

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812

JUNE 9, 2016

VOL. 374 NO. 23

Genomic Classification and Prognosis in Acute Myeloid Leukemia

Elli Papaemmanuil, Ph.D., Moritz Gerstung, Ph.D., Lars Bullinger, M.D., Verena I. Gaidzik, M.D., Peter Paschka, M.D., Nicola D. Roberts, B.Sc., Nicola E. Potter, Ph.D., Michael Heuser, M.D., Felicitas Thol, M.D., Niccolo Bolli, M.D., Ph.D., Gunes Gundem, Ph.D., Peter Van Loo, Ph.D., Inigo Martincorena, Ph.D., Peter Ganly, B.M., B.Ch., Ph.D., Laura Mudie, B.Sc., Stuart McLaren, B.Sc., Sarah O'Meara, B.Sc., Keiran Raine, M.Sc., David R. Jones, M.Sc., Jon W. Teague, B.Sc., Adam P. Butler, B.Sc., Mel F. Greaves, Ph.D., Arnold Ganser, M.D., Konstanze Döhner, M.D., Richard F. Schlenk, M.D., Hartmut Döhner, M.D., and Peter J. Campbell, M.B., Ch.B., Ph.D.

ABSTRACT

BACKGROUND

Recent studies have provided a detailed census of genes that are mutated in acute myeloid leukemia (AML). Our next challenge is to understand how this genetic diversity defines the pathophysiology of AML and informs clinical practice.

METHODS

We enrolled a total of 1540 patients in three prospective trials of intensive therapy. Combining driver mutations in 111 cancer genes with cytogenetic and clinical data, we defined AML genomic subgroups and their relevance to clinical outcomes.

RESULTS

We identified 5234 driver mutations across 76 genes or genomic regions, with 2 or more drivers identified in 86% of the patients. Patterns of co-mutation compartmentalized the cohort into 11 classes, each with distinct diagnostic features and clinical outcomes. In addition to currently defined AML subgroups, three heterogeneous genomic categories emerged: AML with mutations in genes encoding chromatin, RNA-splicing regulators, or both (in 18% of patients); AML with *TP53* mutations, chromosomal aneuploidies, or both (in 13%); and, provisionally, AML with *IDH2*^{R172} mutations (in 1%). Patients with chromatin-spliceosome and *TP53*-aneuploidy AML had poor outcomes, with the various class-defining mutations contributing independently and additively to the outcome. In addition to class-defining lesions, other co-occurring driver mutations also had a substantial effect on overall survival. The prognostic effects of individual mutations were often significantly altered by the presence or absence of other driver mutations. Such gene-gene interactions were especially pronounced for *NPM1*-mutated AML, in which patterns of co-mutation identified groups with a favorable or adverse prognosis. These predictions require validation in prospective clinical trials.

CONCLUSIONS

The driver landscape in AML reveals distinct molecular subgroups that reflect discrete paths in the evolution of AML, informing disease classification and prognostic stratification. (Funded by the Wellcome Trust and others; ClinicalTrials.gov number, NCT00146120.)

From the Cancer Genome Project, Wellcome Trust Sanger Institute (E.P., M.G., N.D.R., N.B., G.G., P.V.L., I.M., L.M., S.O., K.R., D.R.J., J.W.T., A.P.B., P.J.C.), and the European Bioinformatics Institute, European Molecular Biology Laboratory (EMBL-EBI) (M.G.), Hinxton, the Centre for Evolution and Cancer, Institute of Cancer Research, London (N.E.P., M.F.G.), and the Department of Haematology, University of Cambridge, Cambridge (N.B.) — all in the United Kingdom; the Departments of Epidemiology and Biostatistics and Cancer Biology, the Center for Molecular Oncology and the Center for Hematologic Malignancies, Memorial Sloan Kettering Cancer Center, New York (E.P.); the Department of Internal Medicine III, Ulm University, Ulm (L.B., V.I.G., P.P., K.D., R.F.S., H.D.), and the Department of Hematology, Hemostasis, Oncology, and Stem Cell Transplantation, Hannover Medical School, Hannover (M.H., F.T., A.G.) — both in Germany; the Division of Hematology, Fondazione IRCCS, Istituto Nazionale dei Tumori, and Department of Oncology and Onco-Hematology, University of Milan, Milan (N.B.); the Department of Human Genetics, University of Leuven, Leuven, Belgium (P.V.L.); and the Department of Pathology, University of Otago, Christchurch, New Zealand (P.G., P.J.C.). Address reprint requests to Dr. Campbell at the Cancer Genome Project, Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire CB10 1SA, United Kingdom, or at pc8@sanger.ac.uk.

Drs. Papaemmanuil and Gerstung and Drs. H. Döhner and Campbell contributed equally to this article.

This article was last updated on July 12, 2016, at NEJM.org.

N Engl J Med 2016;374:2209-21.

DOI: 10.1056/NEJMoa1516192

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N ENGL J MED 374;23 NEJM.ORG JUNE 9, 2016

The New England Journal of Medicine

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ACUTE MYELOID LEUKEMIA (AML) IS characterized by clonal expansion of undifferentiated myeloid precursors, resulting in impaired hematopoiesis and bone marrow failure. Although many patients with AML have a response to induction chemotherapy, refractory disease is common, and relapse represents the major cause of treatment failure.¹

Cancer develops from somatically acquired driver mutations, which account for the myriad biologic and clinical complexities of the disease. A classification of cancers that is based on causality is likely to be durable, reproducible, and clinically relevant. This is already evident in the case of AML, for which there has been a progressive shift from a morphologic classification scheme to one informed by causative genomic changes.²⁻⁴ Systematic studies of the genomic landscape of AML, such as analyses of data from the Cancer Genome Atlas⁵ (TCGA), have generated a catalogue of leukemia genes that is increasingly comprehensive. It is therefore an opportune time to revisit the possibility of an AML classification scheme that is fully genomic.

With whole-genome sequencing, AML emerges as a complex, dynamic disease.⁵⁻⁸ There are many leukemia genes, most of which are infrequently mutated, and patients typically have more than one driver mutation.⁵ The disease evolves over time, with multiple competing clones coexisting at any time.⁵⁻⁸ These discoveries are revealing the biologic intricacies of AML, but how they inform clinical practice is unclear.

Here we report a comprehensive study of leukemia genes in three clinical trials of intensive AML treatment, acknowledging that the landscape of AML in older patients may be underrepresented. The structure of driver mutations identifies nonoverlapping subgroups of patients, allowing a fully genomic classification of AML. Beyond class, we explore patterns of gene–gene co-occurrences and investigate how such compound genotypes are related to clinical outcomes.

METHODS

STUDY PARTICIPANTS, TREATMENT, AND OVERSIGHT

We obtained samples from patients participating in three prospective multicenter clinical trials of the German–Austrian AML Study Group: AML-HD98A, AML-HD98B, and AMLSG-07-04 (see the Supplementary Appendix, available with the full text of this article at NEJM.org).⁹⁻¹¹ In AML-

HD98A, patients 18 to 65 years of age received induction chemotherapy consisting of idarubicin, cytarabine, and etoposide (ICE); high-risk patients were offered allogeneic stem-cell transplantation, intermediate-risk patients a stem-cell allograft (if a matched related donor was available) or intensive chemotherapy, and low-risk patients intensive chemotherapy. AMLSG-07-04 had a similar design, but patients 18 to 61 years of age were randomly assigned to receive ICE or ICE plus all-*trans* retinoic acid (ATRA) as induction therapy. In AML-HD98B, patients 58 to 84 years of age were randomly assigned to induction therapy with ICE or ICE plus ATRA, with further therapy dictated by the response (see Figs. S1 and S2 in the Supplementary Appendix). The median follow-up period was 5.9 years (Table S1 in the Supplementary Appendix). All patients provided written informed consent.

The last two authors guided the analysis and data interpretation.

GENETIC STUDIES

Genetic profiling included cytogenetic analyses and sequencing of 111 genes (Table S2 in the Supplementary Appendix). Sequencing data have been deposited in the European Genome-Phenome Archive (www.ebi.ac.uk/ega) under accession number EGAS00001000275. We based our analysis on variants that we classified as driver mutations, using widely accepted genetic criteria.¹² Recurrent fusion genes, aneuploidies, and leukemia gene mutations, including base substitutions and small (<200-bp) insertions or deletions (indels), were all included as drivers.

STATISTICAL ANALYSIS

We used Bayesian Dirichlet processes¹³ to establish classification rules that partitioned patients into subgroups, minimizing overlap between categories. The Dirichlet process defines an infinite prior distribution for the number and proportions of clusters in a mixture model, fitted with the use of the Markov chain Monte Carlo method.

We modeled overall survival using Cox proportional-hazards methods with the study variables treated as random effects¹⁴ (see the Methods section in the Supplementary Appendix). Random effects are modeled as independently distributed normal variables with identical mean and variance across eight variable categories: point mutations (58 variables), fusion genes (8), copy-

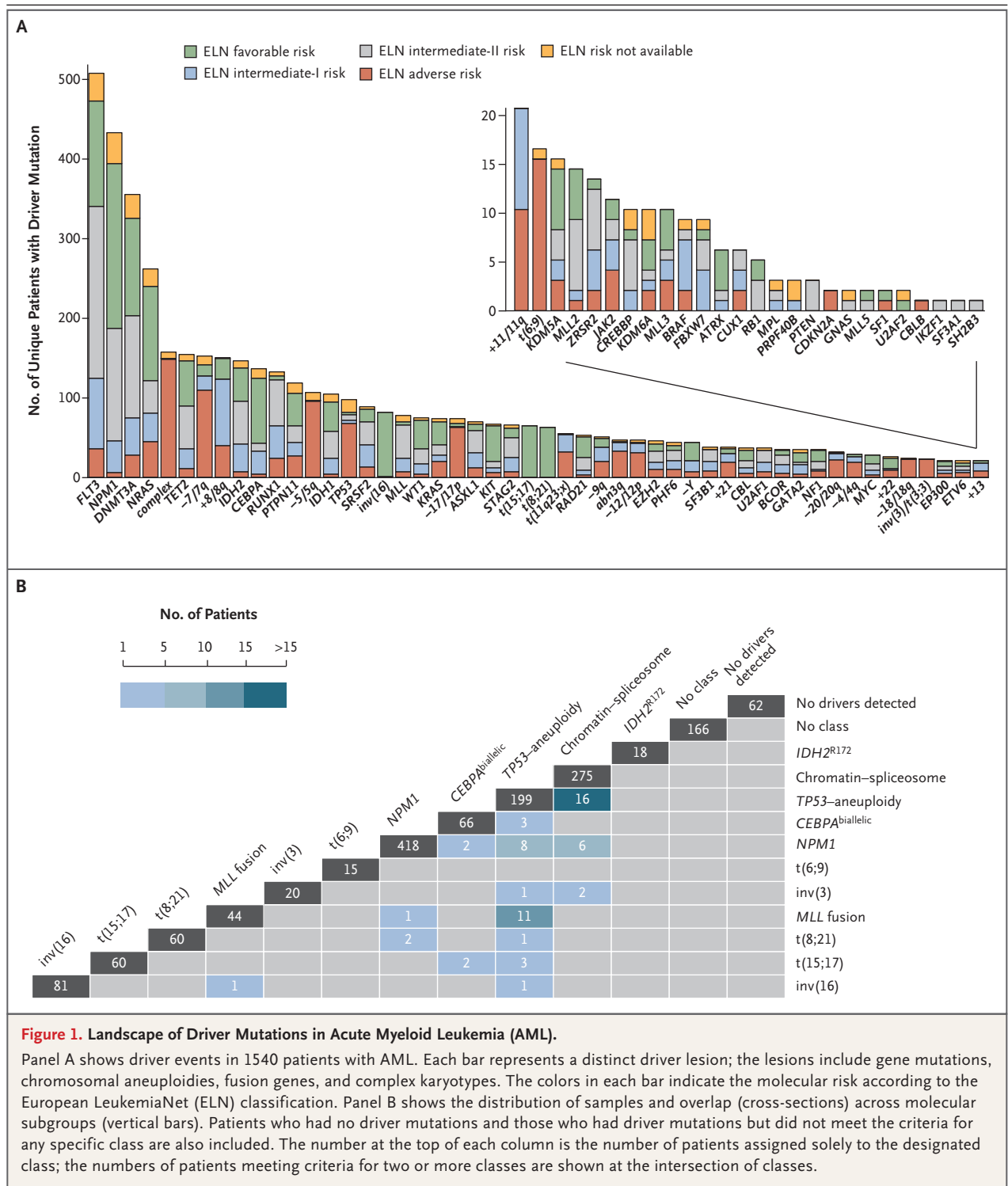


Figure 1. Landscape of Driver Mutations in Acute Myeloid Leukemia (AML).

Panel A shows driver events in 1540 patients with AML. Each bar represents a distinct driver lesion; the lesions include gene mutations, chromosomal aneuploidies, fusion genes, and complex karyotypes. The colors in each bar indicate the molecular risk according to the European LeukemiaNet (ELN) classification. Panel B shows the distribution of samples and overlap (cross-sections) across molecular subgroups (vertical bars). Patients who had no driver mutations and those who had driver mutations but did not meet the criteria for any specific class are also included. The number at the top of each column is the number of patients assigned solely to the designated class; the numbers of patients meeting criteria for two or more classes are shown at the intersection of classes.

number alterations (18), clinical variables (11), demographic characteristics (2), treatment (3), nuisance (4; nuisance variables are other or missing variables, such as the trial a patient was enrolled in, the year a patient entered the clinical

trial, and whether cytogenetic data were missing), and gene–gene interaction terms, defined as nonadditive effects on survival between two genes when both are mutated (126). We used an expectation-maximization algorithm to estimate

the shared means and variances; maximum a posteriori estimates were used for the effects of individual variables.

RESULTS

DRIVER-MUTATION LANDSCAPE OF AML

We identified 5234 driver mutations involving 76 genes or regions in 1540 patients (Fig. 1A, and Tables S5 and S6 in the Supplementary Appendix), with mutation frequencies that were consistent with those in previous studies^{4,5} (Fig. S2c in the Supplementary Appendix). Point mutations accounted for 73% of all drivers (3824 of 5234) (Fig. S2d in the Supplementary Appendix) and were often enriched in patients with AML classified as intermediate risk according to current guidelines.³ We identified at least 1 driver mutation in 1478 of 1540 samples (96%), and 2 or

more driver mutations in 86% of samples (Fig. S2e in the Supplementary Appendix). The mutation landscape in this cohort, including novel hot-spot mutations in *MYC*, is described in detail in the Results S1 section in the Supplementary Appendix.

Although patterns of co-mutation and mutual exclusivity have been described previously in AML,^{4,5} our sample size afforded a more comprehensive analysis, which identified many new pairwise gene–gene correlations (see the Results S2 section, Tables S7 and S8, and Fig. S3a in the Supplementary Appendix). We also found surprising differences in patterns of co-mutation for hotspots within genes. *NPM1*, for example, preferentially associated with *NRAS*^{G12V} but not with *NRAS*^{Q61}. Distinct patterns of co-mutation were also observed for *IDH2*^{R140} and *IDH2*^{R172} and for *FLT3* internal tandem duplication (*FLT3*^{ITD})

Table 1. Proposed Genomic Classification of Acute Myeloid Leukemia (AML).

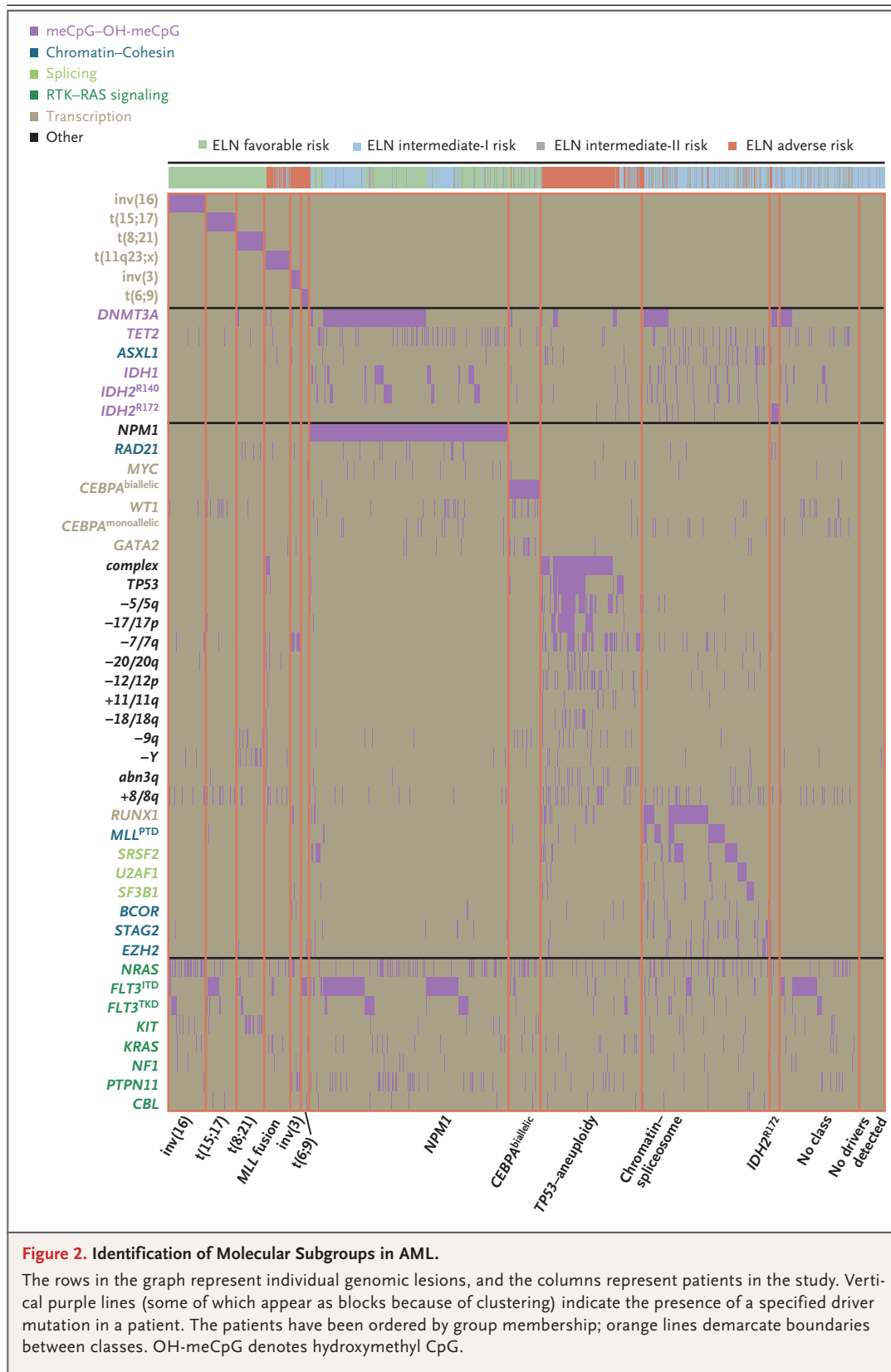
Genomic Subgroup	Frequency in the Study Cohort (N=1540)	Most Frequently Mutated Genes*
	no. of patients (%)	gene (%)
AML with <i>NPM1</i> mutation	418 (27)	<i>NPM1</i> (100), <i>DNMT3A</i> (54), <i>FLT3</i> ^{ITD} (39), <i>NRAS</i> (19), <i>TET2</i> (16), <i>PTPN11</i> (15)
AML with mutated chromatin, RNA-splicing genes, or both†	275 (18)	<i>RUNX1</i> (39), <i>MLL</i> ^{PTD} (25), <i>SRSF2</i> (22), <i>DNMT3A</i> (20), <i>ASXL1</i> (17), <i>STAG2</i> (16), <i>NRAS</i> (16), <i>TET2</i> (15), <i>FLT3</i> ^{ITD} (15)
AML with <i>TP53</i> mutations, chromosomal aneuploidy, or both‡	199 (13)	Complex karyotype (68), –5/5q (47), –7/7q (44), <i>TP53</i> (44), –17/17p (31), –12/12p (17), +8/8q (16)
AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); <i>CBFB–MYH11</i>	81 (5)	inv(16) (100), <i>NRAS</i> (53), +8/8q (16), +22 (16), <i>KIT</i> (15), <i>FLT3</i> ^{TKD} (15)
AML with biallelic <i>CEBPA</i> mutations	66 (4)	<i>CEBPA</i> ^{biallelic} (100), <i>NRAS</i> (30), <i>WT1</i> (21), <i>GATA2</i> (20)
AML with t(15;17)(q22;q12); <i>PML–RARA</i>	60 (4)	t(15;17) (100), <i>FLT3</i> ^{ITD} (35), <i>WT1</i> (17)
AML with t(8;21)(q22;q22); <i>RUNX1–RUNX1T1</i>	60 (4)	t(8;21) (100), <i>KIT</i> (38), –Y (33), –9q (18)
AML with <i>MLL</i> fusion genes; t(x;11)(x;q23)§	44 (3)	t(x;11q23) (100), <i>NRAS</i> (23)
AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); <i>GATA2</i> , <i>MECOM</i> (<i>EVI1</i>)	20 (1)	inv(3) (100), –7 (85), <i>KRAS</i> (30), <i>NRAS</i> (30), <i>PTPN11</i> (30), <i>ETV6</i> (15), <i>PHF6</i> (15), <i>SF3B1</i> (15)
AML with <i>IDH2</i> ^{R172} mutations and no other class-defining lesions	18 (1)	<i>IDH2</i> ^{R172} (100), <i>DNMT3A</i> (67), +8/8q (17)
AML with t(6;9)(p23;q34); <i>DEK–NUP214</i>	15 (1)	t(6;9) (100), <i>FLT3</i> ^{ITD} (80), <i>KRAS</i> (20)
AML with driver mutations but no detected class-defining lesions	166 (11)	<i>FLT3</i> ^{ITD} (39), <i>DNMT3A</i> (16)
AML with no detected driver mutations	62 (4)	
AML meeting criteria for ≥2 genomic subgroups	56 (4)	

* Genes with a frequency of 15% or higher are shown in descending order of frequency. Key contributing genes in each class are shown in boldface type.

† Classification in this subgroup requires one or more driver mutations in *RUNX1*, *ASXL1*, *BCOR*, *STAG2*, *EZH2*, *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, or *MLL*^{PTD}. In the presence of other class-defining lesions — namely, inv(16), t(15;17), t(8;21), t(6;9), *MLL* fusion genes, or complex karyotype or driver mutations in *TP53*, *NPM1*, or *CEBPA*^{biallelic} — two or more chromatin–spliceosome mutations are required.

‡ Classification in this subgroup requires *TP53* mutation, complex karyotype, or in the absence of other class-defining lesions, one or more of the following: –7/7q, –5/5q, –4/4q, –9q, –12/12p, –17/–17p, –18/18q, –20/20q, +11/11q, +13, +21, or +22.

§ Multiple fusion partners for *MLL* were found, with the clinical implications depending on the specific fusion partner.



and *FLT3* tyrosine kinase domain (*FLT3*^{TKD}) mutations (Fig. S3b). Thus, the functional consequences of hotspot mutations within genes may not be equivalent.

Leukemias often comprise heterogeneous mixtures of subclones,^{6-8,15} but samples to date have been too small to define common patterns of evolution. We used allele fractions of point mutations to infer clonal relationships^{5,7,16,17} (see the Results S3 section in the Supplementary Appendix). Mutations in *DNMT3A*, *ASXL1*, *IDH1/2*, and *TET2*, genes that encode epigenetic modifiers, were often acquired earliest (Fig. S4a in the Supplementary Appendix). These genes are frequently mutated in elderly persons with clonal hematopoiesis and confer an increased risk of hematologic cancers.¹⁸⁻²² In our study population with AML, mutations in these genes were typically in the founding clone and were almost never found in isolation, suggesting that they are not sufficient for overt leukemia (Fig. S4b in the Supplementary Appendix). Mutations in receptor tyrosine kinase–RAS pathway genes typically occurred late²³⁻²⁵ and frequently more than once in the same patient (Fig. S5 in the Supplementary Appendix). *NPM1* mutations were usually secondary events, often occurring after *DNMT3A*, *IDH1*, or *NRAS* mutations (Fig. S6 and Table S9 in the Supplementary Appendix). These data suggest that development of AML follows specific and ordered evolutionary trajectories.

IMPLICATIONS OF GENOMIC STRUCTURE FOR CLASSIFICATION OF AML

In the 2008 World Health Organization (WHO) classification, molecular groups in adult AML include t(15;17), t(8;21), inv(16)–t(16;16), t(6;9), inv(3)–t(3;3), *MLL* fusion genes, and provisionally, *CEBPA* or *NPM1* mutations.²⁶ In our cohort, AML in 736 patients (48%) would not be classified according to these genomic lesions, even though 96% of the patients had driver mutations. The characterization of many new leukemia genes, multiple driver mutations per patient, and complex co-mutation patterns prompted us to reevaluate genomic classification of AML from the beginning.

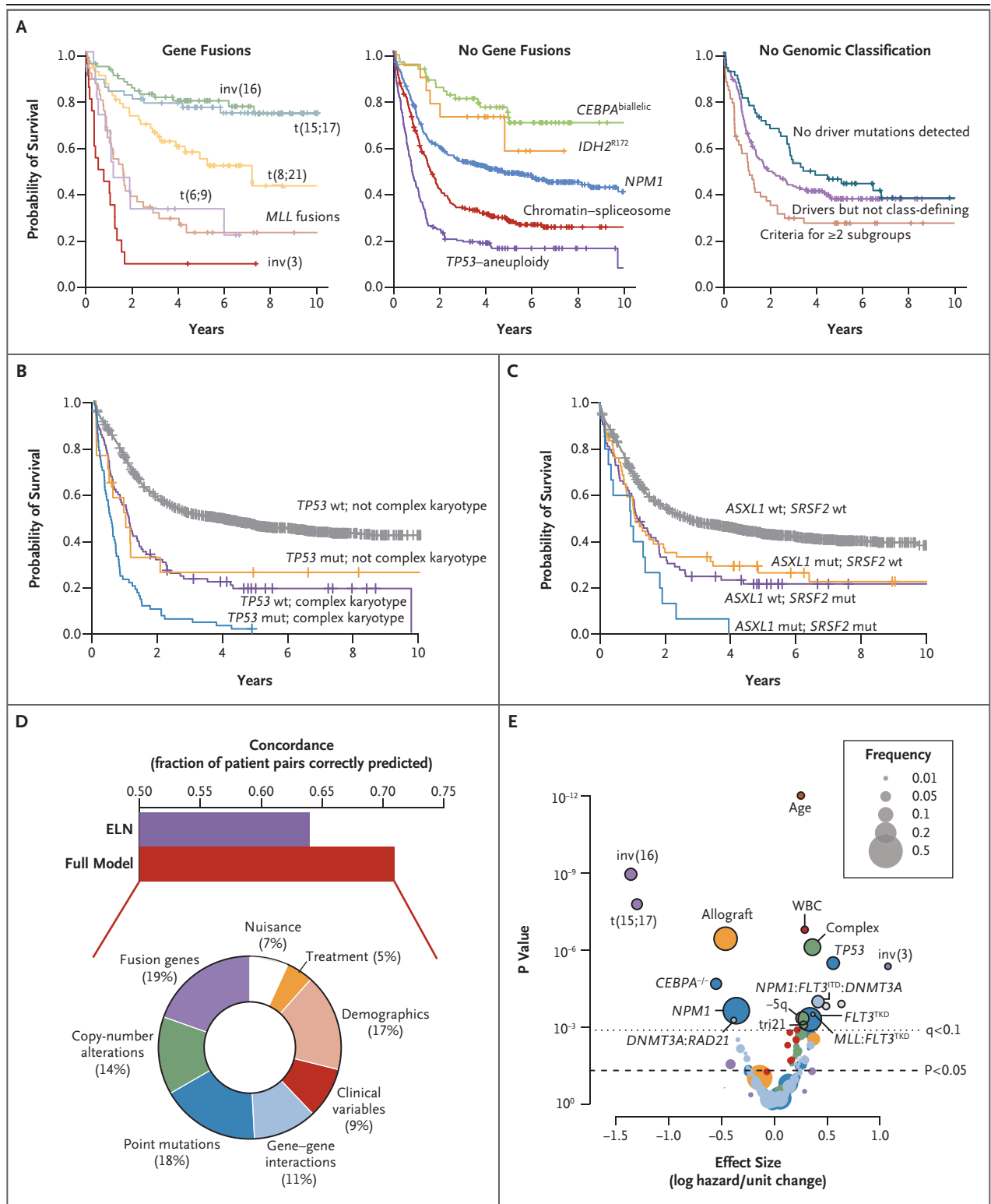
We developed a Bayesian statistical model to compartmentalize AML into mutually exclusive subtypes on the basis of patterns of co-mutation (see the Methods section and Fig. S7 in the Supplementary Appendix). From this model, we defined simple rules to generate 11 subgroups or

Figure 3 (facing pages). Molecular Subclassification and Overall Survival.

Panel A shows Kaplan–Meier curves for overall survival among patients in the 11 genomically defined subgroups and patients who did not have a straightforward classification. Panel B shows Kaplan–Meier curves for overall survival with the additive and independent prognostic effects of *TP53* mutation and complex karyotype (*TP53* mutation, 17 patients; complex karyotype, 89; and *TP53* mutation with complex karyotype, 70). Panel C shows Kaplan–Meier curves for overall survival with the additive and independent prognostic effects of *ASXL1* and *SRSF2* mutations (*ASXL1* mutation, 55 patients; *SRSF2* mutation, 74; and *ASXL1* and *SRSF2* mutations, 15). In Panels B and C, wt denotes wild type, and mut mutation. The bar chart in Panel D shows concordance estimates for overall survival with the use of variables selected in our model (concordance, approximately 71%) as compared with a model using variables considered in the ELN guidelines (concordance, approximately 64%). The doughnut chart shows the relative proportion of explained variance in overall survival in the full model that is accounted for by different categories of predictor variables. Clinical variables are performance status, splenomegaly, bone marrow blasts, and blood counts. Demographic variables are age and sex. Nuisance variables are other variables (e.g., which trial a patient was enrolled in, what year a patient entered the clinical trial, and whether cytogenetic data were missing). The volcano plot in Panel E shows the incremental contribution to the effect size (expressed as the logarithmic hazard on the x axis; positive values indicate a worsening effect) versus P values (expressed on an inverted logarithmic scale on the y axis) for each of the 228 variables included in the random-effects model. The circles above the dotted line represent 18 variables with a q value of less than 0.1; the size of each circle corresponds to the frequency of the variable, as indicated in the box. The incremental contribution of age is shown for every 10 years of age, and the incremental contribution of the white-cell count (WBC) is shown for each increase of 1×10^9 cells per liter. The colors of the circles correspond to the colors shown in the doughnut chart in Panel D.

classes of AML (Table 1, Fig. 1B, and Fig. 2; and Results sections S4 through S7 in the Supplementary Appendix). We found that inv(16), t(15;17), t(8;21), inv(3), t(6;9), and *MLL* fusions each represent small, individual subgroups ($\leq 5\%$ of the study cohort), confirming the WHO classification. *NPM1*-mutated AML and *CEBPA*^{biallelic} AML were also identified as distinct subgroups. *NPM1*-mutated AML was the largest class in our cohort (accounting for 27% of the cohort), with 73% of patients (319 of 436) also carrying mutations in DNA methylation or hydroxymethylation genes (*DNMT3A*, *IDH1*, *IDH2*^{R140}, and *TET2*).

The second largest subgroup, accounting for 18% of the cohort, was defined by mutations in



genes regulating RNA splicing (*SRSF2*, *SF3B1*, *U2AF1*, and *ZRSR2*), chromatin (*ASXL1*, *STAG2*, *BCOR*, *MLL*^{PTD}, *EZH2*, and *PHF6*), or transcription (*RUNX1*); we call this the chromatin-spliceosome group. In contrast to the WHO classes of AML, no single genomic lesion defines this

group. In our cohort, it could not be further subdivided because of the overlapping patterns of co-mutations among the defining genes (Fig. 2).

Mutations in *TP53*, complex karyotype alterations, cytogenetically visible copy-number alterations (aneuploidies), or a combination, characterize an additional AML subgroup (accounting for 13% of the cohort). This group emerges because specific chromosomal abnormalities are closely correlated with one another and with *TP53* mutations, but both are mutually exclusive with other class-defining lesions. Since this subtype includes patients with *TP53* mutations, isolated chromosomal-arm losses or gains, or both, it is broader than previously proposed groups, such as “monosomal karyotype AML” and “complex karyotype AML”^{2,3,27,28} (see the Results S5 section in the Supplementary Appendix). Patients in this subgroup were older and had fewer RAS-pathway mutations than patients in other subgroups (Fig. 2, and Fig. S3a in the Supplementary Appendix). This may reflect redundancy between RAS-pathway activation by point mutation and loss of RAS regulators by chromosomal aneuploidies.²⁹

Finally, our analysis identified a subgroup of AML with *IDH2*^{R172} mutations, accounting for 1% of the cohort. Unlike *IDH2*^{R140} mutations, which show strong co-mutation with *NPM1* (odds ratio for co-mutation, 3.6; $P=5\times 10^{-10}$), *IDH2*^{R172} mutations are mutually exclusive with *NPM1* (odds ratio for co-mutation, 0.06; $P=4\times 10^{-5}$) and other class-defining lesions. *IDH2*^{R172} AML is associated with gene-expression and DNA-methylation profiles that differ from the profiles for other *IDH* mutations and that lead to more severe aberrations in metabolic activity,^{30,31} adding further support for it as a distinct entity.

Under this schema, 1236 of the 1540 patients with driver mutations (80%) were unambiguously classified in a single subgroup, and 56 patients (4%) met criteria for two or more categories (Fig. 1B). These mostly fell in the *TP53*–aneuploidy and chromatin–spliceosome classes. A total of 166 patients (11%) with driver mutations remained unclassified, potentially harboring mutations in drivers not sequenced here⁶ or class-defining mutations that were missed. Notably, 105 of 166 unclassified patients had two or more driver mutations, with *DNMT3A*, *TET2*, *IDH1*, *FLT3*, and *NRAS* observed most frequently.

We applied the classification scheme developed here to the independent cohort that was

evaluated for the Cancer Genome Atlas (TCGA),⁵ which included many older patients with AML. The absence of overlap among subgroups was replicated, and the relative frequencies were equivalent to those in our cohort (see the Results S8 section in the Supplementary Appendix).

CLINICAL IMPLICATIONS OF GENOMIC CLASSIFICATION OF AML

Although a genomic classification does not presuppose clinical relevance, its foundation on causal mutations could plausibly provide a bridge from molecular to clinical features of disease. We found considerable differences in clinical presentation and overall survival across the genomic subgroups (Fig. 3A, and Fig. S8 and the Results S5 section in the Supplementary Appendix). For subgroups defined by fusion genes, *NPM1* and *CEBPA*^{biallelic} mutations, survival curves were as expected. Despite its more inclusive definition, the *TP53*–aneuploidy subgroup had dismal outcomes, as previously described.^{3,26,28,32}

As compared with other groups, patients in the chromatin–spliceosome group were older, with lower white-cell and blast counts, lower rates of response to induction chemotherapy, higher relapse rates, and a poor long-term clinical outlook (Fig. S9 in the Supplementary Appendix). Under current guidelines,³ 84% of patients in this subgroup (232 of 275) would be classified as being at intermediate risk, whereas their outcomes are in fact similar to those for patients in subgroups of AML with adverse outcomes, such as patients with *MLL* fusion genes (except for *MLLT3*–*MLL* fusion) or t(6;9) (Fig. 3A). In this group, 9% of patients had antecedent chronic myeloid disorders,³³ and 91% of the patients in this subgroup had a diagnosis of de novo AML (Fig. S10 in the Supplementary Appendix). Evaluation of the morphologic features in bone marrow specimens from 1064 patients in the cohort showed dysplastic features in 139 patients, 55 of whom were molecularly assigned to the chromatin–spliceosome group (Fig. S11 in the Supplementary Appendix). The same chromatin and splicing factors are also frequently mutated in high-risk myeloproliferative neoplasms³⁴ and myelodysplastic syndromes (MDS),^{17,35} suggesting that this subgroup could transcend conventional diagnostic boundaries³⁶ between acute and high-risk chronic myeloid disorders.

Although the number of patients in the

Table 2. Driver Mutations with the Strongest Effect on Overall Survival and Other Class-Defining Lesions in AML.*

Variable	Frequency in Study Cohort (N=1540) no. of patients (%)	Hazard Ratio for Death (95% CI)	P Value	q Value	Hazard Ratio for Interaction Terms
Main effects					
inv(3), GATA2, MECOM(EVI1)	23 (1)	2.9 (1.8–4.7)	9×10^{-6}	0.0003	
TP53	98 (6)	1.7 (1.4–2.2)	7×10^{-6}	0.0002	
Complex karyotype	159 (10)	1.4 (1.2–1.7)	2×10^{-6}	7×10^{-5}	
BRAF	9 (1)	1.4 (1.1–1.8)	0.009	0.06	
SRSF2	89 (6)	1.4 (1.1–1.7)	0.003	0.03	
FLT3 ^{ITD}	341 (22)	1.4 (1.2–1.7)	0.0008	0.01	
+21	39 (3)	1.3 (1.1–1.6)	0.001	0.02	
–5/5q	107 (7)	1.3 (1.1–1.5)	0.0007	0.01	
–17/17p	74 (5)	1.3 (1.1–1.5)	0.003	0.03	
+13	21 (1)	1.3 (1.1–1.5)	0.004	0.03	
–7	88 (6)	1.3 (1.1–1.5)	0.003	0.03	
–9q†	53 (3)	1.2 (1.1–1.5)	0.01	0.08	
+22†	26 (2)	1.2 (1.1–1.4)	0.008	0.06	
NPM1	436 (28)	0.7 (0.6–0.9)	0.0004	0.007	
CEBPA ^{biallelic}	73 (5)	0.6 (0.4–0.7)	4×10^{-5}	0.001	
t(15;17), PML–RARA	65 (4)	0.3 (0.2–0.4)	5×10^{-8}	4×10^{-6}	
inv(16), CBFβ–MYH11	82 (5)	0.3 (0.2–0.4)	4×10^{-9}	4×10^{-7}	
Gene–gene interactions					
NPM1–FLT3 ^{ITD} –DNMT3A	93 (6)	1.5 (1.2–1.9)	0.0002	0.004	1.1 for NPM1–FLT3 ^{ITD} , 1.0 for DNMT3A–NPM1, 1.2 for DNMT3A–FLT3, 1.1 for DNMT3A
MLL ^{PTD} –FLT3 ^{TKD}	10 (1)	1.4 (1.2–1.8)	0.0005	0.008	1.2 for MLL ^{PTD} , 1.1 for FLT3 ^{TKD}
DNMT3A–IDH2 ^{R140}	47 (3)	1.4 (1.1–1.8)	0.007	0.05	1.1 for DNMT3A, 1.0 for IDH2 ^{R140}
STAG2–IDH2 ^{R140}	11 (1)	0.8 (0.6–0.9)	0.01	0.09	0.9 for STAG2, 1.0 for IDH2 ^{R140}
NPM1–FLT3 ^{TKD}	53 (3)	0.7 (0.6–0.9)	0.009	0.06	0.7 for NPM1, 1.1 for FLT3 ^{TKD}
DNMT3A–RAD21	19 (1)	0.7 (0.5–0.9)	0.0008	0.01	1.1 for DNMT3A, 1.0 for RAD21
Other class-defining lesions					
t(x;11), not MLLT3–MLL	37 (2)	1.4 (1.0–2.1)	0.06	0.2	
ASXL1	70 (5)	1.3 (1.0–1.6)	0.04	0.2	
ZRSR2	13 (1)	1.3 (1.0–1.7)	0.04	0.2	
RUNX1	133 (9)	1.1 (0.9–1.3)	0.5	0.8	
t(9;11), MLLT3–MLL	18 (1)	0.8 (0.4–1.4)	0.5	0.7	
IDH2 ^{R172}	39 (3)	0.8 (0.6–1.0)	0.07	0.2	
t(8;21), RUNX1–RUNX1T1	63 (4)	0.7 (0.4–1.0)	0.03	0.2	

* The strongest effect on survival was defined as $q < 0.1$. The effects reported in this table do not take into account the maintenance of favorable outcomes in the context of core-binding leukemias. We did not include interactions between fusion genes and copy-number alterations in this analysis, because they are mutually exclusive for the most part and we wanted to limit the number of variables in the model. See Table S10 in the Supplementary Appendix for the full model.

† A +22 lesion frequently co-occurs with inv(16) and is associated with an especially favorable outcome; –9q frequently co-occurs with t(8;21) but does not appear to affect outcomes.

IDH2^{R172} subgroup was small, the long-term outcomes in this group were broadly similar to those in patients with *NPM1*-mutated AML (Fig. 3A).³⁷ Patients in whom no driver mutations were detected had lower blast and white-cell counts and better outcomes (Fig. 3A, and Fig. S12a in the Supplementary Appendix).

INFLUENCE OF CO-OCCURRING MUTATIONS ON CLINICAL OUTCOMES

Overall survival was correlated with the number of driver mutations (Fig. S12b in the Supplementary Appendix), independent of age and the white-cell count ($P=8\times 10^{-12}$). One possible explanation for this finding is that driver mutations other than class-defining lesions influence clinical outcomes. For example, despite the frequent co-occurrence of a *TP53* mutation and a complex karyotype, they were correlated independently and additively with survival in our cohort (Fig. 3B). Similarly, mutations in chromatin, splicing, and transcriptional regulators are frequently associated with low survival rates, and co-mutation among these genes typically results in even lower survival rates (Fig. 3C, and Fig. S13 in the Supplementary Appendix).

We developed multivariate models to explore the relative contributions of genetic, clinical, and diagnostic variables to overall survival. Using the full model, we could correctly rank approximately 71% of patients for overall survival (vs. 64% with models using only variables in the European LeukemiaNet criteria) (Fig. 3D). Genomic features were the most powerful predictors, accounting for about two thirds of explained variation, with the other third contributed by demographic, clinical, and treatment variables (Fig. 3D). Among genomic factors, fusion genes, copy-number alterations, and point mutations were broadly equivalent. These overall findings were replicated in the TCGA cohort of patients with AML⁵ (see the Results S8 section and Fig. S14 in the Supplementary Appendix).

Although a number of genomic variants are significant predictors of overall survival ($P\leq 0.01$) (Table 2 and Fig. 3E), many more genes show a somewhat weaker correlation with outcome (Table S10 in the Supplementary Appendix). The prognostic effects of class-defining lesions have mostly been described before, but we note the independent deleterious effects of *TP53* mutations and the chromatin-spliceosome genes, such as *SRSF2* and *ASXL1*. *BRAF* mutations are indepen-

Figure 4 (facing page). Influence of Gene–Gene Interactions on Overall Survival.

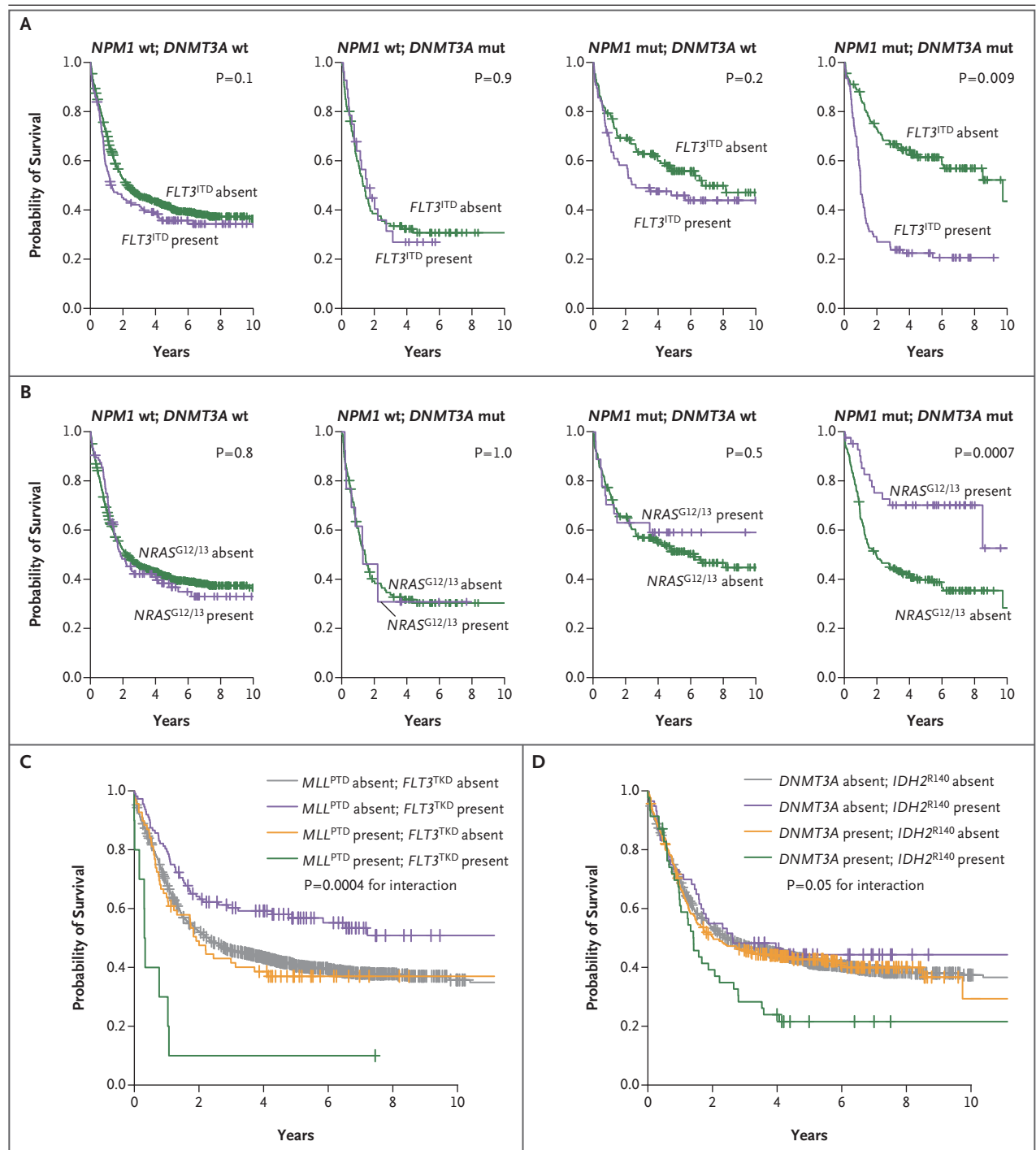
Panel A shows Kaplan–Meier curves for overall survival according to the presence or absence of *FLT3*^{ITD}. The deleterious prognostic effect of *FLT3*^{ITD} was significantly greater when both *DNMT3A* and *NPM1* were mutated, as shown in the graph at the right ($P=0.009$ for three-way interaction in the univariate analysis; $q=0.004$ in the multivariate analysis with correction for multiple hypothesis testing). A total of 28 patients had both *DNMT3A* and *FLT3*^{ITD}, 77 had both *NPM1* and *FLT3*^{ITD}, and 93 had all three mutations. Panel B shows Kaplan–Meier curves for overall survival according to the presence or absence of *NRAS* codon 12/13 mutation. The prognostic effect of *NRAS* codon 12/13 mutation was significantly greater when both *DNMT3A* and *NPM1* carried the driver mutation (in 45 patients), as shown in the graph at the right ($P=0.0007$ for three-way interaction in the univariate analysis). Panel C shows Kaplan–Meier curves for overall survival according to the presence or absence of *MLL*^{PTD} and *FLT3*^{TKD} ($P=0.0004$ for gene–gene interaction in the univariate analysis; $q=0.008$ in the multivariate analysis with correction for multiple hypothesis testing). A total of 69 patients had *MLL*^{PTD}, 112 had *FLT3*^{TKD}, and 10 had both. Panel D shows Kaplan–Meier curves for overall survival according to the presence or absence of driver mutations in *DNMT3A*, *IDH2*^{R140}, or both ($P=0.05$ for gene–gene interaction in the univariate analysis; $q=0.05$ in the multivariate analysis with correction for multiple hypothesis testing). A total of 338 patients had *DNMT3A*, 20 had *IDH2*^{R140}, and 19 had both.

dently associated with a worse prognosis ($P=0.009$, $q=0.06$), and *BRAF* inhibitors might be a useful therapeutic option for patients in this subgroup.

INFLUENCE OF COMPLEX GENE INTERACTIONS ON SURVIVAL

The prognostic effects of *TP53* mutations and complex karyotype (Fig. 3B) and of *ASXL1* and *SRSF2* mutations (Fig. 3C) are examples of additive associations — that is, the deleterious effect of each lesion remains unchanged whether or not another is present, with co-occurrence indicating a particularly dismal prognosis. We found that 11% of explained variation in survival in the cohort could be attributed to gene–gene interactions (Fig. 3D and Table 2), in which the prognostic effect of one gene is significantly altered if another gene is co-mutated. This suggests that the clinical effect of some driver mutations is modified by the wider genomic context in which they occur.

In our data set, this was exemplified by a three-way interaction among *NPM1*, *DNMT3A*, and *FLT3*^{ITD}. This combined genotype represented the



most frequent three-gene co-occurrence in our cohort, identified in 93 of the 1540 patients (6%) ($P<0.0001$). We found that the deleterious effect of *FLT3*^{ITD} was most clinically relevant in patients with concomitant *NPM1* and *DNMT3A* mutations ($P=0.009$ for three-way interaction in the univariate analysis, $q=0.004$ in the multivariate analysis) (Fig. 4A). When present with either *NPM1* or

DNMT3A or with neither of these other genes, the effect of *FLT3*^{ITD} on survival was considerably less pronounced. This observation held true regardless of the ratio of mutant to wild-type *FLT3*^{ITD} (Fig. S15 in the Supplementary Appendix).

In contrast, the *NPM1*–*DNMT3A*–*NRAS*^{G12/13} genotype carried an unexpectedly benign prognosis in our cohort ($P=0.04$ for three-way inter-

action) (Fig. 4B). Previous reports have suggested that *NPM1*–*NRAS* is a favorable association,³⁸ something we find specific to *NPM1*–*DNMT3A*–*NRAS*^{G12/13}. In this cohort, outcomes for patients in the *NPM1* subgroup were strongly dictated by the mutation context in which *NPM1* mutations occurred (i.e., *NRAS*, *IDH1*, *PTPN11*, *FLT3*, and chromatin–spliceosome mutations) (Fig. S15 in the Supplementary Appendix).

Other significant gene–gene interactions were found. In particular, the prognosis was significantly poorer than expected for the co-occurrence of *FLT3*^{TKD} mutations with partial tandem duplications of *MLL* ($q=0.008$) and for the co-occurrence of *DNMT3A* with *IDH2*^{R140} ($q=0.05$) (Fig. 4C and 4D and Table 2).

DISCUSSION

There is a distinction between a system for classifying patients with AML for diagnostic purposes and a system for predicting the outcome of AML. The former should be stable and durable, based on fixed biologic properties of the disease; hence, the focus on causative driver mutations. The latter should be flexible and adaptable, evolving to reflect advances in treatment and often being influenced by changes in disease surveillance.³⁹ For example, the advent of effective *FLT3* and *RAS*-pathway kinase inhibitors will lead to changes in outcome predictions for patients with these mutations but will not fundamentally alter the fact that these variants co-occur with class-defining mutations such as *t*(15;17), *t*(6;9), and *NPM1*.

It is somewhat counterintuitive that *NPM1* emerges as a separate entity, since it appears to be a late driver mutation, is almost never found in isolation, and is associated with a clinical course that is modified depending on the specific co-occurring mutations. Nonetheless, among all the mutations with which it co-occurs, it is the one that most clearly occupies its own distinct niche. Why it occurs late is not clear — it may have transformative effects only in the context of an epigenetic landscape already shaped by initiating mutations such as *DNMT3A*, *TET2*, or *IDH1/2*. *NPM1* mutations are not the only route of transformation these initiating lesions can follow: when *JAK2* mutations occur, myeloproliferative neoplasms result, whereas co-mutation with *SF3B1* leads to MDS with ring sideroblasts.

Beyond the existing WHO subgroups, three

genomic categories of AML emerge from our analysis: chromatin–spliceosome, *TP53*–aneuploidy, and provisionally, *IDH2*^{R172} mutations. The chromatin–spliceosome category represents the second largest subgroup of patients with AML, even in this intensively treated, relatively young cohort. In contrast to classes defined by specific fusion genes, no single gene defines this group. We observed intersecting patterns of mutated chromatin and RNA-splicing regulators, suggesting a plasticity of paths of disease evolution in this subgroup, yet these genes showed minimal overlap with other class-defining lesions. Patients in the chromatin–spliceosome subgroup were, on average, older and had lower blast counts and higher rates of antecedent MDS or dysplasia-related morphologic features than patients in other subgroups. This overlap should not be overstated, however — although 20% of patients had a preceding myeloid disorder or evidence of dysplasia as defined by the WHO, 80% did not have such dysplastic features and presented with intermediate-risk, de novo AML.

The most widely accepted classification and prognostic schemes for AML include cytogenetic lesions together with *NPM1*, *FLT3*^{ITD}, and *CEBPA*.^{3,26} In the short term, *TP53*, *SRSF2*, *ASXL1*, *DNMT3A*, and *IDH2* should be considered for incorporation into prognostic guidelines because they are common and exert a strong influence on clinical outcomes. For AML classification, evaluation of splicing-factor genes *RUNX1*, *ASXL1*, and *MLL*^{PTD} at diagnosis would identify patients in the chromatin–spliceosome group.

In conclusion, we analyzed somatic driver mutations retrospectively in more than 1500 patients with AML, generated a new genetic approach to disease classification with prognostic implications, and obtained similar results for an independent data set from TCGA. Prospective clinical studies are needed for further validation of this schema.

Supported by grants from the Wellcome Trust (077012/Z/05/Z), Bundesministerium für Bildung und Forschung (01GI9981 and 01KG0605), Deutsche Krebshilfe (DKH, 109675), and Deutsche Forschungsgemeinschaft (DFG) for projects B3 and B4 of Sonderforschungsbereich (SFB) 1074. Dr. Papaemmanuil is a Josie Robertson Investigator and the recipient of a European Hematology Association early career fellowship. Dr. Campbell is the recipient of a Wellcome Trust Senior Clinical Research Fellowship (WT088340MA), and Drs. Bullinger and Heuser are the recipients of grants from the DFG (BU 1339/8-1 and HE 5240/6-1). Drs. Potter and Greaves were supported in part by grants from the Kay Kendall Leukaemia Fund (KKL688) and Wellcome Trust (105104/Z/14/Z). The German-Austrian AML Study Group (AMLSG) treatment trials were supported in part by Amgen.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Daniela Weber for managing clinical data, Veronica Teleanu for assisting with cytogenetic data classification, Dr. Sabine Kayser for assisting with morphologic evaluation, and all

the members of the AMLSG (see the Supplementary Appendix for a list of participating institutions and investigators) for participating in this study and providing samples from patients.

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