higher leukocyte counts and higher percentages of bone marrow blasts than the 179 patients without any marker analyzed. However, there was no significant difference between the two subgroups in the primary end point (relapse-free survival,  $P=0.87$ ) or the secondary end points (complete remission,  $P=0.55$ ; overall survival,  $P=0.57$ ).

Of the 693 patients with at least one marker

analyzed, 526 (76%) had a complete remission. Five patients died before the start of postremission therapy. Table 2 lists data from the multivariable analysis for the primary end point and a secondary end point. Figure 2 shows the Kaplan–Meier curves for relapse-free and overall survival, according to genotype.

#### ALLOGENEIC TRANSPLANTATION

A univariable analysis of data for patients with a complete remission, comparing the donor group with the no-donor group, revealed a significantly longer relapse-free survival ( $P=0.009$ ) in the donor group, but this difference did not translate into a significant difference in overall survival ( $P=0.54$ ). The treatment-related mortality rate among the patients who underwent allogeneic transplantation was 21%. To explore the role of allogeneic transplantation according to genotype, we performed an analysis based on indirect assessment with separated tests.<sup>38</sup> Cox regression analyses of relapse-free survival were performed with the use of data from two subgroups: 130 patients with mutant *NPM1* without *FLT3*-ITD, a prognostically favorable subgroup, and 172 patients with other genotypes. Among the patients with mutant *NPM1* without *FLT3*-ITD, there was no benefit for the donor group as compared with the no-donor group (hazard ratio for the risk of relapse or the risk of death during complete remission, 0.92; 95% CI, 0.47 to 1.81), whereas in the subgroup of patients with other, less prognostically favorable genotypes, there was a significant advantage for the donor group (hazard ratio, 0.61; 95% CI, 0.40 to 0.94). Figure 3 shows relapse-free–survival curves, according to donor status, for the patients with mutant *NPM1* without *FLT3*-

ITD and for those with other genotypes. Data for the 62 patients with mutant *CEBPA* were excluded, because there were too few patients for a meaningful statistical analysis.

#### TREATMENT AND SURVIVAL AFTER RELAPSE

In all, 54 patients in the donor group and 240 patients in the no-donor group had a relapse, and a second complete remission was achieved in 25 patients (46%) and 102 patients (42%), respectively. (Details of treatment after relapse are given in Table 3 in the Supplementary Appendix.) Among the patients with a relapse, the median survival at 3 years was 6.1 months in the no-donor group and 7.3 months in the donor group, and the 3-year survival rate was 12% (95% CI, 4 to 23) in the no-donor group and 24% (95% CI, 18 to 30) in the donor group ( $P=0.44$  by the log-rank test for survival). The 3-year survival rate among the 94 patients who received a transplant from an HLA-matched unrelated donor after relapse was 49% (95% CI, 38 to 60).

#### DISCUSSION

Our analysis, based on four prospective clinical trials by the German–Austrian Acute Myeloid Leukemia Study Group, was performed to evaluate the prognostic and predictive value of *NPM1*, *FLT3*, *CEBPA*, *MLL*, and *NRAS* mutations in patients with cytogenetically normal AML. Our results show that, beyond cytogenetic risk classification, molecular genetic markers are clinically significant factors in the response to therapy and survival.

The frequencies of mutations that we found are consistent with those in previous studies.<sup>6,21–34</sup> The clustering of certain mutations supports the concept of different classes of mutations (Fig. 1). Among the hypothetical class II mutations — that is, mutations in *CEBPA*, *MLL*, and *NPM1* that are thought to impair hematopoietic-cell differentiation<sup>18–20</sup> — there was only minimal overlap. Likewise, the class I mutations in *FLT3* and *NRAS*, which confer a proliferation and survival advantage to the cell,<sup>16,17</sup> were largely nonoverlapping. In addition, the associations between the two classes of mutations were not equally distributed. *NPM1* mutations were associated with both types of activating *FLT3* mutations. In contrast, *CEBPA* mutations and *FLT3*-ITD were rarely found concurrently.

The considerable prognostic implications of

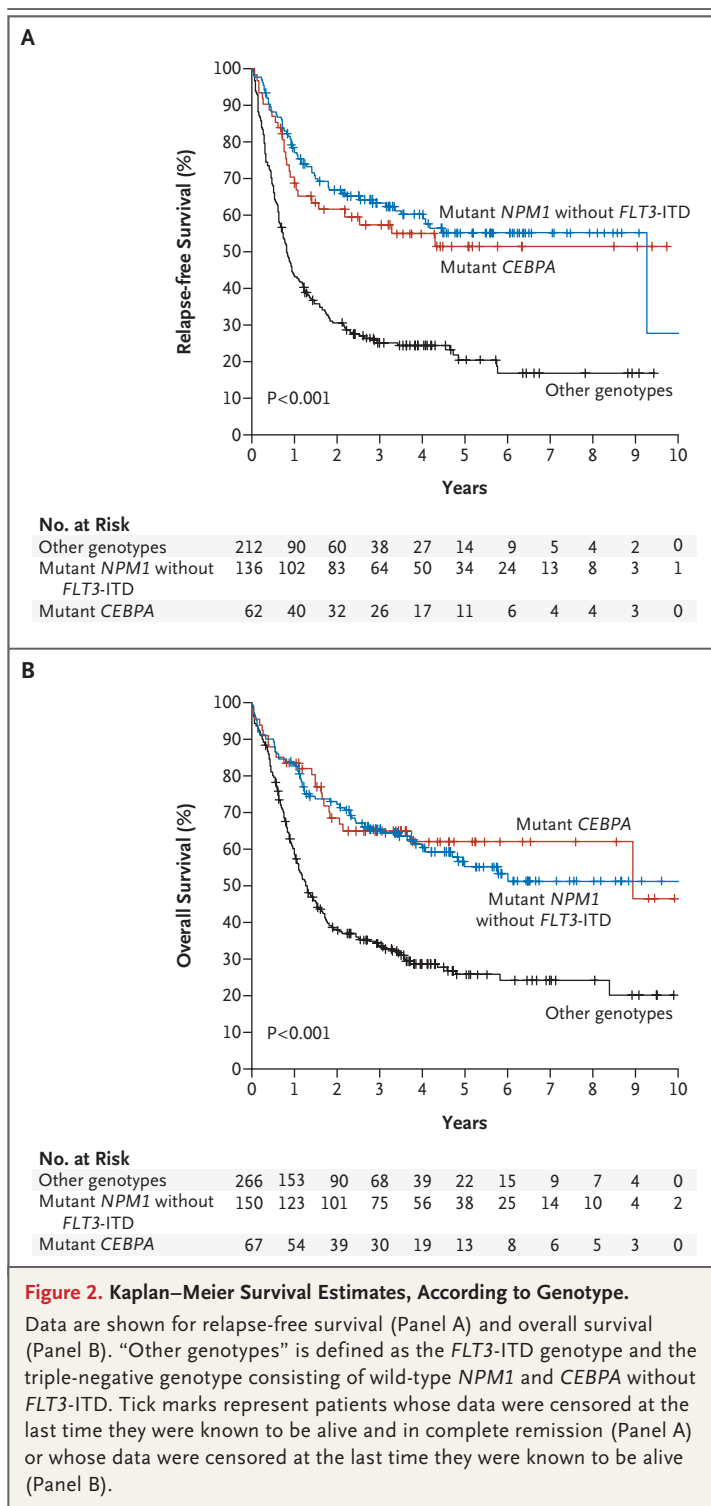
**Table 2. Hazard Ratios for End Points in the 521 Patients with a Complete Remission, According to Risk Factor.**

Risk Factor	Hazard Ratio (95% CI)
<b>Relapse or death during complete remission</b>	
Mutant <i>CEBPA</i>	0.48 (0.30–0.75)
Mutant <i>NPM1</i> without <i>FLT3</i> -ITD	0.44 (0.32–0.61)
<i>MLL</i> -PTD	1.56 (1.00–2.43)
Family donor available	0.60 (0.44–0.82)
<b>Death</b>	
Mutant <i>CEBPA</i>	0.50 (0.30–0.83)
Mutant <i>NPM1</i> without <i>FLT3</i> -ITD	0.51 (0.37–0.70)
10-year increase in age	1.33 (1.16–1.53)

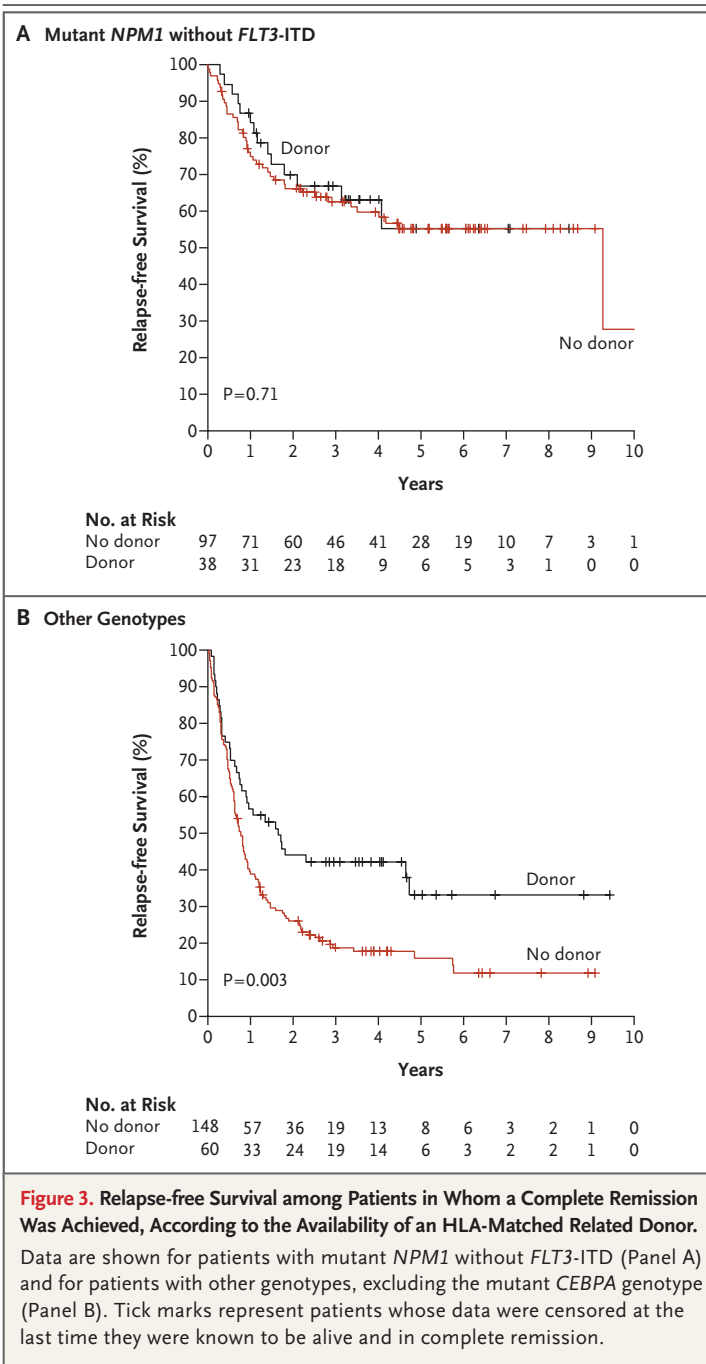
the mutations we analyzed confirm and substantially extend the results of previous studies.<sup>21-34</sup> Logistic-regression analyses showed that the genotype of mutant *NPM1* without *FLT3*-ITD was associated with a complete remission after conventional anthracycline and cytarabine-based induction therapy. Similarly, the mutant *CEBPA* genotype was associated with a complete remission, a correlation that had not been found in previous studies of *CEBPA* as a single genetic marker.<sup>28-30</sup> In Cox regression analyses with relapse-free and overall survival as end points, the genotype of mutant *NPM1* without *FLT3*-ITD and the mutant *CEBPA* genotype again appeared to be associated with a favorable outcome. The 4-year rate of overall survival for patients with the mutant *NPM1* genotype without *FLT3*-ITD was 60% and for those with mutant *CEBPA* was 62%. These outcome data are similar to those for patients with core-binding-factor leukemias, which are categorized as diseases with cytogenetically favorable risks.<sup>39-41</sup> In contrast, the subgroups of patients with the *FLT3*-ITD genotype or the triple-negative genotype consisting of wild-type *NPM1* and *CEBPA* without *FLT3*-ITD had similarly poor outcomes, with 4-year rates of relapse-free survival of 24% and 25%, respectively, and 4-year rates of overall survival of 24% and 33%, respectively.

The influence of *FLT3*-TKD mutations on the outcome is unsettled. A negative influence was reported in a meta-analysis,<sup>42</sup> but in a recent study by the Medical Research Council, TKD mutations were associated with a favorable outcome in the entire cohort as well as in patients with cytogenetically normal AML.<sup>43</sup> In our study, *FLT3*-TKD mutations were not significantly associated with the outcome, possibly because other genetic markers, *NPM1* in particular, were considered in the multivariable analysis. Notably, 54% of patients with the mutant *FLT3*-TKD genotype were in the subgroup of patients with the prognostically favorable genotype of mutant *NPM1* without *FLT3*-ITD; in contrast, patients with a *FLT3*-TKD mutation as the sole aberration had a poor outcome.

Among the various clinical and genetic features at presentation, besides genotype, the only significant factor for overall survival in our study was age, and this result was mainly due to the favorable outcome among younger patients who received a stem-cell transplant from a matched unrelated donor after relapse. However, age did



not influence relapse-free survival in the donor group or in the no-donor group. In contrast, recently published data from the Dutch–Belgian Hemato-Oncology Cooperative Group and the



Swiss Group for Clinical Cancer Research showed that an age of less than 40 years (the median age of the study population) was associated with a survival advantage for patients who received a transplant from a matched related donor.<sup>44</sup> We found that receipt of an allogeneic transplant from a matched related donor improved relapse-free survival, but this benefit was mitigated, with

respect to overall survival, by the favorable results of receipt of a transplant from an HLA-matched unrelated donor after relapse in the no-donor group.

The type of AML did not influence any of the end points we analyzed, and among patients who had the favorable genotype of mutant *NPM1* without *FLT3*-ITD or the favorable mutant *CEBPA* genotype, the outcome for patients in whom AML developed after a myelodysplastic syndrome or after chemotherapy, radiation therapy, or both and the outcome of those with primary AML were similarly favorable.

We could assess any association of genotypes with the result of postremission therapy,<sup>38</sup> since the four trials we analyzed included assignment to a treatment group according to whether an HLA-matched donor was available. Notably, only patients with none of the favorable genotypes — that is, the patients with the *FLT3*-ITD mutation or the genotype consisting of wild-type *NPM1* and *CEBPA* without *FLT3*-ITD — benefited from an allogeneic transplant performed during the first complete remission (Fig. 3). In contrast, within the subgroup of patients with the favorable genotype of mutant *NPM1* without *FLT3*-ITD, the probability of relapse-free survival did not differ according to whether a related donor was available. Similarly, no benefit of an allogeneic transplant has been shown in patients with core-binding-factor leukemias.<sup>39-41</sup> In our analysis of cytogenetically normal AML, however, the number of patients with the mutant *CEBPA* genotype was too small to draw conclusions regarding the value of related-donor transplantation during the first complete remission. In a recent study, Gale et al.<sup>45</sup> found no beneficial effect of allogeneic transplantation in patients with *FLT3*-ITD. In contrast, we focused on subgroups of patients with cytogenetically normal AML who had unfavorable genotypes, not only the *FLT3*-ITD genotype but also the triple-negative genotype consisting of wild-type *NPM1* and *CEBPA* without *FLT3*-ITD. In the cohort reported on by Gale et al., the rate of allogeneic transplantation during the first period of complete remission was only 63% (173 of 273 patients), and treatment-related mortality was as high as 30%, whereas in our cohort, these values were 82% and 21%, respectively.

Our data provide a basis for refining the risk classification of AML. Cytogenetically normal AML involving the genotype of mutant *NPM1*

without *FLT3*-ITD or the mutant *CEBPA* genotype should no longer be classified as intermediate-risk leukemia but rather should be classified as favorable-risk leukemia, together with the core-binding-factor AMLs. We recommend that screening for *NPM1*, *FLT3*, and *CEBPA* mutations be part of the initial workup for newly diagnosed AML. Patients with mutant *NPM1* without *FLT3*-ITD may not benefit from related-donor transplantation as first-line treatment. In contrast, transplantation involving a related donor — and possibly that in-

volving an unrelated donor — should be explored further in patients with the unfavorable genotype *FLT3*-ITD or the unfavorable genotype consisting of wild-type *NPM1* and *CEBPA* without *FLT3*-ITD, at least while no successful targeted therapies are available.

Supported by grants from the Bundesministerium für Bildung und Forschung (01GI9981 and 01KG0605), the Deutsche José Carreras Leukämie-Stiftung (DJCLS R06/06v), and the Else Kröner-Fresenius-Stiftung (P38/05//A49/05//F03).

No potential conflict of interest relevant to this article was reported.

#### APPENDIX

Members of the German–Austrian Acute Myeloid Leukemia Study Group were as follows: **German Centers:** *Klinikum Augsburg, Augsburg* — G. Schlimok; *Charité, Berlin* — R. Arnold; *Robert-Rössle Klinik, Berlin* — A. Pezzutto; *Universitätsklinikum Bonn, Bonn* — A. Glasmacher; *Universitätsklinikum Düsseldorf, Düsseldorf* — U. Germing; *Krankenhaus Essen-Werden, Essen* — W. Heit; *Universitätsklinikum Frankfurt, Frankfurt* — D. Hoelzer; *Städtische Kliniken Frankfurt am Main-Höchst, Frankfurt* — H.G. Derigs; *Universitätsklinikum Freiburg, Freiburg* — M. Lübbert; *Universitätsklinikum Gießen, Gießen* — H. Pralle; *Wilhelm-Anton-Hospital, Goch* — V. Runde; *Universitätsklinikum Göttingen, Göttingen* — F. Griesinger; *Universität Hamburg, Hamburg* — W. Fiedler; *Allgemeines Krankenhaus Altona, Hamburg* — H. Salwender; *Krankenhaus Siloah, Hannover* — H. Kirchner; *Universitätsklinikum Heidelberg, Heidelberg* — M. Hensel; *Universitätsklinikum Hamburg-Saar, Hamburg* — F. Hartmann; *Städtisches Klinikum Karlsruhe, Karlsruhe* — J.T. Fischer; *Universitätsklinikum Kiel, Kiel* — M. Kneba; *Wissenschaftlicher Service Pharma, Langenfeld* — A. Hinke; *Caritas-Krankenhaus Lebach, Lebach* — S. Kremers; *Technische Universität München, Munich* — K. Götzte; *Klinikum München-Schwabing, Munich* — C. Waterhouse; *Klinikum Oldenburg, Oldenburg* — F. del Valle; *Caritas-Klinik St. Theresia, Saarbrücken* — A. Matzdorff; *Bürgerhospital Stuttgart, Stuttgart* — W. Grimminger; *Katharinenhospital Stuttgart, Stuttgart* — H.G. Mergenthaler; *Krankenhaus der Barmherzigen Brüder, Trier* — H. Kirchen; *Universitätsklinikum Tübingen, Tübingen* — P. Brossart; *Universitätsklinikum Ulm, Ulm* — L. Bergmann; *Klinikum Wuppertal, Wuppertal* — Aruna Raghavachar; **Austrian Centers:** *Universitätsklinikum Innsbruck, Innsbruck* — A. Petzer; *Hanuschkrankenhaus, Vienna* — E. Koller; **Belgian Center:** *Universitair Ziekenhuis Gent, Gent* — L. Noens.

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