Classification and Personalized Prognosis in Myeloproliferative Neoplasms


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Classification and Personalized Prognosis in Myeloproliferative Neoplasms


BACKGROUND
Myeloproliferative neoplasms, such as polycythemia vera, essential thrombocytemia, 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 thrombocytemia 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. (Fundied by the Wellcome Trust and others.)
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eyeloproliferative 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. 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, but uncertainty clouds where and how to draw dividing lines among them.

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. Driver mutations correlate with phenotype and prognosis, and mutation order can also influence phenotype. 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. Driver mutations are increasingly important in predicting clinical outcomes, but large, well-characterized cohorts are necessary for accurate prognostic models. Studies have suggested that this promise extends to myeloproliferative neoplasms, 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). Additional patients underwent whole-exome sequencing, as reported previously.

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. 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. 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,
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 polyclthemia 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 S515 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.

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 JAK226–28); JAK2 R867 in 2 patients with essential thrombocythemia (associated with familial thrombocythemia29); 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
**Classification and Prognosis in Myeloproliferative Neoplasms**

**A Recurrently Mutated Genes and Chromosomal Abnormalities**

**B PPM1D Mutations**

**C Clonal Structures of PPM1D Mutations**

**D Noncanonical Mutations of JAK2 and MPL**

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**Legend:**
- Missense
- In frame
- Frameshift (+1 bp)
- Frameshift (+2 bp)
- Gain
- LOH
- Loss
- Nonsense
- Splice or other

**Noncanonical Mutations of JAK2 and MPL**

- PV
- ET
- MF
- JAK2 V617F
- CALR mutated
- MPL mutated
- Triple negative

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**Reported gain of function**

**Somatic acquisition shown**

**Reported in familial thrombocytosis**

**Associated with uniparental disomy**

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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, 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, 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, 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. 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 SF3BJ and...
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

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B Frequency of Driver Mutations or Chromosomal Changes According to Age at Diagnosis

JAK2, CALR, or MPL

Calibration and Prognosis in Myeloproliferative Neoplasms

C Associations between Genetic or Demographic Features, ET vs. PV

Higher Odds of ET

- Chr9pLOH
- JAK2 clone >50%
- NFE2
- Age >60 yr
- Male sex
- rs12340895 (G)
- rs10758658 (A)
- rs11042125 (T)
- rs409801 (C)
- rs2236496 (C)

Higher Odds of PV

- <0.001
- <0.001
- 0.004
- 0.006
- <0.001
- 0.47
- 0.001
- 0.09

Odds Ratio for Polycythemia Vera

D Associations between Genetic or Demographic Features, CP vs. MF

Higher Odds of CP

- NRAS
- ZR5R2
- Chr7q
- U2AF1
- SRSF2
- EZH2
- ASXL1
- CBL
- Chr1p
- JAK2 clone >50%
- Chr9p
- Age >60 yr
- Male sex
- rs11104870 (T)
- rs13219787 (A)

Higher Odds of MF

- <0.001
- 0.004
- <0.001
- 0.004
- 0.09
- <0.001
- 0.11
- <0.001
- <0.001
- 0.064
- 0.02
- 0.006
- <0.001
- 0.008

Odds Ratio for Myelofibrosis

E Log Odds of Gene Occurring 2nd in Gene Pair

Least Likely

- MPL
- CALR
- SH2B3
- SF3B1
- CUX1

Most Likely

- DNM31A
- JAK2
- U2AF1
- IDH1
- SRSF2
- TET2
- RUNX1
- EZH2
- ASXL1
- NFI
- TP53
- IDH2
- CBL
- PPM1D
- NFE2
- KRAS
- NRAS
- PTPN11
DNMT3A are also associated with age-related clonal hematopoiesis,\textsuperscript{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 thrombocytopenia 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.\textsuperscript{13,14}

**GENOMIC SUBGROUPS IN MYELOPROLIFERATIVE NEOPLASMS**

Hematologic cancers may be subclassified according to driver mutations that distinguish subgroups of patients,\textsuperscript{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,\textsuperscript{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 vs. the JAK2-heterozygous subgroup, 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 thrombocytopenia 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 thrombocytopenia 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-
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 thrombocytemia 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...
### A Transition States and Contributing Factors

- **Chronic Phase** (N=1599) -> **Myelofibrosis** (N=276) -> **Death** (N=403)

- **MF transformation** (N=62) -> **AML transformation** (N=31)

- **Other clinical factor**
  - Age
  - Male sex

- **Cytogenetic factor**
  - **ASXL1**
  - **DNMT3A**
  - **U2AF1**

- **Other genetic factor**
  - **JAK2**
  - **CALR**

### B Actual vs. Predicted Event-free Survival (EFS) among Patients with Chronic-Phase Disease or with Myelofibrosis

#### Chronic Phase
- **Training Cohort** (N=1599)
  - 20-yr prediction
  - 10-yr prediction

- **External Validation** (N=325, 47% with genomic data)
  - 20-yr prediction
  - 10-yr prediction

#### Myelofibrosis
- **Training Cohort** (N=276)
  - 10-yr prediction
  - 5-yr prediction

- **External Validation** (N=190, 58% with genomic data)
  - 10-yr prediction
  - 5-yr prediction
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. 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. 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. 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
Classification and Prognosis in Myeloproliferative Neoplasms

Overall survival

Worst predicted EFS

Best predicted EFS

Death in MF

Alive in MF

Death from AML after MF

Death in MF

Death from AML after MF

Alive in CP

Death in CP

Death from AML after CP

Alive in CP

Death from AML after CP

Years since Diagnosis

Probability of Event

1.0

0.8

0.6

0.4

0.2

0.0

0

5

10

15

20

25

A Outcomes in Patient with Essential Thrombocytemia

B Outcomes in Patient with Myelofibrosis

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 thrombocytopenia, but simply retaining the distinction between polycythemia vera and essential thrombocytopenia, 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), the Dynamic IPSS (DIPSS), the high molecular risk category for myelofibrosis, and the International Prognostic Score for Essential Thrombocytopenia score (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/mypn-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 thrombocytopenia 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. Likewise, the subgroup of myeloproliferative neoplasms with mutations in genes regulating chromatin and RNA splicing is mirrored in both the myelodysplastic syndrome and acute myeloid leukemia. Patients with myeloproliferative neoplasms in this group typically had myelofibrosis, although some had essential thrombocytopenia 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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