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ORIGINAL ARTICLE

Classification and Personalized Prognosis in Myeloproliferative Neoplasms

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ABSTRACT

BACKGROUND

Myeloproliferative neoplasms, such as polycythemia vera, essential thrombocythemia, and myelofibrosis, are chronic hematologic cancers with varied progression rates. The genomic characterization of patients with myeloproliferative neoplasms offers the potential for personalized diagnosis, risk stratification, and treatment.

METHODS

We sequenced coding exons from 69 myeloid cancer genes in patients with myeloproliferative neoplasms, comprehensively annotating driver mutations and copy-number changes. We developed a genomic classification for myeloproliferative neoplasms and multistage prognostic models for predicting outcomes in individual patients. Classification and prognostic models were validated in an external cohort.

RESULTS

A total of 2035 patients were included in the analysis. A total of 33 genes had driver mutations in at least 5 patients, with mutations in *JAK2*, *CALR*, or *MPL* being the sole abnormality in 45% of the patients. The numbers of driver mutations increased with age and advanced disease. Driver mutations, germline polymorphisms, and demographic variables independently predicted whether patients received a diagnosis of essential thrombocythemia as compared with polycythemia vera or a diagnosis of chronic-phase disease as compared with myelofibrosis. We defined eight genomic subgroups that showed distinct clinical phenotypes, including blood counts, risk of leukemic transformation, and event-free survival. Integrating 63 clinical and genomic variables, we created prognostic models capable of generating personally tailored predictions of clinical outcomes in patients with chronic-phase myeloproliferative neoplasms and myelofibrosis. The predicted and observed outcomes correlated well in internal cross-validation of a training cohort and in an independent external cohort. Even within individual categories of existing prognostic schemas, our models substantially improved predictive accuracy.

CONCLUSIONS

Comprehensive genomic characterization identified distinct genetic subgroups and provided a classification of myeloproliferative neoplasms on the basis of causal biologic mechanisms. Integration of genomic data with clinical variables enabled the personalized predictions of patients' outcomes and may support the treatment of patients with myeloproliferative neoplasms. (Funded by the Wellcome Trust and others.)

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Myeloproliferative neoplasms are clonal hematopoietic disorders comprising polycythemia vera, which is characterized by red-cell overproduction; essential thrombocythemia, which involves elevated platelet counts; and myelofibrosis, which is defined by bone marrow fibrosis.[1] Polycythemia vera and essential thrombocythemia are chronic-phase myeloproliferative neoplasms, whereas myelofibrosis represents advanced disease that is diagnosed either initially or after the diagnosis of essential thrombocythemia or polycythemia vera. Current classification schemes distinguish among the subtypes of myeloproliferative neoplasms according to clinical and laboratory features,[2-5] but uncertainty clouds where and how to draw dividing lines among them.[6,7]

Biologically, the development of myeloproliferative neoplasms is driven by mutations in *JAK2*, *CALR*, or *MPL*. Many patients have additional drivers that span a wide range of cancer genes, with patient-to-patient variation in the genetic and clonal landscape.[8,9] Driver mutations correlate with phenotype and prognosis,[10-12] and mutation order can also influence phenotype.[13,14] This complex genetic landscape probably contributes to heterogeneity in diagnostic features and outcomes in patients with myeloproliferative neoplasms.

In blood cancers, a progressive shift is under way, from clinical and morphologic classification schemes to those that are based on genomics.[15] Driver mutations are increasingly important in predicting clinical outcomes, but large, well-characterized cohorts are necessary for accurate prognostic models.[16] Studies have suggested that this promise extends to myeloproliferative neoplasms,[10,17] but larger cohorts and comprehensive gene sequencing are needed in order to provide definitive answers.

METHODS

STUDY SAMPLES

We analyzed samples that were obtained from patients after they provided written informed consent and after ethics approval from relevant authorities was obtained. Details regarding the cohort, disease classification, and diagnostic review are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org. Tumor DNA was derived from blood granulocytes, bone marrow mononuclear cells, or whole blood. The majority of patients did not have matched germline samples sequenced. We use the term "myelofibrosis" to encompass both primary myelofibrosis and myelofibrosis that evolved from essential thrombocythemia or polycythemia vera.

No commercial support was involved in this study. See the Supplementary Appendix for details regarding patient cohorts and samples.

SEQUENCING AND ANALYSES

We designed custom RNA baits to capture the full coding sequence of 69 genes, single-nucleotide polymorphisms for copy number profiling, and germline loci that have been associated with myeloproliferative neoplasms (Tables S1 and S2 in the Supplementary Appendix).[18-20] Additional patients underwent whole-exome sequencing, as reported previously.[8]

CLINICAL VARIABLES

Laboratory and clinical data from diagnosis were incorporated into prognostic models. The median duration between diagnosis and sample acquisition was 49 days. The median follow-up was 93.8 months (range, <1 to 523) from diagnosis and 72.0 months (range, <1 to 360) from DNA sampling.

STATISTICAL ANALYSIS

We estimated the timing of mutation acquisition using Bradley–Terry models of pairwise comparisons of clonal fractions.[13] We used a Bayesian network analysis and Dirichlet processes to identify genetic associations and subgroups. Random-effects Cox proportional-hazards multistate modeling was used for outcome predictions (see the Supplementary Appendix).

RESULTS

SPECTRUM OF GENOMIC CHANGES IN MYELOPROLIFERATIVE NEOPLASMS

Targeted sequencing for the full coding sequence of 69 genes and genomewide copy-number information was undertaken in 1887 patients, and 148 patients underwent whole-exome sequencing, as reported previously.[8] The cohort of 2035 patients included 1321 patients with essential thrombocythemia, 356 with polycythemia vera, 309 with myelofibrosis, and 49 with other diagnoses of myeloproliferative neoplasms (Table S3 in the Supplementary Appendix). A total of 33 genes had driver mutations in at least 5 patients (Fig. 1A, and Tables S4 and S5 in the Supplementary Appendix). Mutations in *JAK2*, *MPL*, and *CALR* accounted for 1831 driver mutations and were the sole abnormality in 45% of the patients. A total of 1075 driver mutations were identified across other genes. Loss of heterozygosity was frequent for *JAK2* V617F, especially in patients with polycythemia vera, but was infrequent for *CALR* and *MPL* (Fig. S1 in the Supplementary Appendix).

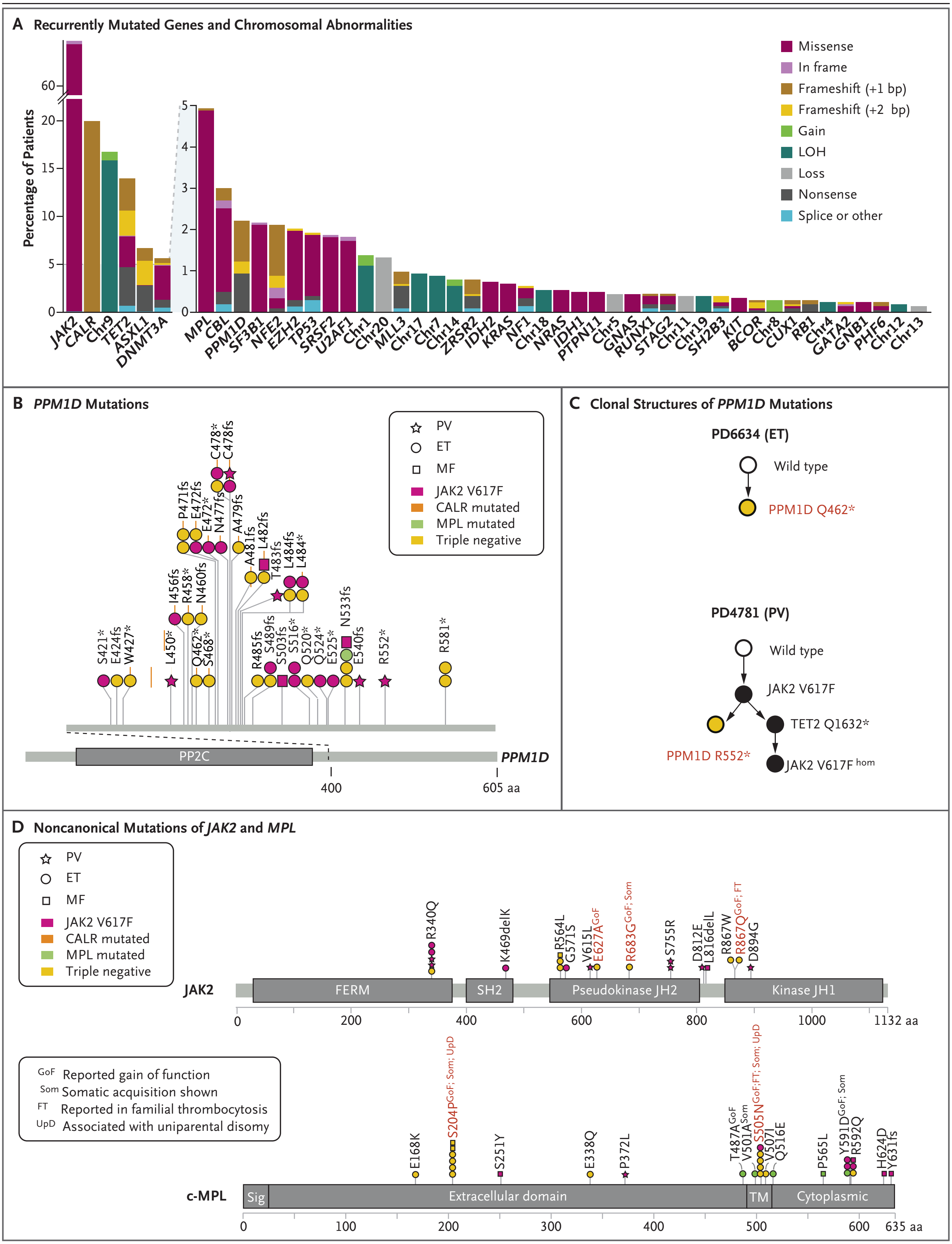
We identified 45 truncating mutations in the terminal exon of *PPM1D* in 38 patients within the cohort (1.9%) (Fig. 1B); thus, *PPM1D* was the eighth most commonly mutated gene in myeloproliferative neoplasms. These mutations have also been detected in solid tumors, blood samples obtained from healthy persons, and patients with breast or ovarian tumors, often after chemotherapy.[21,22] In our cohort, 10 patients had *PPM1D* mutations that were detectable only in a later sample obtained during treatment with hydroxyurea. However, *PPM1D* mutations were also detected at or within 1 month after diagnosis in 20 patients. Analysis of single-cell–derived hematopoietic colonies identified mutated *PPM1D* in a patient with triple-negative essential thrombocythemia (i.e., nonmutated *JAK2*, *CALR*, or *MPL*) but also identified mutated *PPM1D* that was subclonal to *JAK2* V617F in a patient with polycythemia vera (Fig. 1C). These data confirm that *PPM1D* mutations can occur within the myeloproliferative neoplasm clone and be present at diagnosis; thus, their presence does not always indicate age-related clonal hematopoiesis or therapy-related disease evolution.

Mutations in *MLL3* were detected in 20 patients (1.0%) and were predominantly nonsense or frameshift, as has been reported in patients with acute myeloid leukemia (Fig. 1A, and Table S4 in the Supplementary Appendix).[23] Among these 20 patients, 7 patients had triple-negative myeloproliferative neoplasms, which suggests that *MLL3* could be an important tumor-suppressor gene in these patients.

Whether mutations in *JAK2* and *MPL* outside the known hot spots could be relevant to patients with myeloproliferative neoplasms has been unclear.[24,25] We identified noncanonical variants in *JAK2* and *MPL* in 16 patients with triple-negative essential thrombocythemia and in 1 patient with triple-negative myelofibrosis (Fig. 1D). Of these, three groups of variants were likely to be relevant to disease pathogenesis: *JAK2* R683G and *JAK2* E627A in 2 patients with essential thrombocythemia (reported in acute lymphoblastic leukemia in which they activate JAK2[26-28]); *JAK2* R867 in 2 patients with essential thrombocythemia (associated with familial thrombocythemia[29]); and *MPL* S505N and *MPL* S204P in 4 and 5 patients, respectively, with essential thrombocythemia.[24] *MPL* S204P co-occurred with loss of heterozygosity (LOH) at chromosome 1p, which suggests

Figure 1 (facing page). Genomic Landscape of Myeloproliferative Neoplasms.

Panel A shows the frequency of recurrently mutated genes and chromosomal abnormalities in the cohort of 2035 patients. Mutations were stratified according to type (missense, nonsense, affecting a splice site, or other [e.g., stop, gain, or loss]). Insertions and deletions (del) were categorized according to whether they resulted in a shift in the codon reading frame (by either 1 or 2 base pairs [bp]) or were in frame. Chromosomes are indicated by Chr plus a numeral (e.g., Chr9 denotes chromosome 9). Chromosomal gains include whole-chromosome gains (trisomy) and subchromosomal amplifications. Chromosomal losses include whole-chromosome deletions (monosomy) and subchromosomal deletions. Loss of heterozygosity (LOH) was predominantly copy-number neutral, but in some cases, chromosome losses could not be ruled out. Panel B shows the site within the gene and protein consequence of *PPM1D* mutations. Colored shapes represent the characteristics of the patient who had the specific mutation (shapes indicate the subtype of myeloproliferative neoplasm, and colors the phenotypic driver). A triple-negative finding indicates nonmutated *JAK2*, *CALR*, and *MPL*. The term aa denotes amino acid, ET essential thrombocythemia, fs frameshift, MF myelofibrosis, PP2C protein phosphatase 2C domain, and PV polycythemia vera. Panel C shows clonal structures of two patients with *PPM1D* mutations determined by genotyping of hematopoietic colonies derived from peripheral-blood mononuclear cells. Each circle represents a group of hematopoietic colonies that share the same genotype: wild type (white), other driver mutations (black), and *PPM1D* mutated (yellow). Wild-type colonies are represented at the top of each diagram, with subsequent mutant subclones shown below. Somatic mutations acquired in each subclone are indicated beside respective nodes and represent those that were acquired in addition to mutations present in earlier subclones. The term hom denotes homozygous. Panel D shows the site within the gene and protein consequence of noncanonical mutations of *JAK2* and *MPL*. The V617F and exon 12 mutations in *JAK2* and W515 mutations in *MPL* are not shown. Mutations highlighted in red are likely to be relevant to disease pathogenesis, with previous studies having shown somatic acquisition, familial inheritance, or functional consequences for the specific variants (see box of abbreviations). FERM denotes the 4.1–ezrin–radixin–moesin domain, SH2 Src homology 2, Sig signal, and TM transmembrane.


A Recurrently Mutated Genes and Chromosomal Abnormalities
Percentage of Patients
60
20
15
10
5
0
5
4
3
2
1
0
JAK2
CALR
Chr9
TET2
ASXL1
DNMT3A
MPL
CBL
PPM1D
SF3B1
NFE2
EZH2
TP53
SRSF2
U2AF1
Chr1
Chr20
MLL3
Chr17
Chr7
Chr14
ZRSR2
IDH2
KRAS
NF1
Chr18
NRAS
IDH1
PTPN11
Chr5
GNAS
RUNX1
STAG2
Chr11
Chr19
SH2B3
KIT
BCOR
Chr8
CUX1
RB1
Chr4
GATA2
GNB1
PHF6
Chr12
Chr13
Missense
In frame
Frameshift (+1 bp)
Frameshift (+2 bp)
Gain
LOH
Loss
Nonsense
Splice or other
B PPM1D Mutations
PV
ET
MF
JAK2 V617F
CALR mutated
MPL mutated
Triple negative
C478*
C478fs
P471fs
E472fs
E472*
N477fs
A479fs
A481fs
L482fs
T483fs
L484fs
L484*
I456fs
R458*
N460fs
S421*
E424fs
W427*
L450*
Q462*
S468*
R485fs
S489fs
S503fs
S516*
Q520*
Q524*
E525*
N533fs
E540fs
R552*
R581*
PP2C
PPM1D
400
605 aa
C Clonal Structures of PPM1D Mutations
PD6634 (ET)
Wild type
PPM1D Q462*
PD4781 (PV)
Wild type
JAK2 V617F
TET2 Q1632*
PPM1D R552*
JAK2 V617F hom
D Noncanonical Mutations of JAK2 and MPL
PV
ET
MF
JAK2 V617F
CALR mutated
MPL mutated
Triple negative
R340Q
K469delK
R564L
G571S
V615L
E627A GoF
R683G GoF; Som
S755R
D812E
L816delL
R867W
R867Q GoF; FT
D894G
JAK2
FERM
SH2
Pseudokinase JH2
Kinase JH1
0
200
400
600
800
1000
1132 aa
GoF Reported gain of function
Som Somatic acquisition shown
FT Reported in familial thrombocytosis
UpD Associated with uniparental disomy
E168K
S204P GoF; Som; UpD
S251Y
E338Q
P372L
T487A GoF
V501A Som
S505N GoF; FT; Som; UpD
V507I
Q516E
P565L
Y591D GoF; Som
R592Q
H624D
Y631fs
c-MPL
Sig
Extracellular domain
TM
Cytoplasmic
0
100
200
300
400
500
600
635 aa

a clonal advantage to acquired homozygosity for this variant.

FACTORS INFLUENCING CLASSIFICATION INTO DISEASE SUBTYPES

Currently, patients with myeloproliferative neoplasms are classified as having essential thrombocythemia, polycythemia vera, or myelofibrosis on the basis of clinical and laboratory criteria,[2-5] but the biologic factors underlying these distinctions are incompletely understood. The number of driver mutations per patient was higher in those with myelofibrosis than in those with polycythemia vera or essential thrombocythemia (Fig. 2A), as previously reported,[8] and increased according to the age of the patient (Fig. 2B).

The distinction between *JAK2* V617F–mutated essential thrombocythemia and polycythemia vera rests on whether the red-cell mass or hematocrit is elevated. We found that acquired driver mutations correlated with hematologic variables (Fig. S2 in the Supplementary Appendix) and were the strongest determinants of a patient with *JAK2* V617F–mutated chronic-phase disease receiving a diagnosis of essential thrombocythemia as compared with polycythemia vera, although germline genetic background and demographic factors also contributed (Fig. 2C, and Fig. S2 in the Supplementary Appendix). LOH at chromosome 9p (9pLOH), causing *JAK2* V617F homozygosity, or a high *JAK2* V617F allele burden correlated with polycythemia vera, as did mutated *NFE2*, a transcription factor critical to erythroid differentiation.

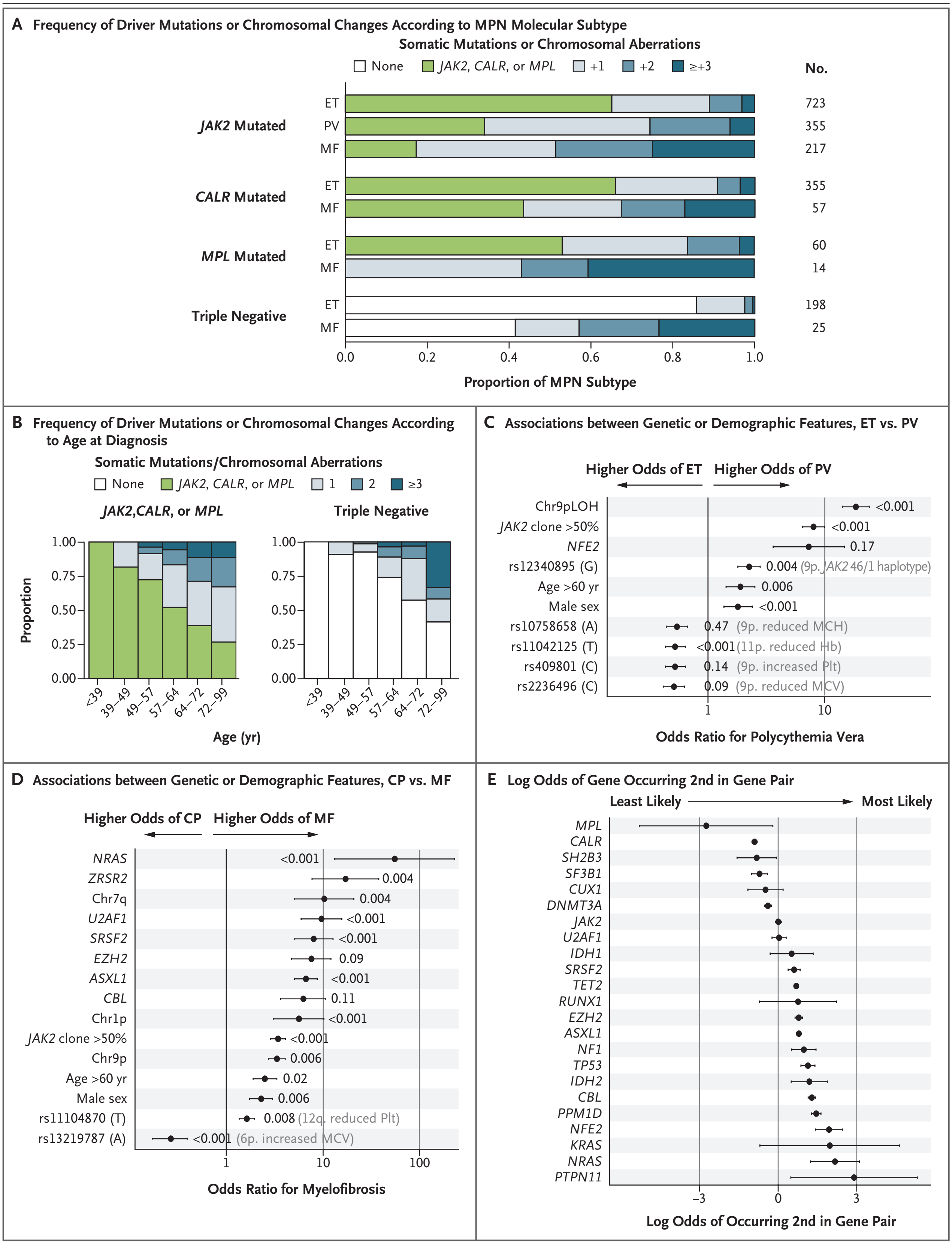
Germline polymorphisms that have been associated with red-cell variables in the general population were distributed unevenly, with alleles associated with lower hemoglobin level and higher platelet counts being enriched in patients with essential thrombocythemia (Fig. 2C). Furthermore, the *JAK2* 46/1 haplotype, which is known to increase the predisposition to myeloproliferative neoplasms,[18] correlated with polycythemia vera (odds ratio, 2.3; 95% confidence interval [CI], 1.7 to 3.3; P=0.004), possibly through increasing odds of *JAK2* V617F homozygosity by 9pLOH (odds ratio, 2.7; 95% CI, 2.0 to 3.9; P<0.001). Older age and male sex also increased the odds of polycythemia vera. These data show that the location of any chronic-phase disease on the hemoglobin and red-cell mass continuum is influenced by many factors and that any arbitrary threshold to label patients' disease as being one subtype or the other will not distinguish among patients with different underlying biologic factors.

Mutations in spliceosome components, epigenetic regulators, and the RAS pathway were strongly associated with accelerated phase (myelofibrosis), as compared with chronic-phase disease (essential thrombocythemia or polycythemia vera), as were male sex, older age, and germline loci associated with platelet count and red-cell variables (Fig. 2D).

The order in which mutations are acquired in myeloproliferative neoplasms has previously been shown to influence disease phenotype.[1314] *CALR* and *MPL* mutations occurred more commonly early in disease, whereas mutations in *NRAS*, *TP53*, *PPM1D*, and *NFE2* were acquired significantly later in disease (Fig. 2E, and Fig. S3 in the Supplementary Appendix). Some of the earlier-occurring mutations in genes such as *SF3B1* and

Figure 2 (facing page). Factors Affecting Disease Classification at Presentation and Timing of Somatic Mutations.

Histograms show the frequency of driver mutations or chromosomal changes (gains, losses, or LOH) that were identified in different molecular subtypes of myeloproliferative neoplasm (MPN) (excluding 24 patients with >1 detectable phenotypic driver mutation) (Panel A) and according to the age of the patient at diagnosis (Panel B). Forest plots showing the associations between genetic or demographic features and presentation with essential thrombocythemia (ET) as compared with polycythemia vera (PV) in patients with *JAK2* V617F mutations (Panel C) and the presentation in chronic-phase (CP) disease as compared with MF across patients with *JAK2*, *CALR*, or *MPL* mutations (Panel D). Significant associations from univariate analyses after correction for multiple hypothesis testing are shown. P values were derived from logistic-regression modeling, identifying independent associations. Causes of reductions in the hemoglobin (Hb) level, mean corpuscular hemoglobin (MCH) level, mean corpuscular volume (MCV), and platelet (Plt) count are indicated. Of 671 patients who had more than one somatic mutation, the order of mutation acquisition of at least one pair of mutations was determined in 271 patients (40%) (Panel E). These ordered pairings were used to determine the relative probabilities of a gene occurring first or second for a given pairing with the use of Bradley–Terry modeling, which provided an estimate of the overall timing of mutation acquisition. The horizontal axis shows the log odds of a gene occurring second in a gene pair. For example, as compared with *JAK2*, *PPM1D* mutations have a log odds of 1.45 and therefore are $e^{1.45}$, or 4.3, times more likely to occur second in the pair. Any pair of genes can be assessed in this manner by calculating the exponential of the difference in log odds for gene A and gene B. The error bars indicate 95% confidence intervals.


A Frequency of Driver Mutations or Chromosomal Changes According to MPN Molecular Subtype
Somatic Mutations or Chromosomal Aberrations
None
JAK2, CALR, or MPL
+1
+2
≥+3
No.
JAK2 Mutated
ET 723
PV 355
MF 217
CALR Mutated
ET 355
MF 57
MPL Mutated
ET 60
MF 14
Triple Negative
ET 198
MF 25
0.0
0.2
0.4
0.6
0.8
1.0
Proportion of MPN Subtype
B Frequency of Driver Mutations or Chromosomal Changes According to Age at Diagnosis
Somatic Mutations/Chromosomal Aberrations
None
JAK2, CALR, or MPL
1
2
≥3
JAK2,CALR, or MPL
Triple Negative
Proportion
1.00
0.75
0.50
0.25
0.00
<39
39–49
49–57
57–64
64–72
72–99
Age (yr)
C Associations between Genetic or Demographic Features, ET vs. PV
Higher Odds of ET
Higher Odds of PV
Chr9pLOH <0.001
JAK2 clone >50% <0.001
NFE2 0.17
rs12340895 (G) 0.004 (9p. JAK2 46/1 haplotype)
Age >60 yr 0.006
Male sex <0.001
rs10758658 (A) 0.47 (9p. reduced MCH)
rs11042125 (T) <0.001 (11p. reduced Hb)
rs409801 (C) 0.14 (9p. increased Plt)
rs2236496 (C) 0.09 (9p. reduced MCV)
1
10
Odds Ratio for Polycythemia Vera
D Associations between Genetic or Demographic Features, CP vs. MF
Higher Odds of CP
Higher Odds of MF
NRAS <0.001
ZRSR2 0.004
Chr7q 0.004
U2AF1 <0.001
SRSF2 <0.001
EZH2 0.09
ASXL1 <0.001
CBL 0.11
Chr1p <0.001
JAK2 clone >50% <0.001
Chr9p 0.006
Age >60 yr 0.02
Male sex 0.006
rs11104870 (T) 0.008 (12q. reduced Plt)
rs13219787 (A) <0.001 (6p. increased MCV)
1
10
100
Odds Ratio for Myelofibrosis
E Log Odds of Gene Occurring 2nd in Gene Pair
Least Likely
Most Likely
MPL
CALR
SH2B3
SF3B1
CUX1
DNMT3A
JAK2
U2AF1
IDH1
SRSF2
TET2
RUNX1
EZH2
ASXL1
NF1
TP53
IDH2
CBL
PPM1D
NFE2
KRAS
NRAS
PTPN11
−3
0
3
Log Odds of Occurring 2nd in Gene Pair

DNMT3A are also associated with age-related clonal hematopoiesis,[30,31] which suggests that some myeloproliferative neoplasms could arise from an antecedent asymptomatic clone. In patients with multiple mutations, *JAK2* V617F was more commonly a secondary event in patients with essential thrombocythemia and an earlier event in those with polycythemia vera or myelofibrosis (Figs. S4 and S5 in the Supplementary Appendix), a finding that confirms and generalizes observations that had previously been shown for *JAK2* relative to *TET2* or *DNMT3A*.[13,14]

GENOMIC SUBGROUPS IN MYELOPROLIFERATIVE NEOPLASMS

Hematologic cancers may be subclassified according to driver mutations that distinguish subgroups of patients,[32,33,34] with the use of patterns of mutually exclusive or co-mutated genes. In our cohort, driver mutations showed complex patterns of assortment (Fig. S6 in the Supplementary Appendix). We used Bayesian modeling to identify genomic subgroups of myeloproliferative neoplasms with maximum within-group similarity and maximum between-group discrimination.

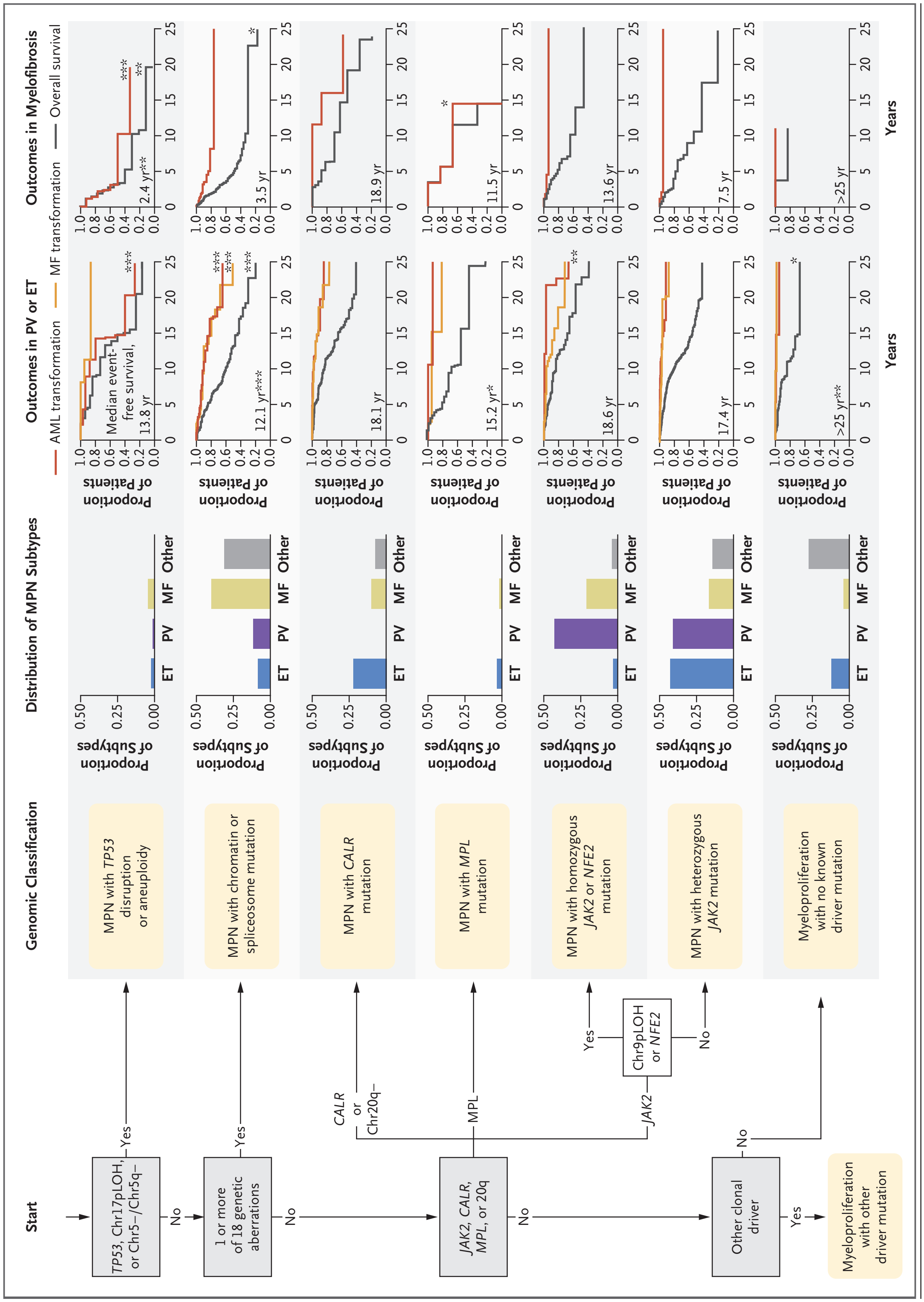
We identified eight genomic subgroups in myeloproliferative neoplasms, defined according to simple rules (Fig. 3, and Fig. S7 in the Supplementary Appendix). *TP53* mutations, often co-occurring with aberrations at chromosome 17p, and deletions at chromosome 5q identified the first subgroup. *TP53* mutations often occur later in disease (Fig. 2E) but dominate the genomic and clinical features of these patients regardless of the initial driver of the myeloproliferative neoplasm. As in patients with other blood cancers with *TP53* mutations,[32,35] these patients have a dismal prognosis with a high risk of transformation to acute myeloid leukemia (hazard ratio vs. the *JAK2*-heterozygous subgroup, 15.5; 95% CI, 7.5 to 31.4; P<0.001) and early death (hazard ratio, 2.4; 95% CI, 1.6 to 3.6; P<0.001).

The second subgroup was defined by the presence of one or more mutations in 16 myeloid cancer genes, especially chromatin and spliceosome regulators, LOH at chromosome 4q, and aberrations in chromosomes 7 and 7q. This subgroup was enriched for patients with myelofibrosis (odds ratio, 6.5; 95% CI, 4.9 to 8.7; P<0.001) and myelodysplastic–myeloproliferative neoplasms (including all seven patients with chronic myelomonocytic leukemia or atypical chronic myeloid leukemia) but also included 8.4% of patients with essential thrombocythemia and 11.5% of those with polycythemia vera. Patients were at increased risk for transformation to myelofibrosis (hazard ratio vs. the *JAK2*-heterozygous subgroup, 5.4; 95% CI, 2.7 to 11.0; P<0.001) and shorter event-free survival, regardless of myeloproliferative neoplasm subtype or phenotypic driver mutation (hazard ratio for disease progression or death, 2.6; 95% CI, 2.1 to 3.2; P<0.001).

Patients who were not identified in the above two subgroups were classified according to their dominant myeloproliferative neoplasm phenotypic driver mutation. Patients with *CALR* mutations, which co-occurred with LOH at chromosome 19p and with deletion at chromosome 20q, or those with *MPL* mutations all presented with essential thrombocythemia or myelofibrosis. Patients with *MPL*-mutated myelofibrosis had an elevated rate of acute myeloid leukemia transformation (hazard ratio vs. the *JAK2*-heterozygous subgroup, 8.6; 95% CI, 1.4 to 49.1; P=0.02), but otherwise the two subgroups had a clinical course that was similar to that in the *JAK2* subgroups. Patients with *JAK2* V617F heterozygosity constituted most of the patients with *JAK2*-

Figure 3 (facing page). Genomic Subgroups in MPN and Phenotypic Characteristics.

According to a Bayesian clustering algorithm (Dirichlet process), patients could be classified into six distinct subgroups on the basis of the presence or absence of mutations and chromosomal abnormalities. The remaining patients either had no detectable genomic changes or had clonal markers that were not defining for one of the six groups. The flowchart shows the logic that allows patients to be classified into the total of eight groups. Proportions of patients with essential thrombocythemia (ET), polycythemia vera (PV), myelofibrosis (MF, either primary or after chronic-phase disease), or other MPN diagnoses are shown, as are rates of overall survival and myelofibrotic or leukemic transformation among patients in the individual subgroups. The 18 genetic aberrations involved *EZH2*, *IDH1*, *IDH2*, *ASXL1*, *PHF6*, *CUX1*, *ZRSR2*, *SRSF2*, *U2AF1*, *KRAS*, *NRAS*, *GNAS*, *CBL*, Chr7/7qLOH, Chr4qLOH, *RUNX1*, *STAG2*, and *BCOR*. Patients who had more than one mutation across *JAK2*, *CALR*, and *MPL* and deletion at chromosome 20q could belong to more than one classification. In patients who had myeloproliferation with other driver mutations, other diagnoses should be considered, depending on the nature of the genetic aberration. Chromosome 9pLOH was judged to be present if detectable at a 10% clonal fraction. The number of asterisks indicates the P value (*P<0.05, **P<0.01, and ***P<0.001) for the comparison with patients with MPN with heterozygous *JAK2* mutation.


Start
TP53, Chr17pLOH, or Chr5−/Chr5q−
Yes
No
1 or more of 18 genetic aberrations
Yes
No
JAK2, CALR, MPL, or 20q
CALR or Chr20q−
MPL
JAK2
Chr9pLOH or NFE2
Yes
No
No
Other clonal driver
No
Yes
Myeloproliferation with other driver mutation
Genomic Classification
MPN with TP53 disruption or aneuploidy
MPN with chromatin or spliceosome mutation
MPN with CALR mutation
MPN with MPL mutation
MPN with homozygous JAK2 or NFE2 mutation
MPN with heterozygous JAK2 mutation
Myeloproliferation with no known driver mutation
Distribution of MPN Subtypes
Proportion of Subtypes
0.50
0.25
0.00
ET
PV
MF
Other
Outcomes in PV or ET
AML transformation
MF transformation
Overall survival
Proportion of Patients
1.0
0.8
0.6
0.4
0.2
0.0
Median event-free survival, 13.8 yr
12.1 yr***
18.1 yr
15.2 yr*
18.6 yr
17.4 yr
>25 yr**
***
***
***
***
**
*
Outcomes in Myelofibrosis
2.4 yr***
***
**
3.5 yr
*
18.9 yr
11.5 yr
*
13.6 yr
7.5 yr
>25 yr
0
5
10
15
20
25
Years

mutated essential thrombocythemia but also some of the patients with polycythemia vera or myelofibrosis; these patients had generally favorable outcomes. The subgroup of patients with *JAK2* homozygosity was enriched for patients with *NFE2* mutations and for patients with polycythemia vera. Myelofibrosis transformations occurred more frequently in this subgroup (hazard ratio vs. the *JAK2*-heterozygous subgroup, 3.0; 95% CI, 1.3 to 6.6; P=0.007).

A seventh subgroup (36 patients [1.8%]) had identifiable driver mutations but none of the class-defining drivers identified above. This included patients with mutations in genes such as *TET2* and *DNMT3A* that are not disease-specific or with mutations in genes that have been associated with other myeloid cancers (such as *KIT* in systemic mastocytosis). The eighth subgroup (192 patients [9.4%]) had no detectable driver mutations and may have included patients with either reactive thrombocythemia or myeloproliferative neoplasms with unidentified drivers. Patients were typically young and female and had received a diagnosis of essential thrombocythemia. This subgroup had particularly benign outcomes; only 1 patient (0.5%) had myelofibrosis transformation and 2 (1%) had acute myeloid leukemia transformation during a median follow-up of 8.0 years (hazard ratio for disease progression or death vs. the *JAK2*-heterozygous subgroup, 0.56; 95% CI, 0.38 to 0.78; P=0.005).

We applied our proposed classification scheme to an external cohort of 270 patients with myeloproliferative neoplasms (137 patients with essential thrombocythemia, 14 with polycythemia vera, and 119 with myelofibrosis) that had sufficient genomic characterization so that our flowchart could be applied. The subgroup proportions were similar in the two cohorts (Fig. S7 in the Supplementary Appendix).

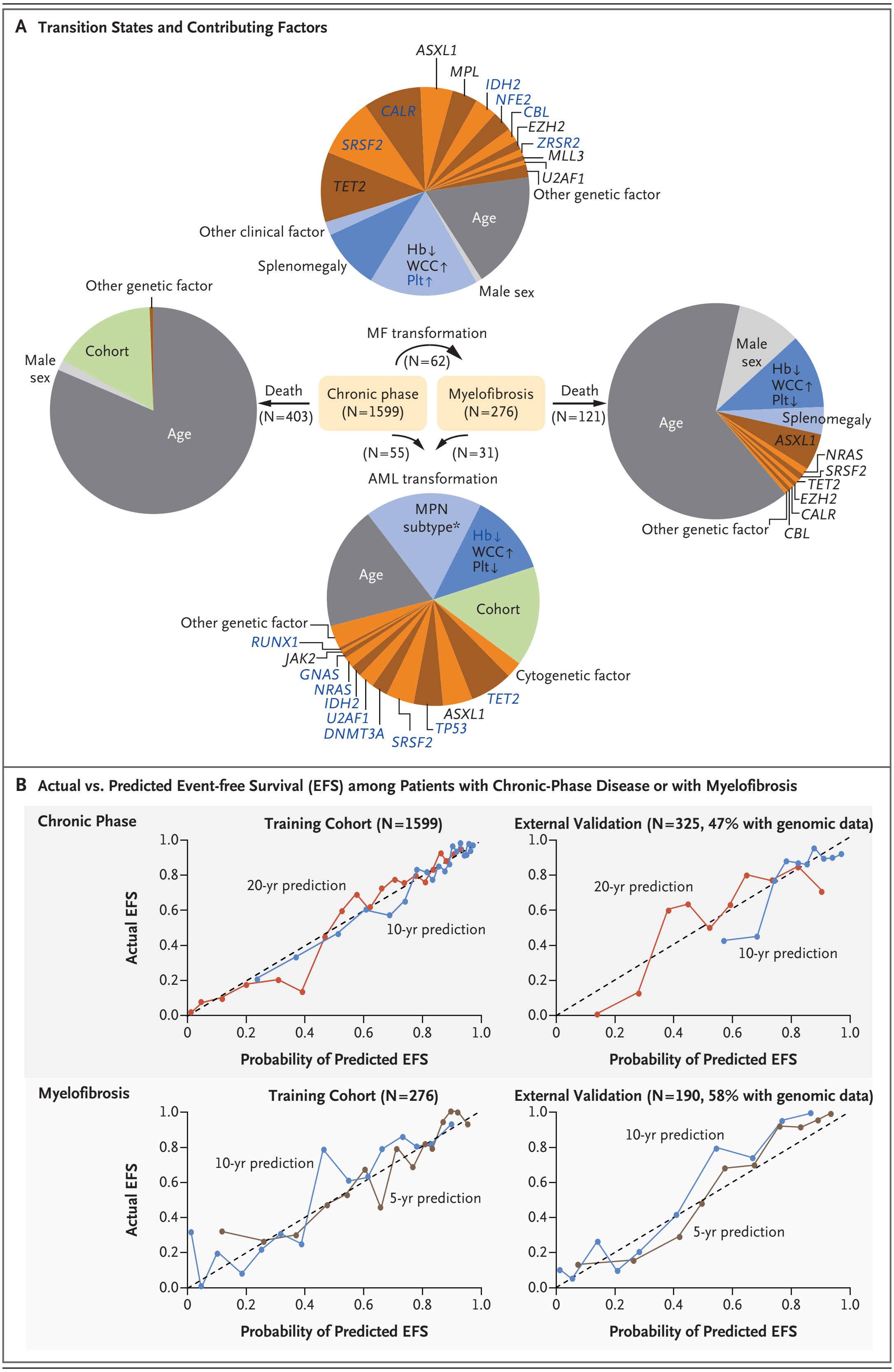
FACTORS INFLUENCING DISEASE PROGRESSION

A key determinant of the treatment of patients with myeloproliferative neoplasms is the predicted prognosis. For example, patients who are expected to have a benign future clinical course would probably benefit from treatments that are aimed at minimizing thrombotic risk, and those who are expected to have progression to leukemia or myelofibrotic bone marrow failure could be candidates for intensive therapy or clinical trials of new agents. We developed multivariate statistical models, incorporating 63 clinical and genomic variables, that estimated a patient's probability of transition between stages of disease — namely, chronic-phase disease (essential thrombocythemia or polycythemia vera), advanced-phase disease (myelofibrosis), acute myeloid leukemia, and death.

We determined the fraction of explained variation for each outcome that was attributable to different prognostic factors (Fig. 4A). Death in

Figure 4 (facing page). Modeling Outcome in Patients.

Panel A shows the transition states during a patient's disease and the factors contributing to the risk of each transition. Patients may have presented with either chronic-phase disease (polycythemia vera, essential thrombocythemia, or unclassifiable MPN) or myelofibrosis (MF), as represented by the two central, rounded rectangles. The patient may have subsequently remained alive in these disease states or, alternatively, could have transitioned to one of four states: death in chronic-phase disease, death in MF, MF transformation of chronic-phase disease, and acute myeloid leukemia (AML) transformation of either chronic-phase disease or MF. Individual models were created for each of these four disease-state transitions and combined into a single multistate model allowing for the prediction of probability of being in each disease state occurring at any time point in the future (up to 25 years after diagnosis), as calculated on an individual patient basis. Pie charts show the variables that contributed most to the predicted risk for each of the four transitions. These show the effect on disease transitions of both rare variables with a strong effect and common variables with a milder effect. Variables with a hazard ratio of more than 2.0 are shown in blue type. The numbers of patients with chronic-phase disease or MF are shown alongside the numbers of patients who transitioned to other states. Patients may have transitioned more than once during their clinical course (e.g., from chronic-phase disease to MF and then to AML). The risk of AML transformation was highest among patients with MF. WCC denotes white-cell count; the arrows by the clinical variables indicate whether the value increased (up arrow) or decreased (down arrow). Panel B shows the model predictions, as compared with the actual event-free survival (EFS), among patients. Comparisons of the actual EFS with the predicted EFS derived from multistate random-effects Cox proportional-hazards modeling for patients with chronic-phase disease and MF, for both the training cross-validation cohort and the external validation cohort, are shown. Each cohort was split into equally sized subgroups of patients, and each subgroup is represented by a data point plotted according to the observed and predicted EFS. Overall, the models show good correlation between predicted and actual outcomes for both the training and external validation cohorts at several time points (brown indicates the EFS at 5 years, blue at 10 years, and red at 20 years). The dashed line indicates points at which predicted outcomes perfectly match observed outcomes.



A Transition States and Contributing Factors

B Actual vs. Predicted Event-free Survival (EFS) among Patients with Chronic-Phase Disease or with Myelofibrosis the chronic phase was influenced predominantly by age, with genomic features having little predictive power — a finding that suggests that once cytoreduction has achieved adequate control of blood counts, causes of death are dominated by those that would also occur in the general population.[36] These would, therefore, not be well predicted by the specific genomic features of the myeloproliferative neoplasm.

By contrast, genomic features played a substantial role in predicting progression from chronic-phase disease to myelofibrosis and to acute leukemia transformation (Fig. 4A). *CALR* mutations were independently associated with an increased risk of myelofibrotic transformation, as previously reported.[37] Mutations in epigenetic regulators, splicing factors, and RAS signaling were all associated with myelofibrotic and leukemic transformation — some of these associations have been identified previously.[10-12] Whether mutations were clonal or subclonal had little effect on prognosis (see the Supplementary Appendix). Clinical features of the disease, such as anemia, splenomegaly, or thrombocytosis, still retained independent predictive power for transformation events, which suggests that these variables reflect important features of the disease state that are not captured in the genomic landscape. Outcomes in patients with myelofibrosis did not significantly differ on the basis of whether the myelofibrosis was primary or occurred after essential thrombocythemia or polycythemia vera.

PERSONALLY TAILORED PROGNOSIS

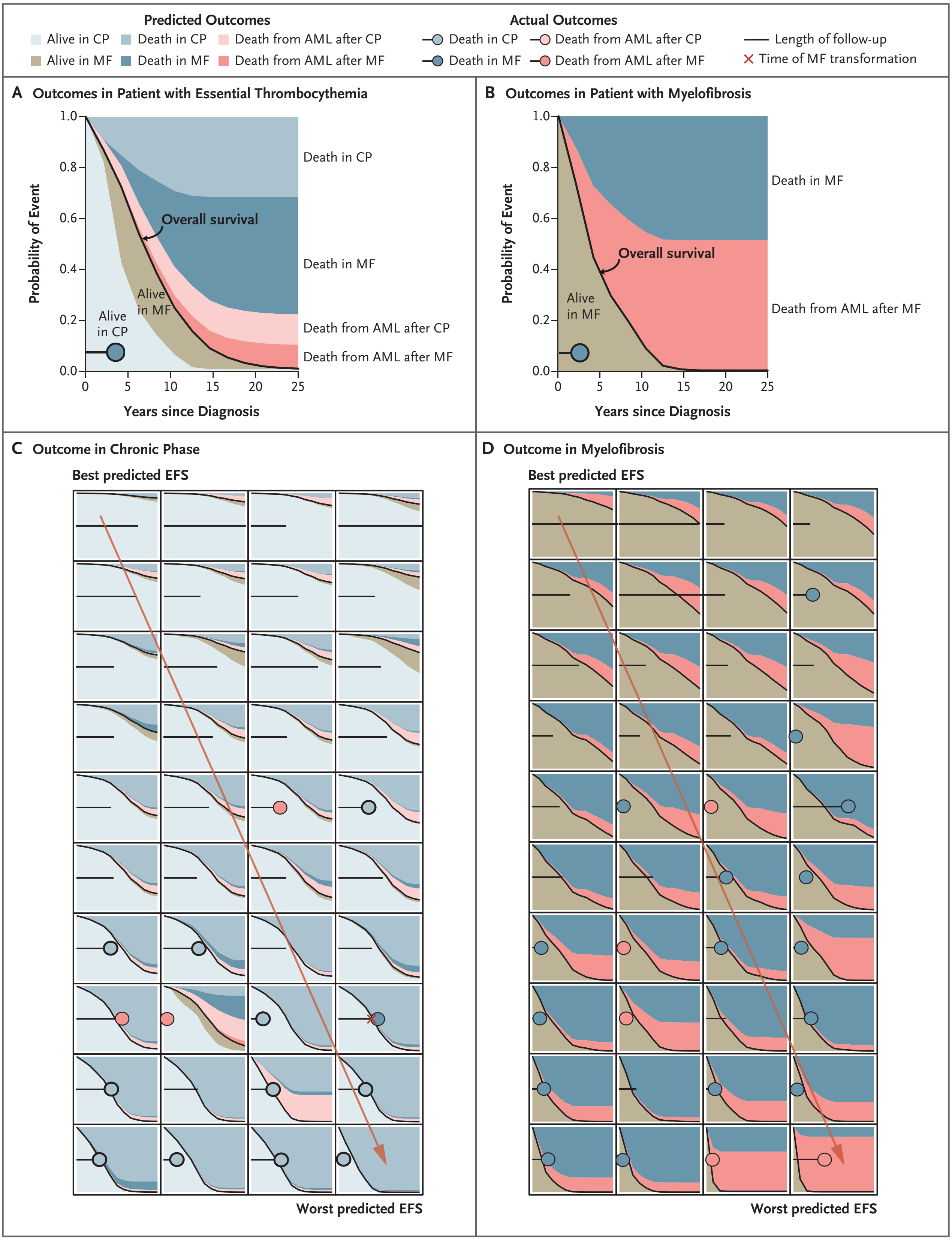
Current prognostic models for myeloproliferative neoplasms, which are focused on myelofibrosis, use simple scoring systems and group patients into broad prognostic categories. Many factors influence clinical outcomes, with a wide range of effect sizes, which means that current schemes discard information that is relevant to prognosis. We explored whether our multivariate, multistate prognostic models could generate accurate predictions for individual patients.

The usefulness of personally tailored predictions can be assessed in two ways: do the predictions usefully distinguish among patients according to prognosis, and are the predictions more informative than conventional schemas? Regarding the first question, not only is our model able to generate a wide range of specific risk predictions (regarding long-term survival, death in chronic-phase disease, and myelofibrotic and leukemic transformation) but they correlate well with observed outcomes (Figs. 4B and 5, and Fig. S8 and Tables S6 and S7 in the Supplementary Appendix), both in cross-validation of an internal cohort and in an external validation cohort of 515 patients with myeloproliferative neoplasms (137 patients with essential thrombocythemia, 188 with polycythemia vera, and 190 with myelofibrosis).

Internal cross-validation showed concordances of 76 to 86% for overall survival, event-free

Figure 5 (facing page). Personalized Predictions of Outcomes in Patients.

Panels A and B show example tiles that represent personalized predicted outcomes in individual patients. Panel A shows the predicted outcomes of a 79-year-old woman who presented with essential thrombocythemia (ET) with a hemoglobin level of 104 g per liter, a white-cell count of 8400 per cubic millimeter, and a platelet count of 2,300,000 per cubic millimeter, and mutated *CALR*, *SRSF2*, and *IDH2* along with LOH in chromosome 18q. For such a patient presenting with chronic-phase (CP) disease (PV or ET), the model incorporates all clinical, demographic, laboratory, and genomic variables to predict the overall probabilities over time of being alive in CP, dying in CP, being alive in myelofibrosis (MF) after CP, dying in MF after CP, transitioning to AML from CP, and transitioning to AML from MF after CP. The varying probabilities of each of these transitions can be judged from the vertical axis and their respective Kaplan–Meier curves over a 25-year period shown along the horizontal axis. The black curve shows the predicted Kaplan–Meier curve of overall survival. This patient transitioned to MF and died within 5 years; this outcome is shown along the bottom of the plot, where the length of the horizontal black line shows the duration of follow-up and the cause of death is indicated by the shading of the circle. For a patient who presented with MF, as shown in Panel B, the same model predicts the probabilities of being alive in MF, dying in MF, and transitioning to AML over a period of 25 years. Panel B shows the predicted and actual outcomes of a 57-year-old man with MF who had a hemoglobin level of 125 g per liter, a white-cell count of 27,000 per cubic millimeter, and a platelet count of 119,000 per cubic millimeter, and mutated *TET2*, *ASXL1*, *CBL*, and *BCOR* along with deletion in chromosomes 7q and 11q. This patient died in MF within 2 years. All patients with chronic-phase disease or MF who had either a disease event (death or disease progression) or had more than 10 years of follow-up (>5 years for patients with MF) were ranked according to their overall predicted EFS. The predicted and actual outcomes for 40 individual patients with CP (Panel C) and MF (Panel D) showing how patients in the cohort may be distinguished in terms of EFS and cause of death.


Predicted Outcomes
Alive in CP
Death in CP
Death from AML after CP
Alive in MF
Death in MF
Death from AML after MF
Actual Outcomes
Death in CP
Death from AML after CP
Death in MF
Death from AML after MF
Length of follow-up
Time of MF transformation
A Outcomes in Patient with Essential Thrombocythemia
Probability of Event
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
Years since Diagnosis
Death in CP
Overall survival
Death in MF
Alive in MF
Alive in CP
Death from AML after CP
Death from AML after MF
B Outcomes in Patient with Myelofibrosis
Probability of Event
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
Years since Diagnosis
Death in MF
Overall survival
Alive in MF
Death from AML after MF
C Outcome in Chronic Phase
Best predicted EFS
Worst predicted EFS
D Outcome in Myelofibrosis
Best predicted EFS
Worst predicted EFS

survival, and transformation to acute leukemia as well as good performance on absolute predictive accuracy (Fig. 4B, and Tables S6 and S7 in the Supplementary Appendix). Concordances were similar in the external cohort, despite the fact that patients in the external cohort received diagnoses at another center, were evaluated by different pathologists who used different diagnostic criteria, and underwent sequencing at a different facility with the use of a different gene panel from the training cohort (Fig. 4B). Thus, the model provides considerable discriminatory power that accurately generalizes to other real-world cohorts. Owing to the existence of different diagnostic criteria, the model does not rely heavily on the exact classification label of the patient's disease. Indeed, removing the distinction between polycythemia vera and essential thrombocythemia, but simply retaining the distinction between myelofibrosis and chronic-phase disease, did not reduce the predictive accuracy of the model (Fig. S9 in the Supplementary Appendix).

Our model showed superior performance to current major prognostic schemas in clinical use, such as the International Prognostic Scoring System (IPSS),[38] the Dynamic IPSS (DIPSS),[39] the high molecular risk category for myelofibrosis,[10] and the International Prognostic Score for Essential Thrombocythemia score[40] (Fig. S9 and Tables S6 and S7 in the Supplementary Appendix). Furthermore, we identified substantial heterogeneity in disease outcomes within individual prognostic categories of current prognostic schemas (shown for DIPSS in Fig. S10 in the Supplementary Appendix); this was especially prominent for intermediate-risk patients and allowed for more informative predictions in a group with otherwise uncertain outcomes. This means that not so many patients need be screened before some emerge as having an increased risk of poor outcomes; the numbers needed to test across different scenarios are shown in Table S8 in the Supplementary Appendix. The inclusion of mutations and chromosomal changes beyond *JAK2*, *CALR*, and *MPL* improved the predictive power of prognostic models (Tables S6 and S7 in the Supplementary Appendix).

We have implemented a free, user-friendly online calculator of individualized patient outcomes (https://cancer.sanger.ac.uk/mpn-multistage/) that enables the exploration of data from patients in our cohort, and the generation of new patient predictions according to available clinical, laboratory and genomic features. Further validation of our model with the use of additional cohorts of patients with myeloproliferative neoplasms will be important, given the bias toward including patients with essential thrombocythemia in this study.

DISCUSSION

A major challenge is how we use our understanding of the pathogenic complexity of myeloproliferative neoplasms to identify groups of patients with shared causative biologic factors of disease, such that existing and new therapies can be targeted to the most appropriate patients. Current classification of myeloproliferative neoplasms is hampered by disease heterogeneity within, and clinical overlap between, subtypes. A genomic classification has the virtue of identifying patients with shared causative biologic factors, is stable over time, and does not rely on blood-count thresholds for assigning particular disease labels.

Of the eight subgroups of myeloproliferative neoplasms identified, the subgroup with *TP53* mutations was genomically unstable and had poor outcomes; this same subgroup, with similar clinical implications, has been identified in acute myeloid leukemia and other hematologic cancers.[32,35] Likewise, the subgroup of myeloproliferative neoplasms with mutations in genes regulating chromatin and RNA splicing is mirrored in both the myelodysplastic syndrome[34] and acute myeloid leukemia.[32] Patients with myeloproliferative neoplasms in this group typically had myelofibrosis, although some had essential thrombocythemia or polycythemia vera, and shared a relatively poor prognosis (as seen in patients with the myelodysplastic syndrome or acute myeloid leukemia). This raises the possibility that these driver mutations define a myeloid cancer in older patients that transcends traditional diagnostic categories.

Our model accurately identified a minority of patients with chronic-phase myeloproliferative neoplasms who were at substantial risk for disease progression. Such patients could be considered for clinical trials of new therapeutic agents, since they are the most likely to benefit and the trials would be more efficient if higher-risk patients are preferentially enrolled. Our model also accurately identified the majority of patients with chronic-phase disease who seemingly had a benign outlook at diagnosis. In such patients, experimental therapy would be unnecessary, and a conservative treatment strategy that is based on cytoreduction and reduction of vascular risk will suffice to give long-term, event-free survival. Myeloproliferative neoplasms continue to evolve, however, and it would be informative to evaluate the opportunities offered by serial genomic profiling to update treatment choices if high-risk genomic changes emerge or if therapy drives further evolution.

Comprehensive gene sequencing of patients with blood cancers is becoming increasingly accessible and routine. The integration of clinical data with diagnostic genome profiling may provide prognostic predictions that are personally tailored to individual patients. Regarding patients with myeloproliferative neoplasms, such information will empower the clinician and support complex decisions around the choice and intensity of therapy, recruitment into clinical trials, and long-term clinical outlook.

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APPENDIX

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