DATA SUPPLEMENT

TET2 mutations improve the new European LeukemiaNet risk classification of acute myeloid leukemia: A Cancer and Leukemia Group B study

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PATIENTS AND METHODS

Treatment Protocols, Sample Collection and Sample Preparation

Patients with cytogenetically normal acute myeloid leukemia (CN-AML) younger than 60 years were treated on Cancer and Leukemia Group B (CALGB) trials 9621 or 19808. Patients enrolled on CALGB 19808 (n=96) were randomly assigned to receive induction chemotherapy with cytarabine, daunorubicin, and etoposide with or without PSC-833 (valspodar), a multidrug resistance protein inhibitor.[1] On achievement of complete remission (CR), patients were assigned to intensification with high-dose cytarabine and etoposide for stem-cell mobilization followed by myeloablative treatment with busulfan and etoposide supported by autologous peripheral blood stem-cell transplantation. Patients enrolled on CALGB 9621 (n=87) were treated similarly to those on CALGB 19808, as previously reported.[2,3]

Older patients (≥60 years) were all treated with cytarabine/daunorubicin-based induction therapy followed by cytarabine-based consolidation therapy. Patients on CALGB 8525 (n=23) were treated with induction chemotherapy consisting of cytarabine in combination with daunorubicin and were randomly assigned to consolidation with different doses of cytarabine followed by maintenance treatment.[4] Patients on CALGB 8923 (n=22) were treated with induction chemotherapy consisting of cytarabine in combination with daunorubicin and were randomly assigned to receive postremission therapy with cytarabine alone

or in combination with mitoxantrone.[5] Patients on CALGB 9420 (n=6) and 9720 (n=109) received induction chemotherapy consisting of cytarabine in combination with daunorubicin and etoposide, with (CALGB 9420) or with/without (CALGB 9720) the multidrug resistance protein modulator PSC-833.[6,7] Patients on CALGB 9420 received postremission therapy with cytarabine (2 g/m^2/d) alone, and patients on CALGB 9720 received a single cytarabine/daunorubicin consolidation course identical to the induction regimen and were then randomly assigned to low-dose recombinant interleukin-2 maintenance therapy or none.[8] Patients on CALGB 10201 (n=75) received induction chemotherapy consisting of cytarabine and daunorubicin, with or without the *BCL2* antisense oblimersen sodium. The consolidation regimen included two cycles of cytarabine (2 g/m^2/d) with or without oblimersen.[9] The frequency of *TET2* mutations did not vary significantly between study protocols when analyzed separately for younger (*P*=.84) and older (*P*=.71) patients.

None of the protocols included allogeneic stem cell transplantation for CN-AML patients in first CR. Patients who enrolled on the treatment protocols also provided written informed consent to participate in the companion protocols CALGB 8461 (prospective cytogenetic companion), CALGB 9665 (leukemia tissue bank) and CALGB 20202 (molecular studies in acute myeloid leukemia), which involved collection of pretreatment bone marrow (BM) aspirates, blood samples, and buccal swabs. Mononuclear cells from BM and blood samples were enriched through Ficoll-Hypaque gradient centrifugation, and cryopreserved

for later use. Genomic DNA and total RNA were extracted as described previously.[10] Genomic DNA from buccal swabs was isolated using the Qiagen DNEasy Blood & Tissue kit (Qiagen, Hilden, Germany). All coding exons of the longest known *TET2* transcript variant (GenBank accession no. NM_001127208) were amplified from genomic DNA by polymerase chain reaction and analyzed by direct sequencing. Primer sequences are available upon request. Exon numbering follows the GenBank reference record.

Gene- and MicroRNA-Expression Profiling

To establish a signature of genes differentially expressed between *TET2*-mutated and *TET2* wild-type (*TET2*-wt) patients, we evaluated gene-expression profiles obtained using Affymetrix HG-U133plus2.0 oligonucleotide microarrays (Affymetrix, Santa Clara, CA). Details regarding sample preparation and array hybridization have been published previously.[11,12] Briefly, summary measures of gene expression were computed for each probe-set using the robust multichip average method, which incorporates quantile normalization of arrays. Expression values were logged (base 2) before analysis. A filtering step was performed to remove probe-sets that did not display significant variation in expression across arrays. In this procedure, a χ^2 test was used to test whether the observed variance in expression of a probe-set was significantly larger than the median observed variance in expression for all probe-sets, using α=.01 as the significance level. A total of 24,995 probe-sets passed the filtering criterion. Gene-expression signatures were then analyzed separately in the ELN

favorable-risk (41 *TET2*-mutated and 93 *TET2*-wt patients) and intermediate-I-risk (28 *TET2*-mutated and 121 *TET2*-wt patients) categories. Normalized expression values were compared between *TET2*-mutated and *TET2*-wt patients, and a univariable significance level of .001 was used to identify differentially expressed probe-sets. A global test of significance based on a permutation procedure was performed to determine whether or not the number of differentially expressed probe sets was more than expected by chance; if not, no signature is reported for the comparison.

For microRNA microarrays (The Ohio State University custom microRNA array version 4.0), signal intensities were calculated for each spot, with an adjustment made for local background. Spots that were flagged due to low signal-to-noise ratio on more than 75% of arrays were excluded from analysis. Signal intensities were log-transformed and quantile normalization was performed on arrays using spots for all human and mouse microRNA probes represented on the array. Log-signal intensities from replicate spots (ie, spots representing the same probe) were averaged. For each microRNA probe, an adjustment was made for batch effects (ie, differences in expression related to the batch in which arrays were hybridized). Further analysis was limited to 460 unique human probes that passed the filtering criterion. Separate microRNA signatures were obtained in the ELN favorable-risk (28 *TET2*-mutated and 50 *TET2*-wt patients) and in the intermediate-I-risk (21 *TET2*-mutated and 79 *TET2*-wt patients) subgroups. A comparison of microRNA expression was made between *TET2*-mutated and

TET2-wt patients, using a univariable significance level of .005 to identify differentially expressed microRNA probes. A global test of significance based on a permutation procedure was performed to determine whether or not the number of differentially expressed probes was more than expected by chance; if not, no signature is reported for the comparison. All microarray analyses were performed using BRB-ArrayTools Version 3.8.1, developed by Richard Simon, DSc, and Amy Peng Lam.

Definition of Clinical Endpoints

Clinical endpoints were defined, in accordance with generally accepted criteria,[13] as follows. CR required a BM aspirate with cellularity greater than 20% and maturation of all cell lines, less than 5% blasts and no Auer rods; in the peripheral blood, an absolute neutrophil count of ≥1,500/μL, platelet count of ≥100,000/μL, and no leukemic blasts; and no evidence of extramedullary leukemia, all of which had to persist for at least 1 month. Relapse was defined by the presence of ≥5% BM blasts, or circulating leukemic blasts, or the development of extramedullary leukemia. Overall survival (OS) was measured from the date of study entry until the date of death, and patients alive at last follow-up were censored. Disease-free survival (DFS) was measured from the date of CR until the date of relapse or death; patients alive and in complete remission were censored at last follow-up. Event-free survival (EFS) was measured from the date of study entry until induction failure, relapse or death; patients alive and in complete remission were censored at last follow-up.

Multivariable Analyses

Multivariable logistic regression models were generated for attainment of CR, and proportional hazards models were constructed for EFS, DFS and OS, using a limited backwards elimination procedure. Since we found a significant interaction between *TET2* mutation status and European LeukemiaNet (ELN) risk group assignment for all four endpoints, this interaction term was included in all multivariable models. Variables considered for model inclusion and evaluated in univariable models were: *TET2* mutation status, ELN risk group assignment (based on the presence/absence of *FLT3* internal tandem duplications [*FLT3*-ITD], and *NPM1* and *CEBPA* mutations), *TET2* mutation-by-ELN risk group interaction, *WT1* mutation status, *FLT3* tyrosine kinase domain mutation status, *MLL* partial tandem duplication status, age group (<60 *v* ≥60 years), sex, race, hemoglobin, platelet count, and white blood count (WBC). Age was considered as a dichotomous rather than as a continuous variable, based on an analysis of Martingale residuals for the continuous age regressed against survival. Additionally, the use of an age cut-off at 60 years allowed us to account for the fact that patients below and above that age were enrolled on different treatment protocols. Variables significant at α=.20 from the univariable analyses were considered for multivariable analyses. For the time-to-event endpoints, the proportional hazards assumption was checked for each variable individually. If the proportional hazards assumption was not met for a particular variable, then an artificial time-dependent covariate was included in all models that contained that variable.

RESULTS

Spectrum of TET2 *Mutations in CN-AML and Analysis of Matched Germline DNA Samples*

Overall, 141 variations from the reference sequence were found in the *TET2* coding sequence and adjacent splice sites in 104 of 427 patients (Table A1). Sixty-two patients had a single, heterozygous *TET2* sequence variation, while 42 patients had more than one variation. We observed a striking heterogeneity of *TET2* sequence variations: only 5 specific variations occurred in more than one patient in our cohort.

As in previous reports,[14-16] we did not identify “hot spots” for nonsense and frame shift variations, which were distributed across all coding exons. Of 102 nonsense, frame shift and splice site variations, matched buccal swab or remission BM DNA samples were available for 72. Seventy-one variations were found to be somatic mutations, whereas only one nonsense change was also detected in the corresponding germline sample. However, the same change (c.4393C>T; p.Arg1465X) was confirmed to be a somatically acquired mutation in three other patients. All nonsense, frame shift and splice site mutations were therefore included in further analyses.

After excluding known single nucleotide polymorphisms listed in the dbSNP database (available online at http://www.ncbi.nlm.nih.gov/projects/SNP/) or

detected in a cohort of healthy controls,[14] we detected 37 missense variations, leading to changes of single amino acids. Twenty-eight missense changes clustered within two evolutionarily conserved regions of the *TET2* gene, which encompass codons 1104 to 1478 and 1845 to 2002 (see Fig 1 in the main text).[14] Of these 28 missense variations inside the conserved domains, all 16 for which germline DNA could be evaluated were confirmed to be somatically acquired mutations. Therefore, all missense variations within the two evolutionarily conserved regions were retained in further analyses. In contrast, of the 9 missense variations outside the conserved domains, all 7 that could be tested were present in the germline. To our knowledge, these germline variations, causing changes in single amino acids, have not been described in healthy subjects or cancer patients. It remains undetermined whether they represent innocent polymorphisms or disease-relevant mutations. Therefore, all 9 patients with missense variations outside the two evolutionarily conserved domains were excluded from further analyses.

Applicability of the ELN Risk Classification to Our Patient Cohort

The ELN recently proposed a standardized reporting system which classifies CN-AML patients into favorable-risk and intermediate-I-risk categories based on presence or absence of *CEBPA* mutations, *NPM1* mutations and *FLT3*-ITD.[17] Since this classification scheme is relatively new, we evaluated the prognostic separation achieved by applying the ELN risk categories to our cohort of CN-AML patients. Patients in the favorable-risk group (n=199) had longer EFS than

patients in the intermediate-I-risk group (n=219) (*P*<.001; 3-year EFS rates, 42% *v* 9%). Favorable-risk patients also had a higher CR rate (85% *v* 65%, *P*<.001), longer DFS (*P*<.001; Fig A4A; 3-year DFS rates, 47% *v* 14%) and longer OS (*P*<.001; Fig A4B; 3-year OS rates, 54% *v* 19%), as compared with the intermediate-I-risk group. These results indicate that the ELN risk classification identified subgroups with significantly different outcomes in our cohort.

Prognostic Relevance of* TET2 *Mutations in Molecular Subsets Within the ELN Favorable-Risk and Intermediate-I-Risk Groups

The ELN favorable-risk group includes CN-AML patients with two distinct genotypes that were previously shown to be associated with favorable outcomes: 1) CN-AML patients with mutated *NPM1* without *FLT3*-ITD, and 2) those with mutated *CEBPA* (Table A2).[18] Consequently, we investigated whether the association of *TET2* mutations with inferior outcomes was consistent across both these genetic subgroups (Table A6). Among patients with mutated *NPM1* without *FLT3*-ITD, there was an association of *TET2* mutations with shorter EFS in comparison with *TET2*-wt patients (*P*=.003). *TET2*-mutated patients also had lower CR rates (*P*=.02), shorter DFS (*P*=.046; Fig A5A), and showed a trend towards shorter OS (*P*=.07; Fig A5B).

Similarly, among *CEBPA*-mutated patients, those with a *TET2* mutation had shorter EFS than *TET2*-wt patients (*P*=.009; Table A6). The CR rate was 75% for *CEBPA*-mutated/*TET2*-mutated and 88% for *CEBPA*-mutated/*TET2*-wt patients

(*P*=.27). *TET2* mutations were associated with shorter DFS (*P*=.03; Fig A5C) and OS (*P*=.003; Fig A5D) among *CEBPA*-mutated patients.

Finally, we also tested for an interaction between age group and *TET2* mutation status among our ELN favorable-risk cases. We found that the association of *TET2* mutations with outcomes did not differ significantly between older and younger patients (EFS, *P*=.98; CR, *P*=.98; DFS, *P*=.97; OS, *P*=.79).

The ELN intermediate-I-risk group comprises all those CN-AML patients that are not assigned to the favorable-risk category. Consequently, patients in the intermediate-I-group may have one of three different genotypes: 1) Mutated *NPM1* and *FLT3*-ITD and wild-type *CEBPA*, or 2) wild-type *NPM1* and *FLT3*-ITD and wild-type *CEBPA*, or 3) wild-type *NPM1* without *FLT3*-ITD and wild-type *CEBPA*. Following the ELN recommendations, we explored the prognostic impact of *TET2* mutations within these three subcategories (Table A7, Figure A6) We did not detect a significant association of *TET2* mutation status and outcomes in any of these subsets, although in some subgroups the number of *TET2*-mutated patients was too small to draw firm conclusions.

Overall, these results demonstrate that *TET2* mutations are associated with inferior outcomes in patients with either of the two genotypes (mutated *NPM1* without *FLT3*-ITD, or mutated *CEBPA*) that, according to the ELN guidelines, qualify a patient for the favorable-risk category. In contrast, exploratory analyses

did not show significant associations of *TET2* mutations with outcomes in the molecular subsets within the intermediate-I-risk group.

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Table A1. List of 141 Sequence Alterations in the Coding Sequence of *TET2* Detected in 104 Patients With Cytogenetically Normal Acute Myeloid Leukemia

UPN	Exon	cDNA Sequence Change	Deduced Change in Protein Sequence	Finding in Matched Buccal Swab or Remission Bone Marrow Genomic DNA Sample
362*	3	c.322T>C	p.Gln108X	not tested
293	3	c.434G>A	p.Ser145Asn	not tested
256*	3	c.519delG	p.Pro174fs	wild-type (buccal swab DNA)
380*	3	c.521delC	p.Pro174fs	wild-type (buccal swab DNA)
50	3	c.590_591insC	p.Val198fs	wild-type (buccal swab DNA)
285*	3	c.640dupT	p.Ser214fs	wild-type (buccal swab DNA)
249*	3	c.688_689delCT	p.Leu230fs	wild-type (buccal swab DNA)
190*	3	c.810_816dupCTCAGGG	p.Gln273fs	wild-type (buccal swab DNA)
342	3	c.941dupG	p.Cys314fs	wild-type (buccal swab DNA)
415	3	c.942T>A	p.Cys314X	not tested
352	3	c.945delC	p.Gln317fs	wild-type (buccal swab DNA)
397	3	c.998delC	p.Pro333fs	not tested
393*	3	c.1034_1037dupAGCT	p. Gly349fs	not tested
286*	3	c.1118_1122delAAAAT	p.Gln373fs	not tested
368*	3	c.1137_1138delTTinsC	p.Tyr380fs	wild-type (buccal swab DNA)
272*	3	c.1219delT	p.Ser407fs	wild-type (buccal swab DNA)
301	3	c.1240C>T	p.Gln414X	wild-type (buccal swab DNA)
337	3	c.1669C>T	p.Gln557X	wild-type (buccal swab DNA)
148	3	c.1747G>T	p.Glu583X	wild-type (buccal swab DNA)
79*	3	c.1755delT	p.S585fs	wild-type (buccal swab DNA)
294	3	c.1842delG	p.Leu615fs	not tested
79*	3	c.1847dupC	p.Pro616fs	wild-type (buccal swab DNA)
28*	3	c.1851delG	p.Arg617fs	wild-type (buccal swab DNA)
44*	3	c.1860C>G	p.Tyr620X	not tested
209*	3	c.2000_2016del17insC	p.His667fs	wild-type (buccal swab DNA)
270	3	c.2083dupA	p.Met695fs	not tested
393*	3	c.2148dupA	p.His717fs	wild-type (buccal swab DNA)
365	3	c.2149_2150del	p.His717fs	not tested
271*	3	c.2176_2179delCAGG	p.Ala727fs	wild-type (buccal swab DNA)
88	3	c.2218C>T	p.Gln740X	wild-type (buccal swab DNA)
318*	3	c.2236C>T	p.Gln746X	wild-type (buccal swab DNA)
313	*3*	*c.2299A>G*	*p.Asn767Asp*	*sequence change detected in buccal swab DNA sample*
184*	3	c.2305delC	p.Gln769fs	wild-type (buccal swab DNA)
114*	3	c.2428C>T	p.Gln810X	wild-type (remission sample)
381*	3	c.2441delG	p.Arg814fs	wild-type (buccal swab DNA)
251*	3	c.2443_2444insAT	p.Arg815fs	wild-type (buccal swab DNA)
360*	3	c.2450_2454del	p.Ser817fs	not tested
72*	3	c.2474C>G	p.Ser825X	wild-type (buccal swab DNA)
358*	3	c.2497delT	p.Ser833fs	not tested
114*	3	c.2527G>T	p.Glu843X	wild-type (remission sample)
153*	3	c.2604T>G	p.Phe868Leu	not tested
401	3	c.2692G>T†	p.Gly898X	not tested
109	3	c.2746C>T†	p.Gln916X	wild-type (buccal swab DNA)

UPN	Exon	cDNA Sequence Change	Deduced Change in Protein Sequence	Finding in Matched Buccal Swab or Remission Bone Marrow Genomic DNA Sample
228	3	c.2746C>T	p.Gln916X	wild-type (buccal swab DNA)
353	3	c.2746C>T	p.Gln916X	wild-type (buccal swab DNA)
373	3	c.2855dupT	p.Leu952fs	wild-type (buccal swab DNA)
120	3	c.2862G>R	p.Trp954X	wild-type (buccal swab DNA)
364*	3	c.2878C>T	p.Gln960X	wild-type (buccal swab DNA)
288	3	c.2896C>T	p.Gln966X	wild-type (buccal swab DNA)
174*	3	c.2918dupG	p.S972fs	wild-type (buccal swab DNA)
386	3	c.2964_2982dup	p.Thr995fs	not tested
59	*3*	*c.3030G>T*	*p.Glu1010Asp*	*sequence change detected in bone marrow sample obtained during complete remission*
282	3	c.3237delAinsCCC†	p.Ala1080fs	not tested
256*	3	c.3344delC	p.Pro1115fs	wild-type (buccal swab DNA)
172*	3	c.3409+1G>A	p.?	wild-type (buccal swab DNA)
309	3c	c.3448delC	p.His1150fs	wild-type (buccal swab DNA)
362*	3c	c.3500+2T>C	p.?	not tested
358*	4	c.3528delG	p.Ile1177fs	not tested
184*	4	c.3535dupA	p.Arg1179fs	wild-type (buccal swab DNA)
187*	4	c.3535dupA	p.Arg1179fs	wild-type (buccal swab DNA)
286*	4	c.3594+3A>G	p.?	not tested
92	5	c.3604A>T	p.Arg1202X	wild-type (buccal swab DNA)
247	5	c.3632G>A	p.Cys1211Tyr	wild-type (buccal swab DNA)
96	5	c.3643dupG	p.Glu1215fs	wild-type (buccal swab DNA)
145	5	c.3646C>T	p.Arg1216X	wild-type (buccal swab DNA)
28*	5	c.3656A>G	p.His1219Arg	wild-type (buccal swab DNA)
272*	5	c.3657C>G	p.His1219Gln	wild-type (buccal swab DNA)
280*	5	c.3729delA	p.Lys1243fs	not tested
44*	5	c.3732_3733delCT	p.Tyr1245fs	wild-type (buccal swab DNA)
115*	5	c.3781C>T	p.Arg1261Cys	not tested, but somatically acquired mutation in two other patients
249*	5	c.3781C>T	p.Arg1261Cys	wild-type (buccal swab DNA)
364*	5	c.3781C>T	p.Arg1261Cys	wild-type (buccal swab DNA)
345	5	c.3787T>C	p.Cys1263Arg	wild-type (buccal swab DNA)
426*	5	c.3787T>G	p.Cys1263Gly	not tested, but somatically acquired mutation at same codon in one other patient
233	5	c.3790G>C†	p.Ala1264Pro	not tested
93	6	c.3812dupG	p.Cys1271fs	wild-type (buccal swab DNA)
231	6	c.3818G>C	p.Cys1273Ser	not tested
419	6	c.3866G>A	p.Cys1289Tyr	wild-type (buccal swab DNA)
284*	6	c.3885delC	p.Tyr1295X	wild-type (buccal swab DNA)
252	6	c.3893G>A	p.Cys1298Tyr	wild-type (buccal swab DNA)
400*	6	c.3893G>A	p.Cys1298Tyr	not tested, but somatically acquired mutation in one other patient
297	6	c.3908G>A	p.Ser1303Asn	not tested
90	6	c.3921delG	p.Arg1307fs	wild-type (buccal swab DNA)
153*	6	c.3954+1G>A	p.?	not tested
271*	7	c.3957delA	p.Glu1320fs	wild-type (buccal swab DNA)

UPN	Exon	cDNA Sequence Change	Deduced Change in Protein Sequence	Finding in Matched Buccal Swab or Remission Bone Marrow Genomic DNA Sample
277	7	c.4005_4009delnsTG	p.Thr1336_Tyr1337delinsAsp	wild-type (buccal swab DNA)
378	7	c.4011_4012insGTAT	p.Lys1338fs	wild-type (buccal swab DNA)
380*	7	c.4011T>A	p.Tyr1337X	wild-type (buccal swab DNA)
185	7	c.4044+1G>C	p.?	wild-type (buccal swab DNA)
72*	7	c.4044+1G>T	p.?	wild-type (buccal swab DNA)
193	7	c.4044+2dupT	p.?	wild-type (buccal swab DNA)
162	8	c.4082G>A†	p.Gly1361Asp	wild-type (buccal swab DNA)
164	8	c.4087A>T	p.Lys1363X	wild-type (buccal swab DNA)
402*	8	c.4097G>A	p.Arg1366His	not tested
55	8	c.4102T>C	p.Phe1368Leu	wild-type (buccal swab DNA)
384	8	c.4128C>G	p.Asp1376Glu	wild-type (buccal swab DNA)
153*	8	c.4138C>T	p.His1380Tyr	not tested
209*	8	c.4144C>T	p.His1382Tyr	wild-type (buccal swab DNA)
172*	8	c.4148delG	p.Arg1383fs	wild-type (buccal swab DNA)
368*	8	c.4167_4168dupGA	p.Asn1390fs	wild-type (buccal swab DNA)
433	8	c.4182+2dupT	p.?	not tested
430	9	c.4183-1G>C	p.?	not tested
360*	9	c.4249G>T	p.Val1417Phe	not tested, but described as somatically acquired mutation in Abdel-Wahab et al. 2009
317	9	c.4317delA	p.Lys1439fs	wild-type (buccal swab DNA)
318*	9	c.4317dupA	p.Arg1440fs	wild-type (buccal swab DNA)
400*	9	c.4317dupA	p.Arg1440fs	not tested, but somatically acquired mutation in one other patient
64	9	c.4393C>T	p.Arg1465X	wild-type (buccal swab DNA)
73	*9*	*c.4393C>T*	*p.Arg1465X*	*sequence change detected in buccal swab DNA sample*
174*	9	c.4393C>T	p.Arg1465X	wild-type (buccal swab DNA)
381*	9	c.4393C>T	p.Arg1465X	wild-type (buccal swab DNA)
280*	9	c.4501C>T	p.Gln1501X	not tested
142	*9*	*c.4513G>A*	*p.Ala1505Thr*	*sequence change detected in buccal swab DNA sample*
11	10	c.4824T>G	p.Tyr1608X	wild-type (buccal swab DNA)
264*	10	c.4887_4936dup	p.Leu1646fs	not tested
251*	10	c.4925delG	p.Cys1642fs	wild-type (buccal swab DNA)
285*	10	c.4977T>G	p.Tyr1659X	wild-type (buccal swab DNA)
308	*10*	*c.5089G>A*	*pGly1697Arg*	*sequence change detected in buccal swab DNA sample*
284*	10	c.5128_5131delACCA	p.Thr1710fs	wild-type (buccal swab DNA)
14	*10*	*c.5152G>T*	*p.Val1718Leu*	*sequence change detected in buccal swab DNA sample*
97	*10*	*c.5152G>T*	*p.Val1718Leu*	*sequence change detected in buccal swab DNA sample*
173	10	c.5162T>A or G>A	p.L1721X	wild-type (buccal swab DNA)
357	10	c.5219T>G	p.Leu1740X	not tested
216	10	c.5283delC	p.His1761fs	wild-type (buccal swab DNA)
402*	10	c.5338delC	p.Leu1780fs	not tested
367	10	c.5374delC	p.His1792fs	wild-type (buccal swab DNA)
17	10	c.5456_5457insGGGAC	p.Ser1820fs	wild-type (buccal swab DNA)

UPN	Exon	cDNA Sequence Change	Deduced Change in Protein Sequence	Finding in Matched Buccal Swab or Remission Bone Marrow Genomic DNA Sample
253	10	c.5482C>T	p.Gln1828X	wild-type (buccal swab DNA)
2	*10*	*c.5497G>A*	*p.Val1833Ile*	*sequence change detected in buccal swab DNA sample*
287	10	c.5540G>A†	p.Trp1847X	not tested
264*	10	c.5603A>C	p.His1868Pro	not tested
183	10	c.5618T>C	p.I1873T	wild-type (buccal swab DNA)
187*	10	c.5627C>T	p.Ala1876Val	wild-type (buccal swab DNA)
245	10	c.5644G>C	p.Ala1882Pro	wild-type (buccal swab DNA)
190*	10	c.5645_5646delCC	p.Ala1882fs	wild-type (buccal swab DNA)
146*	10	c.5665_5670delCCCAAT	p.Pro1889_Asn1890del	not tested
115*	10	c.5675dupA	p.Asn1892fs	not tested
146*	10	c.5681C>A	p.Pro1894His	not tested
154	10	c.5696T>C	p.Leu1899Pro	wild-type (buccal swab DNA)
374	10	c.5750G>A	p.Trp1917X	wild-type (buccal swab DNA)
421	10	c.5885C>T	p.Pro1962Leu	not tested
426*	10	c.5950_5957del	p.Val1984fs	wild-type (buccal swab DNA)

ABBREVIATION: UPN, unique patient number.

NOTE: Mutation nomenclature on the cDNA level follows the recommendations of the Human Genome Variation Society (HGVS) and is based on the reference sequence for *TET2* transcript variant 1 (NCBI GeneBank accession number NM_001127208). Exon numbering is also based on the NCBI reference transcript. The descriptions of changes on the protein level were deduced from the cDNA sequence alterations.

* This patient had more than one sequence alteration.

† This mutation appeared homozygous on the sequencing chromatogram, indicating that either both alleles were affected or the remaining wild-type allele was lost.

Table A2. Clinical and Molecular Characteristics According to *TET2* Mutation Status in 199 Patients with Primary Cytogenetically Normal Acute Myeloid Leukemia Classified in the ELN Favorable-Risk Category

Variable	*TET2* Mutated n=53	*TET2* Wild-Type n=146	*P*
Age, years			<.001
Median	66	56	
Range	29-79	19-81	
Age group, no. (%)			<.001
<60 years	14 (26)	79 (54)	
≥60 years	39 (74)	67 (46)	
Female sex, no. (%)	28 (53)	74 (51)	.87
Race, no. (%)			1.0
White	46 (90)	130 (90)	
Nonwhite	5 (10)	15 (10)	
Hemoglobin, g/dL			.32
Median	9.3	9.5	
Range	6.7-11.9	4.8-13.4	
Platelet count, x10^9/L			.64
Median	60	68	
Range	8-510	7-481	
WBC count, x10^9/L			.04
Median	33.2	23.4	
Range	2.2-450.0	0.9-295.0	
Percentage of blood blasts			.40
Median	46	52	
Range	0-95	0-97	
Percentage of bone marrow blasts			.69
Median	67	65	
Range	11-94	10-98	
FAB category, no. (%)			---
M0	0 (0)	1 (1)	
M1	7 (20)	25 (25)	
M2	12 (34)	30 (30)	
M4	9 (26)	24 (24)	
M5	7 (20)	19 (19)	
M6	0 (0)	2 (2)	
Extramedullary involvement, no. (%)	14 (26)	41 (29)	.73

Variable	*TET2* Mutated n=53	*TET2* Wild-Type n=146	*P*
***IDH1,* no. (%)**			.001
Mutated	1 (2)	28 (19)	
Wild-*type*	51 (98)	118 (81)	
***IDH2,* no. (%)**			<.001
Mutated	1 (2)	30 (21)	
- Codon R140 mutation	1	30	
- Codon R172 mutation	0	0	
Wild-type	51 (98)	116 (79)	
***CEBPA*, no. (%)**			.23
Mutated	20 (38)	42 (29)	
Wild-type†	32 (62)	102 (71)	
***NPM1*, no. (%)**			.46
Mutated	38 (72)	112 (77)	
Wild-type	15 (28)	34 (23)	
***FLT3*-ITD, no. (%)**			.59
Present	6 (11)	13 (9)	
Absent	47 (89)	133 (91)	
Combined *FLT3*-ITD/*NPM1* genotype, no. (%)			.21
NPM1 mutated and no *FLT3*-ITD	34 (64)	108 (74)	
NPM1 wild-type and/or *FLT3*-ITD*	19 (36)	38 (26)	
***FLT3*-TKD, no. (%)**			.63
Present	5 (9)	19 (13)	
Absent	48 (91)	126 (87)	
***WT1*, no. (%)**			1.0
Mutated	3 (6)	10 (7)	
Wild-type	50 (94)	136 (93)	
***MLL*-PTD, no. (%)**			.36
Present	3 (7)	4 (3)	
Absent	39 (93)	128 (97)	

ABBREVIATIONS: ELN, European LeukemiaNet; WBC, white blood count; FAB, French-American-British classification; *FLT3*-ITD, internal tandem duplication of the *FLT3* gene; *FLT3*-TKD, tyrosine kinase domain mutation in the *FLT3* gene; *MLL*-PTD, partial tandem duplication of the *MLL* gene.

* By definition of the ELN favorable-risk category, all of these patients had *CEBPA* mutations.

† By definition of the ELN favorable-risk category, all of these patients had mutated *NPM1* without *FLT3*-ITD.

Table A3. List of Affymetrix Probe-Sets Differentially Expressed Between *TET2*-Mutated and *TET2*-Wild-Type Cytogenetically Normal Acute Myeloid Leukemia Patients Classified in the ELN Favorable-Risk Category

Probe-sets upregulated in *TET2*-mutated patients						
Affymetrix Probe-Set	**Gene Symbol**	**Description**	**Fold Change**	***P***	**Entrez Gene ID**	**Chromosome Band**
232985_s_at	***DPPA4***	developmental pluripotency associated 4	**2.78**	3.40E-04	55211	3q13.13
210254_at	***MS4A3***	membrane-spanning 4-domains, subfamily A, member 3 (hematopoietic cell-specific)	**2.70**	2.59E-04	932	11q12.1
212843_at	***NCAM1***	neural cell adhesion molecule 1	**2.56**	1.00E-07	4684	11q23.1
1554892_a_at	***MS4A3***	membrane-spanning 4-domains, subfamily A, member 3 (hematopoietic cell-specific)	**2.44**	2.36E-04	932	11q12.1
218589_at	***LPAR6***	lysophosphatidic acid receptor 6	**2.44**	4.90E-05	10161	13q14
230550_at	***MS4A6A***	membrane-spanning 4-domains, subfamily A, member 6A	**2.27**	3.64E-04	64231	11q12.1
219666_at	***MS4A6A***	membrane-spanning 4-domains, subfamily A, member 6A	**2.22**	1.02E-04	64231	11q12.1
227388_at	***TUSC1***	tumor suppressor candidate 1	**2.22**	7.90E-06	286319	9p21.2
224356_x_at	***MS4A6A***	membrane-spanning 4-domains, subfamily A, member 6A	**2.17**	8.50E-04	64231	11q12.1
223280_x_at	***MS4A6A***	membrane-spanning 4-domains, subfamily A, member 6A	**2.13**	7.39E-04	64231	11q12.1
227394_at	***NCAM1***	neural cell adhesion molecule 1	**2.13**	< 1E-07	4684	11q23.1
201425_at	***ALDH2***	aldehyde dehydrogenase 2 family (mitochondrial)	**2.08**	1.04E-04	217	12q24.2
223922_x_at	***MS4A6A***	membrane-spanning 4-domains, subfamily A, member 6A	**2.08**	3.92E-04	64231	11q12.1
225331_at	***CCDC50***	coiled-coil domain containing 50	**2.08**	7.50E-06	152137	3q28
203476_at	***TPBG***	trophoblast glycoprotein	**2.00**	8.03E-05	7162	6q14-q15
244297_at	***ANKRD18A***	ankyrin repeat domain 18A	**1.96**	3.93E-04	253650	9p13.1
232725_s_at	***MS4A6A***	membrane-spanning 4-domains, subfamily A, member 6A	**1.92**	5.33E-05	64231	11q12.1
228155_at	***C10orf58***	chromosome 10 open reading frame 58	**1.89**	< 1E-07	84293	10q23.1
206440_at	***LIN7A***	lin-7 homolog A (C. elegans)	**1.85**	4.03E-04	8825	12q21
219651_at	***DPPA4***	developmental pluripotency associated 4	**1.85**	1.53E-04	55211	3q13.13
209205_s_at	***LMO4***	LIM domain only 4	**1.82**	1.48E-04	8543	1p22.3
214953_s_at	***APP***	amyloid beta (A4) precursor protein	**1.82**	8.81E-04	351	21q21.2-q21.3
224435_at	***C10orf58***	chromosome 10 open reading frame 58	**1.82**	1.00E-07	84293	10q23.1
209129_at	***TRIP6***	thyroid hormone receptor interactor 6	**1.79**	6.10E-06	7205	7q22

213931_at	***ID2*** ***ID2B***	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein inhibitor of DNA binding 2B, dominant negative helix-loop-helix protein (pseudogene)	**1.79**	7.04E-04	3398 84099	2p25 3p14.2
228693_at	***CCDC50***	coiled-coil domain containing 50	**1.79**	5.84E-04	152137	3q28
235048_at	***FAM169A***	family with sequence similarity 169, member A	**1.79**	5.02E-05	26049	5q13.3
243546_at	***---***	Transcribed locus Hs.658669	**1.79**	6.80E-06	---	---
218963_s_at	***KRT23***	keratin 23 (histone deacetylase inducible)	**1.75**	3.18E-05	25984	17q21.2
1559034_at	***SIRPB2***	signal-regulatory protein beta 2	**1.72**	4.07E-04	284759	20p13
208782_at	***FSTL1***	follistatin-like 1	**1.72**	7.06E-04	11167	3q13.33
223059_s_at	***FAM107B***	family with sequence similarity 107, member B	**1.72**	9.10E-04	83641	10p13
226713_at	***CCDC50***	coiled-coil domain containing 50	**1.72**	6.54E-04	152137	3q28
206039_at	***RAB33A***	RAB33A, member RAS oncogene family	**1.69**	2.54E-04	9363	Xq26.1
213397_x_at	***RNASE4***	ribonuclease, RNase A family, 4	**1.69**	1.10E-06	6038	14q11.1
221024_s_at	***SLC2A10***	solute carrier family 2 (facilitated glucose transporter), member 10	**1.69**	1.00E-07	81031	20q13.1
226311_at	***---***	---	**1.69**	5.14E-04	---	---
213823_at	***HOXA11***	homeobox A11	**1.67**	1.44E-05	3207	7p15-p14
225123_at	***---***	---	**1.67**	6.02E-04	---	---
204646_at	***DPYD***	dihydropyrimidine dehydrogenase	**1.61**	2.48E-04	1806	1p22
209298_s_at	***ITSN1***	intersectin 1 (SH3 domain protein)	**1.61**	1.30E-06	6453	21q22.1-q22.2
209610_s_at	***SLC1A4***	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	**1.61**	6.13E-05	6509	2p15-p13
213933_at	***PTGER3***	prostaglandin E receptor 3 (subtype EP3)	**1.61**	8.64E-05	5733	1p31.2
219288_at	***C3orf14***	chromosome 3 open reading frame 14	**1.61**	1.11E-04	57415	3p14.2
232686_at	***SIGLECP3***	sialic acid binding Ig-like lectin, pseudogene 3	**1.61**	2.53E-04	284367	19q13.3
235051_at	***CCDC50***	coiled-coil domain containing 50	**1.61**	9.06E-04	152137	3q28
1552388_at	***---***	hypothetical protein FLJ30901	**1.59**	7.96E-04	150378	22q13.31
203231_s_at	***ATXN1***	ataxin 1	**1.59**	9.76E-04	6310	6p23
203548_s_at	***LPL***	lipoprotein lipase	**1.59**	2.46E-04	4023	8p22
213385_at	***CHN2***	chimerin (chimaerin) 2	**1.56**	9.44E-04	1124	7p15.3
219295_s_at	***PCOLCE2***	procollagen C-endopeptidase enhancer 2	**1.56**	2.50E-06	26577	3q21-q24
226925_at	***ACPL2***	acid phosphatase-like 2	**1.56**	4.63E-04	92370	3q23
200771_at	***LAMC1***	laminin, gamma 1 (formerly LAMB2)	**1.54**	6.15E-04	3915	1q31

207496_at	***MS4A2***	membrane-spanning 4-domains, subfamily A, member 2 (Fc fragment of IgE, high affinity I, receptor for; beta polypeptide)	**1.54**	5.99E-04	2206	11q13
210164_at	***GZMB***	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	**1.54**	8.19E-04	3002	14q11.2
226609_at	***DCBLD1***	discoidin, CUB and LCCL domain containing 1	**1.54**	8.18E-04	285761	6q22.1
228805_at	***C5orf25***	chromosome 5 open reading frame 25	**1.54**	2.02E-04	375484	5q35.2
204039_at	***CEBPA***	CCAAT/enhancer binding protein (C/EBP), alpha	**1.52**	1.80E-04	1050	19q13.1
212012_at	***PXDN***	peroxidasin homolog (Drosophila)	**1.52**	7.04E-04	7837	2p25
213915_at	***NKG7***	natural killer cell group 7 sequence	**1.52**	3.74E-05	4818	19q13.41
233518_at	***---***	Homo sapiens cDNA FLJ11493 fis, clone HEMBA1001940.	**1.52**	1.51E-04	---	---
240991_at	***---***	Transcribed locus	**1.52**	9.05E-04	---	---
212607_at	***AKT3***	v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma)	**1.49**	6.70E-05	10000	1q43-q44
212810_s_at	***SLC1A4***	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	**1.49**	5.91E-04	6509	2p15-p13
239950_at	***HOXA11AS***	HOXA11 antisense RNA (non-protein coding)	**1.49**	8.68E-04	221883	7p15.2
239934_x_at	---	Transcribed locus Hs.662980	**1.47**	3.25E-04	---	---
207497_s_at	***MS4A2***	membrane-spanning 4-domains, subfamily A, member 2 (Fc fragment of IgE, high affinity I, receptor for; beta polypeptide)	**1.45**	3.36E-04	2206	11q13
210220_at	***FZD2***	frizzled homolog 2 (Drosophila)	**1.45**	6.05E-05	2535	17q21.1
212811_x_at	***SLC1A4***	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	**1.45**	1.74E-04	6509	2p15-p13
216472_at	***ITSN1***	intersectin 1 (SH3 domain protein)	**1.45**	2.50E-04	6453	21q22.1-q22.2
216628_at	***---***	cDNA DKFZp586A0617 (from clone DKFZp586A0617)	**1.45**	1.06E-05	---	---
219038_at	***MORC4***	MORC family CW-type zinc finger 4	**1.45**	3.21E-05	79710	Xq22.3
228600_x_at	***C7orf46***	chromosome 7 open reading frame 46	**1.45**	2.60E-04	340277	7p15.3
238174_at	---	Transcribed locus Hs.112143	**1.45**	6.88E-04	---	---
1553635_s_at	***TCTEX1D1***	Tctex1 domain containing 1	**1.43**	7.32E-04	200132	1p31.3
202914_s_at	***ARHGEF11***	Rho guanine nucleotide exchange factor (GEF) 11	**1.43**	3.11E-04	9826	1q21
205308_at	***FAM164A***	family with sequence similarity 164, member A	**1.43**	3.22E-05	51101	8q21.12
209297_at	***ITSN1***	intersectin 1 (SH3 domain protein)	**1.43**	6.50E-06	6453	21q22.1-q22.2
213954_at	***FAM169A***	family with sequence similarity 169, member A	**1.43**	1.21E-04	26049	5q13.3
223660_at	***ADORA3***	adenosine A3 receptor	**1.43**	9.07E-04	140	1p13.2
230666_at	***HOXA11AS***	HOXA11 antisense RNA (non-protein coding)	**1.43**	6.87E-04	221883	7p15.2
232724_at	***MS4A6A***	membrane-spanning 4-domains, subfamily A, member 6A	**1.43**	5.74E-04	64231	11q12.1

236215_at	**---**	---	**1.43**	1.10E-06	---	---
228557_at	***L3MBTL4***	l(3)mbt-like 4 (Drosophila)	**1.41**	4.23E-04	91133	18p11.31
233446_at	***ONECUT2***	one cut homeobox 2	**1.41**	2.75E-04	9480	18q21.1-q21.2
202913_at	***ARHGEF11***	Rho guanine nucleotide exchange factor (GEF) 11	**1.39**	2.14E-04	9826	1q21
230292_at	**---**	LOC100131993 similar to hCG2020760	**1.39**	8.12E-04	1E+08	13q14.2
230446_at	---	Transcribed locus Hs.285724	**1.39**	5.75E-04	---	---
239675_at	**---**	hypothetical protein LOC283143	**1.39**	3.25E-04	283143	11q23.3
1557014_a_at	***C9orf122***	chromosome 9 open reading frame 122	**1.37**	2.60E-05	158228	9p13.1
205158_at	***RNASE4***	ribonuclease, RNase A family, 4	**1.37**	1.12E-04	6038	14q11.1
228218_at	***LAMP***	limbic system-associated membrane protein	**1.37**	4.01E-04	---	---
228565_at	***KIAA1804***	mixed lineage kinase 4	**1.37**	3.23E-04	84451	1q42
232309_at	**---**	hypothetical protein LOC202181	**1.37**	6.66E-05	202181	5q35.3
52975_at	***FAM125B***	family with sequence similarity 125, member B	**1.37**	1.17E-04	89853	9q33.3
1554636_at	**---**	ENSG00000204636	**1.35**	6.35E-04	---	---
1559500_at	***VPS8***	vacuolar protein sorting 8 homolog (S. cerevisiae)	**1.35**	9.73E-04	23355	3q27.2
218532_s_at	***FAM134B***	family with sequence similarity 134, member B	**1.35**	8.20E-05	54463	5p15.1
234148_at	***LRRC8D***	Homo sapiens leucine rich repeat containing 8 family, member D	**1.35**	7.55E-04	---	---
241808_at	***FAM164A***	family with sequence similarity 164, member A	**1.35**	1.89E-05	51101	8q21.12
1560522_at	**---**	hypothetical protein LOC201477	**1.33**	7.50E-04	201477	18p11.31
201349_at	***SLC9A3R1***	solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1	**1.33**	6.41E-04	9368	17q25.1
203874_s_at	***SMARCA1***	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1	**1.33**	1.92E-05	6594	Xq25
218510_x_at	***FAM134B***	family with sequence similarity 134, member B	**1.33**	5.27E-04	54463	5p15.1
35776_at	***ITSN1***	intersectin 1 (SH3 domain protein)	**1.33**	1.57E-04	6453	21q22.1-q22.2
1556602_at	***SLC19A2***	Homo sapiens solute carrier family 19 (thiamine transporter), member 2	**1.32**	7.75E-04	10560	1q23.3
200770_s_at	***LAMC1***	laminin, gamma 1 (formerly LAMB2)	**1.32**	6.68E-04	3915	1q31
214829_at	***AASS***	aminoadipate-semialdehyde synthase	**1.32**	4.01E-04	10157	7q31.3
220609_at	**---**	hypothetical protein LOC202181	**1.32**	5.96E-05	202181	5q35.3
221828_s_at	***FAM125B***	family with sequence similarity 125, member B	**1.32**	1.74E-04	89853	9q33.3
239265_at	***TMEM20***	transmembrane protein 20	**1.32**	3.78E-04	159371	10q23.33
240877_x_at	***AMOTL1***	angiomotin like 1	**1.32**	2.28E-05	154810	11q14.3

242045_at	***ANKRD18A***	Ankyrin repeat domain 18A	**1.32**	3.59E-04	253650	9p13.1
244752_at	***ZNF438***	zinc finger protein 438	**1.32**	2.99E-04	220929	10p11.23-p11.22
203143_s_at	***KIAA0040***	KIAA0040	**1.30**	4.42E-05	9674	1q24-q25
204457_s_at	***GAS1***	growth arrest-specific 1	**1.30**	6.36E-04	2619	9q21.3-q22
208493_at	***HOXA11***	homeobox A11	**1.30**	6.44E-04	3207	7p15-p14
213555_at	***RWDD2A***	RWD domain containing 2A	**1.30**	4.20E-04	112611	6q14.2
239911_at	***ONECUT2***	one cut homeobox 2	**1.30**	5.75E-04	9480	18q21.1-q21.2
242956_at	***IDH1***	isocitrate dehydrogenase 1 (NADP+), soluble	**1.30**	2.00E-04	3417	2q33.3
51158_at	***FAM174B***	family with sequence similarity 174, member B	**1.30**	4.05E-05	400451	15q26.1
1552343_s_at	***PDE7A***	phosphodiesterase 7A	**1.28**	9.90E-06	5150	8q13
212552_at	***HPCAL1***	hippocalcin-like 1	**1.28**	4.50E-04	3241	2p25.1
214255_at	***ATP10A***	ATPase, class V, type 10A	**1.28**	5.71E-04	57194	15q11.2
221880_s_at	***FAM174B***	family with sequence similarity 174, member B	**1.28**	1.58E-04	400451	15q26.1
228040_at	***---***	hypothetical locus MGC21881	**1.28**	7.39E-04	389741	9q21.11
229743_at	***ZNF438***	zinc finger protein 438	**1.28**	5.22E-04	220929	10p11.23-p11.22
232664_at	***---***	hypothetical gene FLJ12334 supported by AK022396; AK097927	**1.28**	9.64E-04	400946	2p24.1
1558685_a_at	***---***	hypothetical protein BC009467	**1.27**	1.15E-04	158960	Xq28
207177_at	***PTGFR***	prostaglandin F receptor (FP)	**1.27**	3.86E-04	5737	1p31.1
209204_at	***LMO4***	LIM domain only 4	**1.27**	1.96E-04	8543	1p22.3
218615_s_at	***TMEM39A***	transmembrane protein 39A	**1.27**	6.93E-04	55254	3q13.33
218796_at	***FERMT1***	fermitin family homolog 1 (Drosophila)	**1.27**	9.32E-04	55612	20p12.3
222880_at	***AKT3***	v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma)	**1.27**	7.35E-04	10000	1q43-q44
226454_at	***MARCH9***	membrane-associated ring finger (C3HC4) 9	**1.27**	4.12E-04	92979	12q14.1
1566426_at	***---***	Transcribed locus Hs.683993	**1.25**	8.86E-04	---	---
215314_at	***ANK3***	ankyrin 3, node of Ranvier (ankyrin G)	**1.25**	9.09E-04	288	10q21.2
226822_at	***STOX2***	storkhead box 2	**1.25**	2.96E-04	56977	4q35.1
230951_at	***---***	Transcribed locus Hs.369232	**1.25**	9.18E-05	---	---
201612_at	***ALDH9A1***	aldehyde dehydrogenase 9 family, member A1	**1.23**	2.48E-04	223	1q23.1
224229_s_at	***AKT3***	v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma)	**1.23**	5.18E-04	10000	1q43-q44
200831_s_at	***SCD***	stearoyl-CoA desaturase (delta-9-desaturase)	**1.22**	9.24E-05	6319	10q24.31

225459_at	***AMOTL1***	angiomotin like 1	**1.22**	9.84E-04	154810	11q14.3
241723_at	***IQGAP2***	IQ motif containing GTPase activating protein 2	**1.22**	9.27E-04	10788	5q13.3
1552788_a_at	***HELB***	helicase (DNA) B	**1.20**	3.39E-04	92797	12q14.3
1554701_a_at	***TBC1D16***	TBC1 domain family, member 16	**1.20**	2.60E-05	125058	17q25.3
231532_at	***NCAM1***	neural cell adhesion molecule 1	**1.19**	5.29E-04	4684	11q23.1
242879_x_at	***---***	---	**1.19**	6.60E-04	---	---
204944_at	***PTPRG***	protein tyrosine phosphatase, receptor type, G	**1.18**	5.70E-05	5793	3p21-p14
227803_at	***ENPP5***	ectonucleotide pyrophosphatase/phosphodiesterase 5 (putative function)	**1.14**	5.08E-04	59084	6p21.1-p11.2

Probe-sets downregulated in *TET2*-mutated patients

Affymetrix Probe-Set	Gene Symbol	Description	Fold Change	*P*	Entrez Gene ID	Chromosome Band
219876_s_at	***GOLGA2L1***	golgi autoantigen, golgin subfamily a, 2-like 1	**0.85**	9.85E-04	55592	12q23.1
222432_s_at	***CCDC47***	coiled-coil domain containing 47	**0.83**	6.73E-04	57003	17q23.3
208066_s_at	***GTF2B***	general transcription factor IIB	**0.83**	3.97E-04	2959	1p22-p21
217836_s_at	***YY1AP1***	YY1 associated protein 1	**0.83**	5.11E-04	55249	1q22
210687_at	***CPT1A***	carnitine palmitoyltransferase 1A (liver)	**0.82**	9.25E-04	1374	11q13.1-q13.2
203598_s_at	***WBP4***	WW domain binding protein 4 (formin binding protein 21)	**0.81**	7.11E-04	11193	13q14.11
225222_at	***HIAT1***	hippocampus abundant transcript 1	**0.81**	1.87E-04	64645	1p21.2
235269_at	***FAM83F***	family with sequence similarity 83, member F	**0.81**	3.26E-05	113828	22q13.1
238035_at	***SP3***	Sp3 transcription factor	**0.81**	7.18E-04	6670	2q31
1554229_at	***C5orf41***	chromosome 5 open reading frame 41	**0.80**	6.60E-04	153222	5q35.1
206003_at	***CEP135***	centrosomal protein 135kDa	**0.80**	3.09E-04	9662	4q12
209861_s_at	***METAP2***	methionyl aminopeptidase 2	**0.80**	8.06E-04	10988	12q22
213546_at	***---***	hypothetical protein DKFZp586I1420	**0.80**	1.54E-04	222161	7p14.3
223085_at	***RNF19A***	ring finger protein 19A	**0.80**	1.15E-04	25897	8q22
208844_at	***VDAC3***	voltage-dependent anion channel 3	**0.79**	5.51E-04	7419	8p11.2
233191_at	***RUFY2***	RUN and FYVE domain containing 2	**0.79**	1.24E-04	55680	10q21.3
1556224_a_at	***ZNF783***	zinc finger family member 783	**0.79**	8.93E-04	155060	7q36.1
203579_s_at	***SLC7A6***	solute carrier family 7 (cationic amino acid transporter, y+ system), member 6	**0.79**	1.87E-04	9057	16q22.1
221781_s_at	***DNAJC10***	DnaJ (Hsp40) homolog, subfamily C, member 10	**0.79**	7.46E-04	54431	2q32.1
229791_at	***LPCAT2***	lysophosphatidylcholine acyltransferase 2	**0.79**	6.02E-04	54947	16q12.2

239973_at	**---**	Transcribed locus Hs.212709	**0.79**	2.61E-04	---	---
220018_at	**CBLL1**	Cas-Br-M (murine) ecotropic retroviral transforming sequence-like 1	**0.78**	7.74E-04	79872	7q22.3-q31.1
1554057_at	**---**	hypothetical LOC645676	**0.77**	4.11E-04	645676	1q22
1558914_at	**PPPDE1**	PPPDE peptidase domain containing 1	**0.77**	8.38E-05	51029	1q44
203578_s_at	**SLC7A6**	solute carrier family 7 (cationic amino acid transporter, y+ system), member 6	**0.77**	2.11E-04	9057	16q22.1
223925_s_at	**LUZP6**	leucine zipper protein 6	**0.77**	6.51E-04	136319	7q33
202693_s_at	**STK17A**	serine/threonine kinase 17a	**0.76**	4.93E-04	9263	7p12-p14
212597_s_at	**HMGXB4**	HMG box domain containing 4	**0.76**	3.31E-04	10042	22q13.1
237626_at	**---**	Transcribed locus Hs.671926	**0.76**	1.29E-04	---	---
244561_at	**---**	Homo sapiens cDNA clone IMAGE:3079901	**0.76**	2.80E-04	---	---
1568665_at	**RNF103**	ring finger protein 103	**0.75**	1.30E-04	7844	2p11.2
202427_s_at	**BRP44**	brain protein 44	**0.75**	8.05E-05	25874	1q24
217845_x_at	**HIGD1A**	HIG1 hypoxia inducible domain family, member 1A	**0.75**	8.05E-05	25994	3p22.1
219201_s_at	**TWSG1**	twisted gastrulation homolog 1 (Drosophila)	**0.75**	9.62E-04	57045	18p11.3
227718_at	**PURB**	purine-rich element binding protein B	**0.75**	2.11E-04	5814	7p13
64488_at	**IRGQ**	immunity-related GTPase family, Q	**0.75**	3.71E-04	126298	19q13.31
205288_at	**CDC14A**	CDC14 cell division cycle 14 homolog A (S. cerevisiae)	**0.75**	2.12E-04	8556	1p21
221595_at	**---**	---	**0.75**	3.64E-05	---	---
204761_at	**USP6NL**	USP6 N-terminal like	**0.74**	2.10E-06	9712	10p13
210150_s_at	**LAMA5**	laminin, alpha 5	**0.74**	5.12E-04	3911	20q13.2-q13.3
221896_s_at	**HIGD1A**	HIG1 hypoxia inducible domain family, member 1A	**0.74**	4.71E-05	25994	3p22.1
227669_at	**BRP44**	brain protein 44	**0.73**	6.33E-05	25874	1q24
37408_at	**MRC2**	mannose receptor, C type 2	**0.72**	6.78E-05	9902	17q23.2
1557030_at	**GAB1**	GRB2-associated binding protein 1	**0.71**	7.31E-04	2549	4q31.21
238164_at	**USP6NL**	USP6 N-terminal like	**0.71**	4.57E-04	9712	10p13
1569025_s_at	**FAM13A**	family with sequence similarity 13, member A	**0.69**	4.52E-04	10144	4q22.1
203593_at	**CD2AP**	CD2-associated protein	**0.69**	1.74E-04	23607	6p12
226278_at	**SVIP**	small VCP/p97-interacting protein	**0.68**	1.13E-05	258010	11p14.2
228442_at	**---**	Transcribed locus Hs.599855	**0.68**	6.04E-04	---	Xp
228654_at	**SPIN4**	spindlin family, member 4	**0.68**	1.49E-04	139886	Xq11.1
230285_at	**SVIP**	small VCP/p97-interacting protein	**0.67**	4.86E-04	258010	11p14.2

230006_s_at	***SVIP***	small VCP/p97-interacting protein	**0.66**	7.40E-06	258010	11p14.2
244110_at	***MLL***	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)	**0.65**	2.51E-04	4297	11q23
1554918_a_at	***ABCC4***	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	**0.65**	3.93E-04	10257	13q32
203196_at	***ABCC4***	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	**0.64**	5.36E-04	10257	13q32
1559266_s_at	***C10orf140***	chromosome 10 open reading frame 140	**0.62**	9.35E-04	387640	10p12.31
203680_at	***PRKAR2B***	protein kinase, cAMP-dependent, regulatory, type II, beta	**0.51**	2.71E-04	5577	7q22
213515_x_at	***HBG1*** ***HBG2***	hemoglobin, gamma A hemoglobin, gamma G	**0.36**	2.00E-04	3047 3048	11p15.5
204848_x_at	***HBG1*** ***HBG2***	hemoglobin, gamma A hemoglobin, gamma G	**0.33**	1.29E-04	3047 3048	11p15.5
206310_at	***SPINK2***	serine peptidase inhibitor, Kazal type 2 (acrosin-trypsin inhibitor)	**0.33**	1.41E-05	6691	4q12
204419_x_at	***HBG1*** ***HBG2***	hemoglobin, gamma A hemoglobin, gamma G	**0.31**	9.59E-05	3047 3048	11p15.5
235700_at	***CT45A1*** ***CT45A2*** ***CT45A3*** ***CT45A4*** ***CT45A5*** ***CT45A6***	cancer/testis antigen family 45, member A1 cancer/testis antigen family 45, member A2 cancer/testis antigen family 45, member A3 cancer/testis antigen family 45, member A4 cancer/testis antigen family 45, member A5 cancer/testis antigen family 45, member A6	**0.28**	3.93E-04	100133581 441519 441520 441521 541465 541466	Xq26.3
1567912_s_at	***CT45A1*** ***CT45A2*** ***CT45A3*** ***CT45A4*** ***CT45A5*** ***CT45A6***	cancer/testis antigen family 45, member A1 cancer/testis antigen family 45, member A2 cancer/testis antigen family 45, member A3 cancer/testis antigen family 45, member A4 cancer/testis antigen family 45, member A5 cancer/testis antigen family 45, member A6	**0.25**	2.66E-04	100133581 441519 441520 441521 541465 541466	Xq26.3

ABBREVIATION: ELN, European LeukemiaNet.

NOTE: Fold change indicates the ratio of average expression values of *TET2*-mutated to *TET2*-wild-type patients.

Table A4. List of microRNA Probes Differentially Expressed Between *TET2*-Mutated and *TET2*-Wild-Type Cytogenetically Normal Acute Myeloid Leukemia Classified in the ELN Favorable-Risk Category

Probes upregulated in *TET2*-mutated patients			
Target microRNA	**Probe Sequence**	**Fold Change**	***P***
hsa-miR-148a	TGAGTATGATAGAAGTCAGTGCACTACAGAACTTTGTCTC	2.04	0.00074
hsa-miR-148b	TCTGAAAGTCAGTGCATCACAGAACTTTGTCTCGAAAGCT	1.61	0.00229
hsa-miR-24	TTTTACACACTGGCTCAGTTCAGCAGGAACAGGAGTCGAG	1.45	0.00428
hsa-mir-640 (prec)	GTGACCCTGGGCAAGTTCCTGAAGATCAGACACATCAGAT	1.35	0.00236
hsa-miR-24	AGTTGGTTTGTGTACACTGGCTCAGTTCAGCAGGAACAGG	1.35	0.00328
hsa-miR-107	GGCATGGAGTTCAAGCAGCATTGTACAGGGCTATCAAAGC	1.33	0.00462
Probes down-egulated in *TET2*-mutated patients			
Target microRNA	**Probe Sequence**	**Fold Change**	***P***
hsa-miR-135a	AATTCACTCTAGTGCTTTATGGCTTTTTATTCCTATGTGA	0.65	0.00028
hsa-miR-186	CTTGTAACTTTCCAAAGAATTCTCCTTTTGGGCTTTCTGG	0.41	0.00161

ABBREVIATION: ELN, European LeukemiaNet.

NOTE: Fold change indicates the ratio of average expression values of *TET2*-mutated to *TET2*-wild-type patients. MicroRNA expression profiles were available only for older patients (≥60 years).

Table A5. List of microRNA Probes Differentially Expressed Between *TET2*-Mutated and *TET2*-Wild-Type Cytogenetically Normal Acute Myeloid Leukemia Classified in the ELN Intermediate-I-Risk Category

Probes upregulated in *TET2*-mutated patients			
Target microRNA	**Probe Sequence**	**Fold Change**	***P***
hsa-miR-331-5p	GAGTTTGGTTTTGTTTGGGTTTGTTCTAGGTATGGTCCCA	1.47	0.00271
hsa-mir-660 (prec)	TGTGTGCATGGATTACAGGAGGGTGAGCCTTGTCATCGTG	1.43	0.00046
hsa-mir-539 (prec)	ATTTATGATGAATCATACAAGGACAATTTCTTTTTGAGTA	1.39	0.00132
hsa-miR-498	AGCTCAGGCTGTGATTTCAAGCCAGGGGGCGTTTTTCTAT	1.35	0.00407
hsa-mir-204 (prec)	AGGCTGGGAAGGCAAAGGGACGTTCAATTGTCATCACTGG	1.32	0.00133
hsa-miR-373*	GGGATACTCAAAATGGGGGCGCTTTCCTTTTTGTCTGTAC	1.30	0.00249
Probes downregulated in *TET2*-mutated patients			
Target microRNA	**Probe Sequence**	**Fold Change**	***P***
hsa-miR-374a	ACATCGGCCATTATAATACAACCTGATAAGTGTTATAGCA	0.69	0.00426
hsa-miR-152	ACTCGGGCTCTGGAGCAGTCAGTGCATGACAGAACTTGGG	0.68	0.00280
hsa-miR-126*	GCTGGCGACGGGACATTATTACTTTTGGTACGCGCTGTGA	0.66	0.00120
hsa-miR-19b	TTCTGCTGTGCAAATCCATGCAAAACTGACTGTGGTAGTG	0.65	0.00177
hsa-miR-590-5p	TAGCCAGTCAGAAATGAGCTTATTCATAAAAGTGCAGTAT	0.63	0.00308
hsa-miR-454	GTTCTGAGTAGTGCAATATTGCTTATAGGGTTTTGGTGTT	0.61	0.00042
hsa-miR-126	TGTGACACTTCAAACTCGTACCGTGAGTAATAATGCGCCG	0.53	0.00218
hsa-miR-126	ACACTTCAAACTCGTACCGTGAGTAATAATGCGCCGTCCA	0.48	0.00031
hsa-miR-126	ACACTTCAAACTCGTACCGTGAGTAATAATGCGCCGTCCA	0.47	0.00035

ABBREVIATION: ELN, European LeukemiaNet.

NOTE: Fold change indicates the ratio of average expression values of *TET2*-mutated to *TET2*-wild-type patients. MicroRNA expression profiles were available only for older patients (≥60 years).

Table A6: Treatment Outcomes According to *TET2* Mutation Status in Subgroups within the ELN Favorable-Risk Category

	Molecular subgroup	Endpoint	*TET2* Mutated	*TET2* Wild-Type	*P*
ELN favorable-risk group n=199	**Mutated *NPM1* without *FLT3*-ITD n=142**		**n=34**	**n=108**	
		Event-free survival			.003
		Median, years	0.8	1.8	
		% event-free at 3 years (95% CI)	21 (19-35)	42 (32-51)	
		Complete remission, no. (%)	25 (74)	99 (90)	.02
		Disease-free survival			.046
		Median, years	1.2	2.1	
		% disease-free at 3 years (95% CI)	24 (10-42)	46 (36-55)	
		Overall survival			.07
		Median, years	1.7	3.8	
		% alive at 3 years (95% CI)	35 (19-51)	54 (44-63)	
	Mutated *CEBPA* n=62		**n=20**	**n=42**	
		Event-free survival			.009
		Median, years	0.5	2.2	
		% event-free at 3 years (95% CI)	20 (6-39)	45 (30-59)	
		Complete remission, no. (%)	15 (75)	37 (88)	.27
		Disease-free survival			.03
		Median, years	0.8	4.1	
		% disease-free at 3 years (95% CI)	27 (8-50)	51 (34-66)	
		Overall survival			.003
		Median, years	1.2	3.6	
		% alive at 3 years (95% CI)	25 (9-45)	55 (39-68)	

ABBREVIATIONS: CI, confidence interval; ELN, European LeukemiaNet.

Table A7: Treatment Outcomes According to *TET2* Mutation Status in Subgroups within the ELN Intermediate-I-Risk Category

	Molecular Subgroup	Endpoint	*TET2* Mutated	*TET2* Wild-Type	*P*
ELN intermediate-I-risk group n=219	**Mutated *NPM1* and *FLT3*-ITD; wild-type *CEBPA* n=103**		**n=24**	**n=79**	
		Event-free survival			.66
		Median, years	0.5	0.6	
		% event-free at 3 years (95% CI)	25 (10-43)	13 (6-21)	
		Complete remission, no. (%)	19 (79)	60 (76)	1.0
		Disease-free survival			.67
		Median, years	0.5	0.6	
		% disease-free at 3 years (95% CI)	32 (13-52)	17 (9-27)	
		Overall survival			.90
		Median, years	0.8	0.8	
		% alive at 3 years (95% CI)	29 (13-48)	24 (15-34)	
	Wild-type *NPM1* and *FLT3*-ITD; wild-type *CEBPA* n=24		**n=6**	**n=18**	
		Event- free survival*			---
		Median, years	0.4	0.2	
		% event-free at 3 years (95% CI)	17 (1-52)	0	
		Complete remission, n (%)	3 (50)	7 (39)	.67
		Disease-free survival†	---	---	---
		Overall survival*			---
		Median, years	0.7	0.8	
		% alive at 3 years (95% CI)	17 (1-52)	0	
	Wild-type *NPM1* without *FLT3*-ITD; wild-type *CEBPA* n=92		**n=12**	**n=80**	
		Event- free survival			.40
		Median, years	0.3	0.6	
		% event-free at 3 years (95% CI)	0	8 (3-15)	
		Complete remission, no. (%)	6 (50)	48 (60)	.54
		Disease-free survival*			---
		Median, years	0.8	1.0	
		% disease-free at 3 years (95% CI)	0	13 (5-23)	
		Overall survival			.79
		Median, years	1.4	1.2	
		% alive at 3 years (95% CI)	8 (1-31)	19 (11-28)	

ABBREVIATIONS: CI, confidence interval; ELN, European LeukemiaNet.

*Insufficient number of events for statistical comparison.

†To few patients to provide median or 3-year disease-free survival.

Figure Legends

Figure A1. Frequency of *TET2* mutations across decades of age in 418 patients with primary cytogenetically normal acute myeloid leukemia. The heights of the bars represent the prevalence of *TET2* mutations in patients aged 18–29 years, 30–39 years, 40–49 years, 50–59 years, 60–69 years, and 70 years or older. The *P*-value for trend, analyzing *TET2* mutation frequency across age groups, was calculated by the Armitage trend test.

Figure A2. Heat map of the gene-expression signature associated with *TET2* mutations in patients with ELN favorable-risk, primary cytogenetically normal acute myeloid leukemia. Rows represent probe-sets, and columns represent individual patients. Patients are grouped by *TET2* mutation status and genes are ordered by hierarchical cluster analysis. Expression values of the probe-sets are represented by color, with green indicating expression less than, and red indicating expression greater than, the median value for the given probe-set. One-hundred-fifty probe-sets were upregulated and 63 probe-sets were downregulated in patients with *TET2* mutations, as compared with *TET2* wild-type patients. Arrows identify genes that are discussed in the text, with vertical bars indicating multiple probe-sets representing the same gene.

Figure A3. Heat maps of the microRNA-expression signatures associated with *TET2* mutations in (A) ELN favorable-risk and (B) ELN intermediate-I-risk patients ≥60 years with primary cytogenetically normal acute myeloid leukemia. Rows represent microRNA probes, and columns represent individual patients. Patients are grouped by *TET2*

mutation status and microRNAs are ordered by hierarchical cluster analysis. *miR-24* is listed twice, and *miR-126* is listed three times, because they are represented by two or three distinct probes in the signature, respectively. Expression values of the probes are represented by color, with green indicating expression less than, and red indicating expression greater than, the median value for the given probe. Among favorable-risk patients, 6 microRNA probes were significantly upregulated and 2 were downregulated in *TET2*-mutated versus *TET2*-wild-type patients. In the intermediate-I-risk group, 6 probes were upregulated in *TET2*-mutated versus *TET2*-wild-type patients, and 9 were downregulated. There was no overlap between the microRNA probes contained in these two signatures. Arrows highlight microRNAs that are discussed in the text.

Figure A4. Outcomes according to the European LeukemiaNet (ELN) risk classification in our cohort of cytogenetically normal acute myeloid leukemia (CN-AML) patients. (A) Disease-free survival and (B) overall survival of CN-AML patients in the ELN favorable- and intermediate-I-risk categories. The favorable-risk group is defined as patients with mutated *CEBPA* and/or mutated *NPM1* without *FLT3*-ITD. All remaining CN-AML patients belong to the intermediate-I-risk category.

Figure A5. Association of *TET2* mutations with survival in molecular subsets of ELN favorable-risk cytogenetically normal acute myeloid leukemia. (A) Disease-free survival and (B) overall survival according to *TET2* mutation status, in patients with mutated *NPM1* without *FLT3*-ITD. (C) Disease-free survival and (D) overall survival according to

TET2 mutation status in patients with mutated *CEBPA*. Abbreviations: mut, mutated; wt, wild-type.

Figure A6. Association of *TET2* mutations with survival in molecular subsets of ELN intermediate-I-risk cytogenetically normal acute myeloid leukemia. (A) Overall survival according to *TET2* mutation status, in patients with mutated *NPM1* and *FLT3*-ITD and wild-type *CEBPA.* (B) Overall survival according to *TET2* mutation status in patients with wild-type *NPM1* without *FLT3*-ITD, and wild-type *CEBPA.* (C) Overall survival according to *TET2* mutation status in patients with wild-type *NPM1* and *FLT3*-ITD, and wild-type *CEBPA*. Abbreviations: mut, mutated; wt, wild-type.

Figure A1

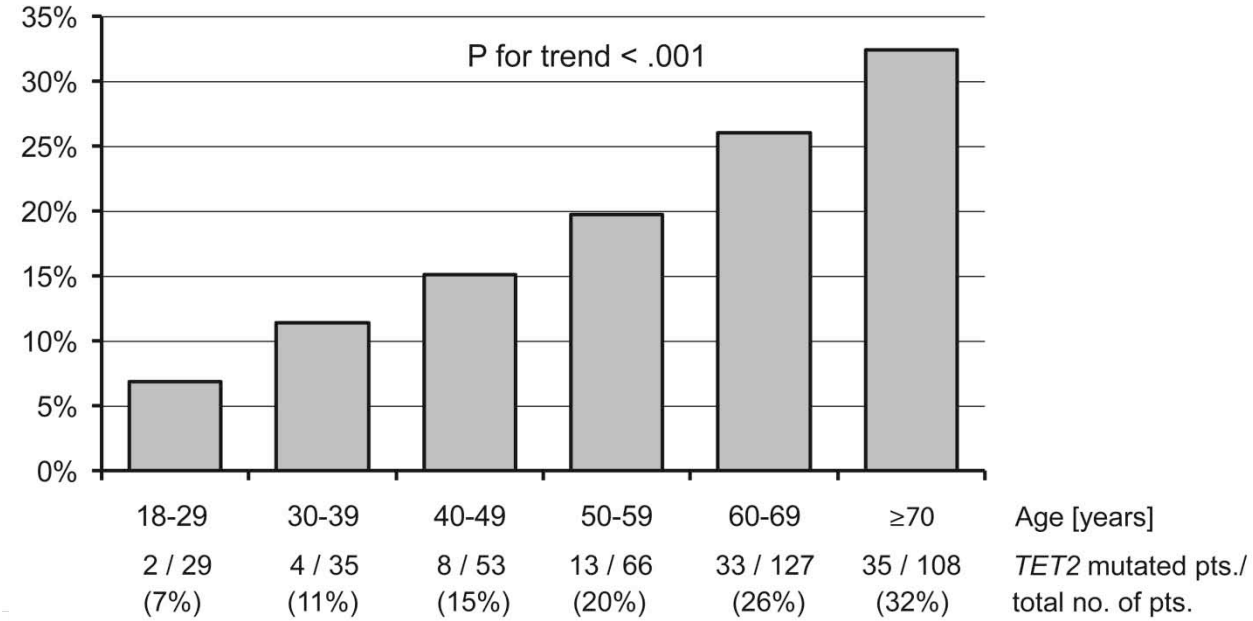

35%
30%
25%
20%
15%
10%
5%
0%
P for trend < .001
18-29
30-39
40-49
50-59
60-69
≥70
Age [years]
2 / 29 (7%)
4 / 35 (11%)
8 / 53 (15%)
13 / 66 (20%)
33 / 127 (26%)
35 / 108 (32%)
TET2 mutated pts./ total no. of pts.

Figure A2

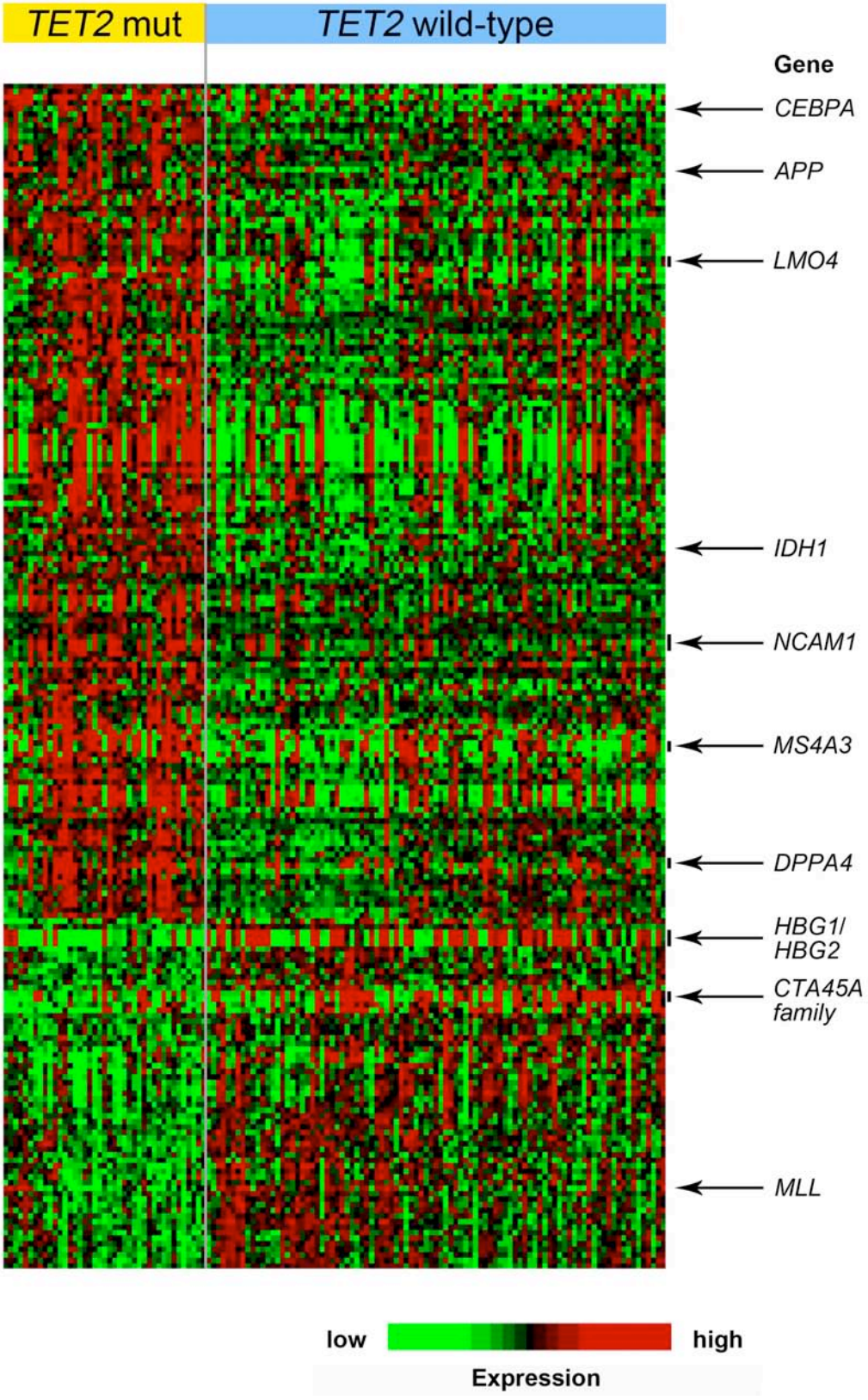

TET2 mut
TET2 wild-type
Gene
CEBPA
APP
LMO4
IDH1
NCAM1
MS4A3
DPPA4
HBG1/
HBG2
CTA45A
family
MLL
low
high
Expression

Figure A3

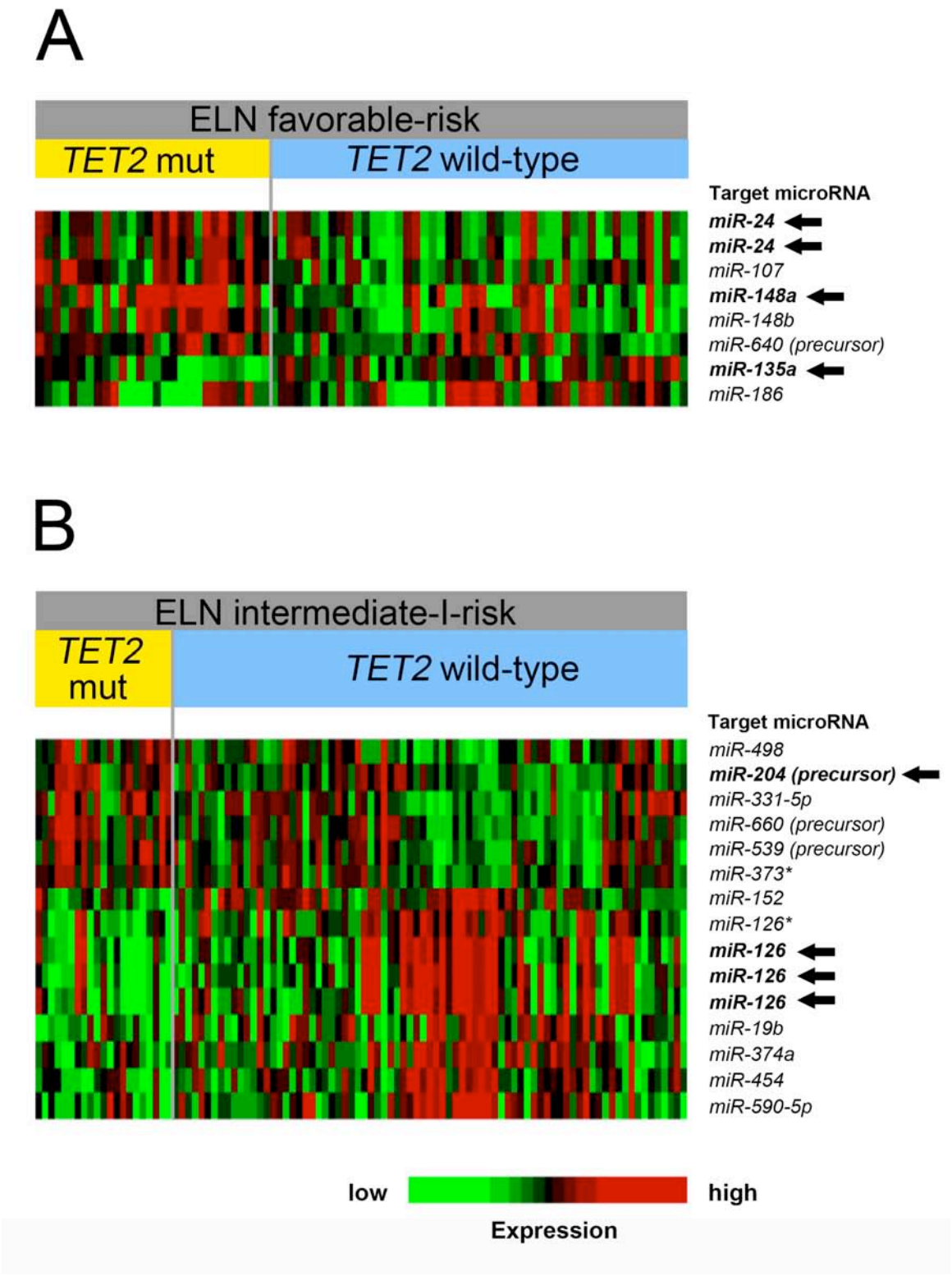

A
ELN favorable-risk
TET2 mut
TET2 wild-type
Target microRNA
miR-24
miR-24
miR-107
miR-148a
miR-148b
miR-640 (precursor)
miR-135a
miR-186
B
ELN intermediate-I-risk
TET2 mut
TET2 wild-type
Target microRNA
miR-498
miR-204 (precursor)
miR-331-5p
miR-660 (precursor)
miR-539 (precursor)
miR-373*
miR-152
miR-126*
miR-126
miR-126
miR-126
miR-19b
miR-374a
miR-454
miR-590-5p
low
high
Expression

Figure A4

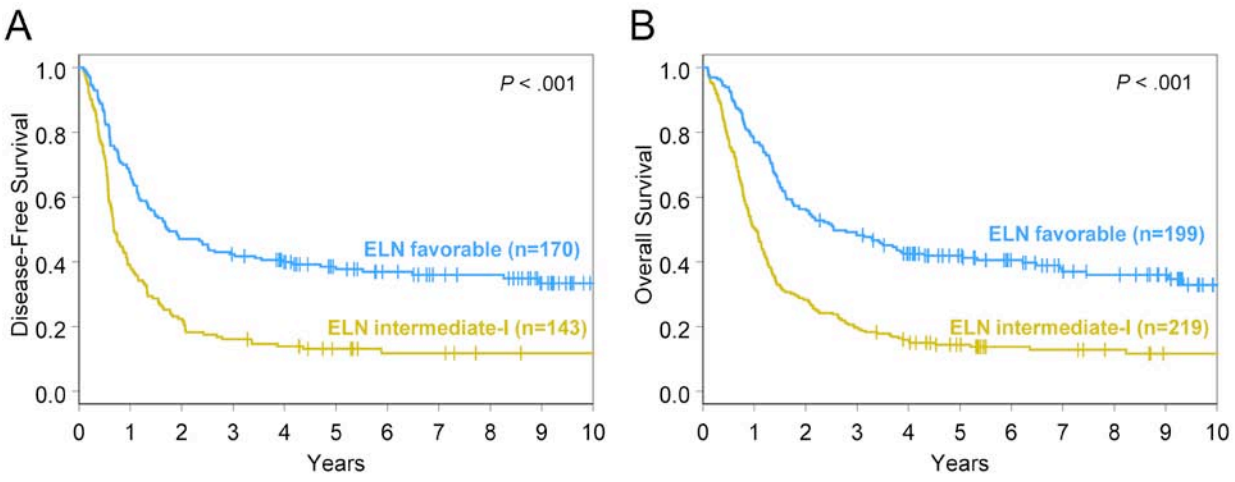

A
Disease-Free Survival
1.0
0.8
0.6
0.4
0.2
0.0
P < .001
ELN favorable (n=170)
ELN intermediate-I (n=143)
0 1 2 3 4 5 6 7 8 9 10
Years
B
Overall Survival
1.0
0.8
0.6
0.4
0.2
0.0
P < .001
ELN favorable (n=199)
ELN intermediate-I (n=219)
0 1 2 3 4 5 6 7 8 9 10
Years

Figure A5

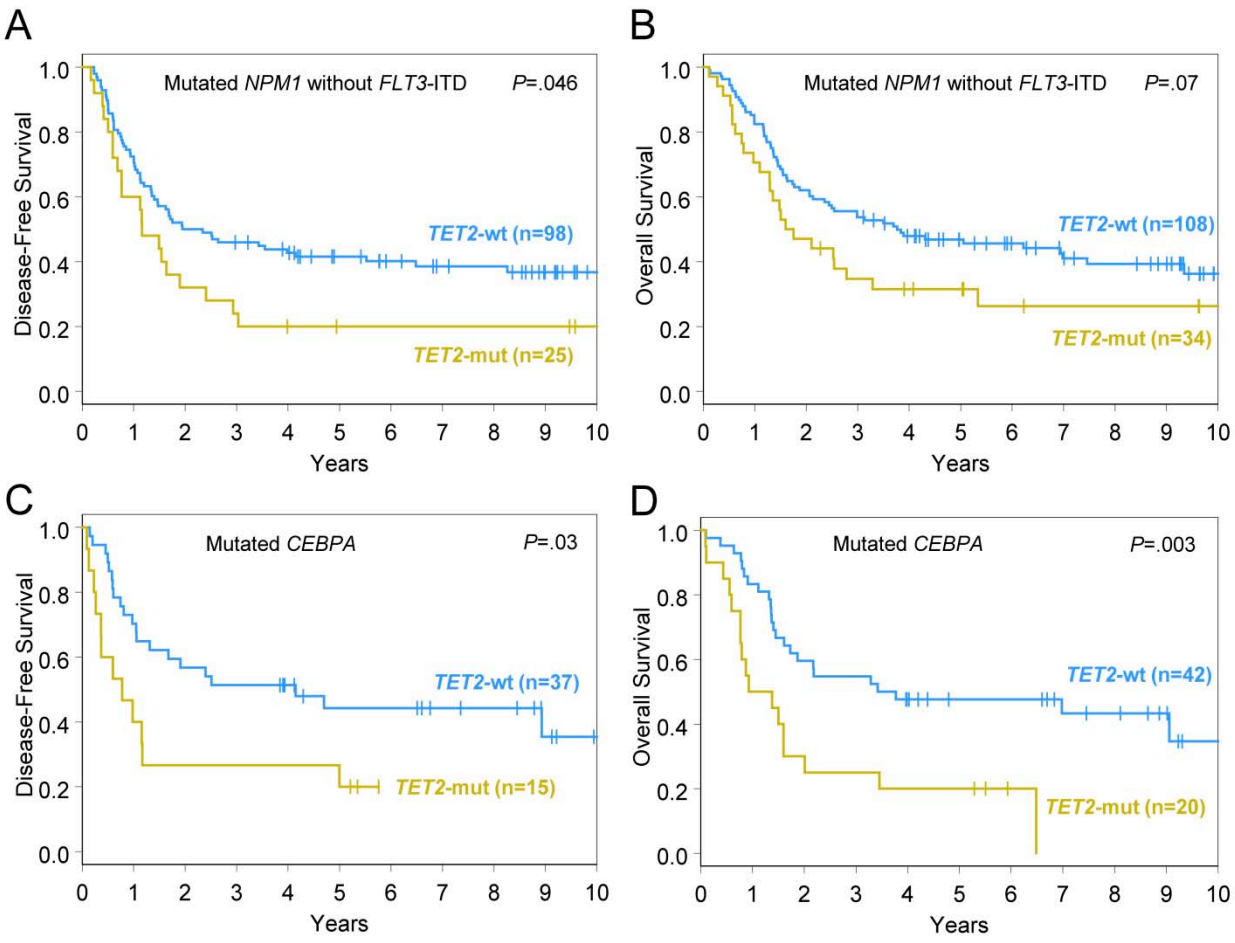
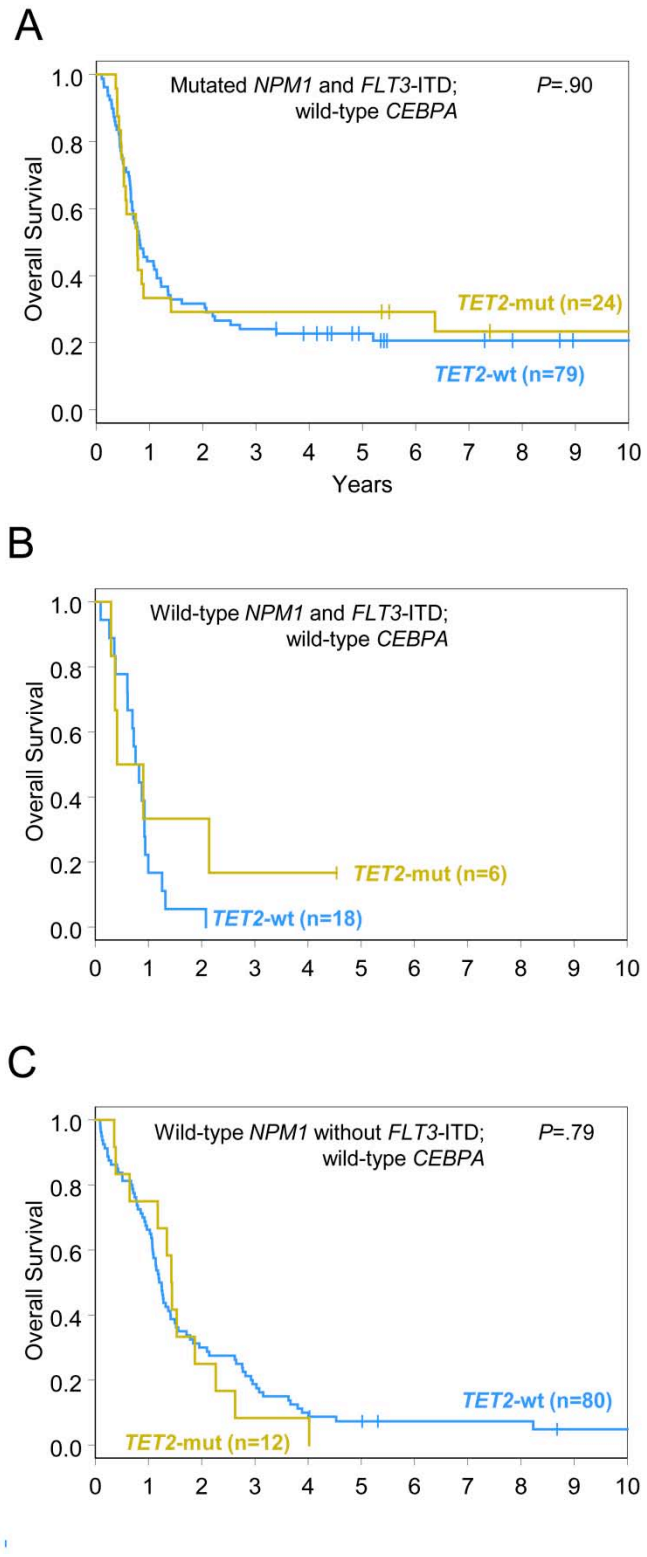

A
Mutated NPM1 without FLT3-ITD
P=.046
Disease-Free Survival
TET2-wt (n=98)
TET2-mut (n=25)
Years
B
Mutated NPM1 without FLT3-ITD
P=.07
Overall Survival
TET2-wt (n=108)
TET2-mut (n=34)
Years
C
Mutated CEBPA
P=.03
Disease-Free Survival
TET2-wt (n=37)
TET2-mut (n=15)
Years
D
Mutated CEBPA
P=.003
Overall Survival
TET2-wt (n=42)
TET2-mut (n=20)
Years

Figure A6


A
Mutated NPM1 and FLT3-ITD; wild-type CEBPA
P=.90
TET2-mut (n=24)
TET2-wt (n=79)
Overall Survival
Years
B
Wild-type NPM1 and FLT3-ITD; wild-type CEBPA
TET2-mut (n=6)
TET2-wt (n=18)
C
Wild-type NPM1 without FLT3-ITD; wild-type CEBPA
P=.79
TET2-wt (n=80)
TET2-mut (n=12)