Disease heterogeneity and personalized prognosis in myeloproliferative neoplasms

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ABSTRACT (250 words)

BACKGROUND. Myeloproliferative neoplasms (MPN), comprising polycythemia vera, essential thrombocythemia and myelofibrosis, are chronic hematological malignancies with variable progression rates. Genomic characterization of MPN patients offers the potential for personalised diagnosis, risk stratification and management.

METHODS. We sequenced coding exons from 69 myeloid cancer genes in 2035 MPN patients, comprehensively annotating driver mutations and copy number changes. We developed a genomic classification for MPNs and multistage prognostic models for predicting individual patient outcomes. Classification and prognostic models were validated on an external cohort.

RESULTS. 33 genes carried driver mutations in >4 patients, with *JAK2*, *CALR* or *MPL* mutations being the sole abnormality in 45% patients. The number of driver mutations increased with age and advanced disease. Driver mutations, germline polymorphisms and demographic variables independently predicted whether patients were diagnosed with essential thrombocythemia versus polycythemia vera, and chronic phase disease versus myelofibrosis. We defined 8 genomic subgroups, exhibiting distinct clinical phenotypes, including diagnostic 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 chronic phase MPN or myelofibrosis. Predicted and observed outcomes correlated well using internal cross-validation and an independent external cohort. Even within individual categories of existing prognostic schemas, our models substantially improved predictive accuracy.

CONCLUSIONS. Comprehensive genomic characterization identifies distinct genetic subgroups and provides an MPN classification based on causal biological mechanisms. Integration of genomic data with clinical parameters enables personalised predictions of patient outcome and will support management of MPN patients.

INTRODUCTION

The myeloproliferative neoplasms (MPNs) are clonal hematopoietic disorders comprising polycythemia vera (PV), characterized by red blood cell over-production; essential thrombocythemia (ET), with elevated platelet counts; and myelofibrosis (MF), defined by bone marrow fibrosis[1]. PV and ET are chronic phase MPNs, while MF represents advanced disease, diagnosed either *de novo* or following ET or PV. Current classification schemes distinguish between MPN subtypes using clinical and laboratory features[2–5], but there is uncertainty and controversy over where and how to draw dividing lines between them[6,7]. This debate is not easily resolved since MPNs exist on a phenotypic continuum, with overlapping distributions of hemoglobin levels, platelet counts and extent of marrow fibrosis.

Biologically, MPNs are driven by cardinal driver mutations in *JAK2*, *CALR* or *MPL*. Many patients have additional drivers spanning a wide range of cancer genes, with patient-to-patient variability in the genetic and clonal landscape[8,9]. Driver mutations correlate with phenotype and prognosis[10–12], and mutation order can also influence disease phenotype[13,14]. This complex genetic landscape likely contributes to heterogeneity in diagnostic features and outcomes in MPNs.

In blood cancers, there has been a progressive shift away from clinical and morphological classification schemes to those based on genomics[15], because such categorization relies on causative disease biology. Driver mutations are increasingly important in predicting clinical outcomes, but large, well-characterized cohorts are necessary for accurate prognostic models[16]. Recent studies have indicated this promise extends to MPNs[10,17], but require larger cohorts and comprehensive gene sequencing to provide definitive answers. We report on a cohort of 2035 patients with long-term follow-up data, sequenced for coding mutations in known myeloid cancer genes, copy number changes and germline polymorphisms.

METHODS

Study samples

Patient samples were obtained following written informed consent and ethics approval. Cohort, disease classification, and diagnostic review details are provided in the supplementary appendix. 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. The external validation cohort comprised 515 patients. We use the term 'myelofibrosis' to encompass both primary MF and post-ET/PV MF.

Sequencing and analyses

Custom RNA bait hybridisation capture for the full coding sequence of 69 genes, genome-wide single nucleotide polymorphisms (SNPs) for copy-number profiling, and germline loci associated with MPN or red cell variation[18–20] (Tables S1-S2) was undertaken in 1887 patients. 148 patients underwent whole-exome sequencing, as reported previously[8]. Further details are provided in the supplementary appendix.

Clinical variables

Baseline laboratory and clinical data from diagnosis were incorporated into prognostic models as detailed in the supplementary appendix. The median duration between diagnosis and sample acquisition was 49 days. Median follow-up was 93.8 months (range 0.03-523) from diagnosis and 72.0 months (range 0.03-360) from time of DNA sampling.

Statistics

Timing of mutation acquisition used Bradley-Terry modelling of pairwise comparisons of clonal fractions in individual patients[13]. Bayesian network analysis and Dirichlet processes identified genetic associations and subgroups. Random-effects Cox proportional hazards multistate modelling was used for outcome prediction, as detailed in the supplementary appendix.

Study conduct

JG and JN gathered and analysed data in collaboration with coauthors, and together with ARG and PJC designed the study and wrote the paper including the first draft. All authors vouch for the data, analyses and publication.

RESULTS

Spectrum of genomic changes in MPNs

The cohort of 2035 patients comprised 1321, 356 and 309 patients with ET, PV and MF respectively and 49 patients with other MPN diagnoses (Table S3). 33 genes carried driver mutations in ≥5 patients (Fig.1A; Tables S4-S5). *JAK2*, *MPL* and *CALR* accounted for 1831 driver mutations, compared to 1075 across other genes. Loss of heterozygosity (LOH) was frequent for *JAK2*^{V617F}, especially in PV, but was infrequent for *CALR* and *MPL* (Fig.S1).

We identified 45 truncating mutations in the terminal exon of *PPM1D* in 38 patients (1.5%, Fig.1B), making *PPM1D* the 8th most commonly mutated gene in MPNs. These mutations have also been detected in solid tumors, and blood from both healthy individuals and patients

with breast/ovarian tumors, often after chemotherapy[21,22]. In our cohort, 10 patients had *PPM1D* mutations emerge during treatment with hydroxycarbamide, having not been present in an earlier sample. However, *PPM1D* mutations were also detected at, or within a month of, diagnosis in 20 cases. Analysis of single-cell derived hematopoietic colonies identified mutated-*PPM1D* in a 'triple-negative' (unmutated-*JAK2*, -*CALR* or -*MPL*) ET patient, but also subclonal to *JAK2*^{V617F} in a PV patient (Fig.1C). These data confirm that *PPM1D* mutations can occur within the MPN clone and be present at diagnosis, not always indicating age-related clonal hematopoiesis or therapy-related disease evolution.

Mutations in *MLL3* (Fig.1A, Table S4) were detected in 20 patients (1%), and were predominantly nonsense or frameshift as reported in AML[23]. Interestingly, seven had triple-negative MPN, suggesting that *MLL3* could be an important tumor suppressor gene in these patients.

There has been interest in whether mutations in *JAK2* and *MPL* outside of known hotspots could be relevant to MPNs[24,25]. We identified non-canonical variants in *JAK2* and *MPL* in 16 patients with triple-negative ET and 1 patient with triple-negative MF (Fig.1D). Of these, three groups of variants are likely relevant to disease pathogenesis: (i) *JAK2*^{R683G} and *JAK2*^{E627A}, reported in acute lymphoblastic leukemia where they result in constitutive JAK2 activation[26–28], were identified in two ET patients, one of whom presented in childhood; (ii) *JAK2*^{R867} was mutated in 2 ET patients and is associated with familial thrombocytosis[29]; (iii) *MPL*^{S505N} and *MPL*^{S204P} were identified in 4 and 5 ET patients respectively[24]. *MPL*^{S204P} co-occurred with 1p-LOH, suggesting a clonal advantage to acquired homozygosity for this variant.

Factors influencing classification into ET, PV or MF

Currently, patients with MPNs are classified as ET, PV or MF based on clinical and laboratory criteria[2–5], but the biology underlying these distinctions is incompletely understood. The number of driver mutations per patient was higher in MF than PV or ET (Fig.2A), as previously reported[8], and increased with age of the patient (Fig.2B).

The distinction between *JAK2*^{V617F}-mutated ET and PV rests on whether red cell mass or hematocrit is elevated. We found that acquired driver mutations correlated with hematological parameters (Fig.S2) and were the strongest determinants of a *JAK2*-mutated chronic phase patient being labeled as ET or PV, although germline genetic background and demographic factors were also relevant (Fig.2C,S2). 9p-LOH, causing *JAK2*^{V617F} homozygosity, or a high *JAK2*^{V617F} allele burden predicted a PV phenotype, as did mutated *NFE2*, a transcription factor critical to erythroid differentiation. Germline polymorphisms associated with red cell

variables in the general population were distributed unevenly between ET and PV, with alleles associated with lower hemoglobin and higher platelets enriched in ET (Fig.2C). Furthermore, the *JAK2* 46/1 haplotype, associated with increased predisposition to MPNs[18], predicted for PV (OR 2.3; $CI_{95\%}$ 1.7-3.3; p<0.001), partly through increased odds of *JAK2*^{V617F} homozygosity via 9p-LOH (OR 2.7; $CI_{95\%}$ 2.0-3.9; p<0.001). Older age and male sex also increased the odds of PV. These data show that the location of any chronic phase patient on the hemoglobin/red cell mass continuum is influenced by many factors, and that the use of any arbitrary threshold to label patients as ET or PV will fail to discriminate between patients with different underlying biological mechanisms.

Mutations in spliceosome components, epigenetic regulators and the RAS pathway were the strongest predictors of accelerated phase (MF) versus chronic phase (ET or PV) disease, as were male sex, older age and germline loci associated with platelet count and red cell parameters within the normal population (Fig.2D).

The order in which mutations are acquired in MPNs has previously been shown to influence disease phenotype[13,14]. *CALR* and *MPL* mutations were more commonly early events, while mutations including *NRAS*, *TP53*, *PPM1D* and *NFE2* were acquired significantly later in disease (Fig.2E,S3). Some of the earlier-occurring mutations in genes such as *SF3B1* and *DNMT3A*, are also associated with age-related clonal hematopoiesis[30,31], suggesting that some MPNs could arise from an antecedent asymptomatic clone. In patients with multiple mutations, *JAK2*^{V617F} was more commonly a secondary event in patients with ET, and an earlier event in those with PV or MF (Fig.S4,S5), confirming and generalizing observations previously shown for *JAK2* relative to *TET2* or *DNMT3A*[13,14].

Genomic subgroups in MPN

Hematological malignancies may be subclassified using driver mutations that distinguish subgroups of patients[32,33], by observing which pairs of genes are either mutually exclusive or co-mutated more frequently than expected. In our cohort, driver mutations showed complex patterns of assortment (Fig.S6). We used Bayesian modelling to identify genomic subgroups of MPNs with maximum within-group similarity and maximum between-group discrimination.

We identified 8 genomic subgroups in MPNs defined by simple rules, with high reproducibility and low ambiguity in classification of individual patients (Fig.3,S7). *TP53* mutations, co-occurring with 17p aberrations and del(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 MPN driver. Mirroring other blood cancers with *TP53* mutations[32,34], these patients have a dismal prognosis with a high risk of AML transformation (Hazard Ratio (HR) 15.5, $CI_{95\%}$ 7.5-31.4, p<0.001; HRs expressed relative to *JAK2*-heterozygous subgroup) and early death (HR 2.4, $CI_{95\%}$ 1.6-3.6, p<0.001, Fig.3).

The second subgroup was defined by the presence of one or more mutations in 16 myeloid cancer genes, especially chromatin and spliceosome regulators, chr4q-LOH and 7/7q aberrations. This subgroup was enriched for patients with MF (OR 6.52, $CI_{95\%}$ 4.9-8.7, p<0.001) and MPN/MDS overlap (including all 7 CMML/atypical CML cases), but also included 8.4% of ET and 11.5% of PV. Patients showed increased risk of MF transformation (HR 5.4, $CI_{95\%}$ 2.7-11.0, p<0.001) and inferior event-free survival (EFS), regardless of MPN subtype or MPN phenotypic driver mutation (HR 2.6, $CI_{95\%}$ 2.1-3.2, p<0.001). Patients with co-operating mutations in epigenome and splicing regulators have also been identified in MDS[35] and AML[32], suggesting that these genes identify groups of patients spanning traditional myeloid disease categories.

Patients not identified in the above 2 subgroups are classified by their dominant MPN phenotypic driver mutation. Patients with *CALR* mutations, significantly associated with 19p-LOH and del(20q), or those with *MPL* mutations, universally presented with ET or MF. Those with *MPL*-mutated MF showed an elevated rate of AML transformation (HR 8.6, $CI_{95\%}$ 1.4-49.1, p=0.02), but otherwise these two subgroups showed similar clinical course to the *JAK2* subgroups. Those with *JAK2*^{V617F} heterozygosity comprised most of the *JAK2*-mutated ET patients, but also some PV and MF patients, and had generally favorable outcomes. The *JAK2*^{V617F} homozygosity subgroup was enriched for *NFE2* mutations and patients with PV. MF transformations occurred more frequently in this subgroup (HR 3.0, $CI_{95\%}$ 1.3-6.6, p=0.007).

A seventh subgroup (36 patients; 1.8%) had identifiable driver mutations, but not one of the class-defining drivers identified above. These included patients with mutations in genes such as *TET2* and *DNMT3A*, that are not disease-specific, and those with mutations associated with other myeloid malignancies (such as *KIT* in systemic mastocytosis). The eighth subgroup (192 patients; 9.4%) had no detectable driver mutations and may include patients with either MPNs carrying unidentified drivers or reactive thrombocytosis. Patients were typically young and female, with a diagnosis of ET. This subgroup had a particularly benign outcome, with only 1 case of MF transformation (0.5%) and 2 of AML transformation (1%) during median follow-up of 8.0 years (HR for EFS: 0.56, $CI_{95\%}$ 0.38-0.78, p=0.005).

We applied our proposed classification scheme to an external cohort of 270 MPN patients (137 ET, 14 PV and 119 MF) that had sufficient genomic characterization to apply our flow-chart. Similar subgroup proportions were observed in the two cohorts (Fig.S7).

Factors influencing disease progression in MPNs

A key determinant of the management of MPN patients is predicted prognosis. Patients expected to have a benign future clinical course should have treatments aimed at minimizing thrombotic risk; those expected to progress to leukemia or myelofibrotic bone marrow failure may be candidates for intensive therapy or clinical trials of novel agents. To explore which variables predict disease progression, we developed a multivariate statistical model that estimates a patient's probability of transition between stages of disease, namely chronic phase (ET or PV), accelerated phase (MF), AML and death.

We determined the fraction of explained variability for each outcome attributable to different prognostic factors (Fig.4A). Death in chronic phase was predominantly influenced by age, with genomic features having little predictive power suggesting 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 MPN.

By contrast, genomic features played a substantial role in predicting progression from chronic phase to MF, and AML transformation (Fig.4A). *CALR* mutations were independently associated with increased risk of myelofibrotic transformation, as previously reported[37]. Mutations in epigenetic regulators, splicing factors and RAS-signaling were all predictive of myelofibrotic and leukemic transformation – some, but not all, of these associations have been identified previously[10–12]. Whether mutations were clonal or subclonal had little impact on prognosis (Supplementary Appendix). Clinical features of the disease, such as anemia, splenomegaly or thrombocytosis, still retained independent predictive power for transformation events suggesting that these variables reflect important features of disease state not captured in the genomic landscape. Outcomes in MF did not significantly differ whether the MF was primary or followed antecedent ET or PV.

Personally tailored prognosis in MPN patients

Current prognostic models for MPNs, focused on MF, use simple scoring systems, grouping patients into broad prognostic categories. As shown above, many factors influence clinical outcomes, with a wide range of effect sizes, meaning that current schemes discard information that is relevant to prognosis. We therefore explored whether our multivariate, multistate prognostic models could be used for individual patient predictions.

The utility of personally tailored predictions can be assessed twofold – do they usefully discriminate between patients 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 predictions (from long-term survival, death in chronic phase, myelofibrotic and leukemic transformation), these correlate well with observed outcomes (Fig.4B, 5, S8. Tables S6-S7), both on internal cross-validation as well as for an externally characterized cohort of 515 MPN patients (137 ET, 188 PV and 190 MF). Internal cross-validation demonstrated concordances of 75%-84% for overall survival (OS), event-free survival (EFS, Fig.4B) and AML transformation, and good performance on absolute predictive accuracy (Tables S6-S7). Concordances were similar for the external validation cohort, despite the external cohort being diagnosed at another center, evaluated by different pathologists using different diagnostic criteria, and sequenced in a different facility using 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. Due to the existence of different diagnostic criteria, the model is not heavily reliant on the exact classification label of the patient. Indeed, removing the distinction between PV and ET, but simply retaining MF versus chronic phase disease, did not reduce the predictive accuracy of the model (Fig.S9).

Our model demonstrated superior performance compared to current major prognostic schemas in clinical use – IPSS[38], DIPSS[39] and High Molecular Risk[10] for MF, and the IPSET score for ET[40] (Fig.S9, Tables.S6,S7). Furthermore, we identified substantial heterogeneity in disease outcomes within individual prognostic categories of current prognostic schemas (shown for DIPSS, Fig.S10); this was especially prominent for 'intermediate risk' patients allowing 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 increased risk of poor outcomes ("numbers needed to test" across different scenarios in Table.S8). Inclusion of mutations and chromosomal changes beyond *JAK2*/*CALR*/*MPL* improved the predictive power of our prognostic models by up to 12% as measured by Brier scores and model concordance.

We have implemented a user-friendly calculator of individualized patient outcome online (https://jg738.shinyapps.io/mpn_app/) enabling exploration of patients in our cohort, and the generation of new patient predictions using available clinical, laboratory and genomic features. Further validation of our model using additional MPN cohorts will be important, given the bias towards ET patients in this study.

Discussion

A major challenge is how we use our emerging understanding of the pathogenic complexity of MPNs to identify groups of patients with shared disease biology, such that existing and novel therapies can be better targeted to the most appropriate individuals. Current classification of MPNs suffers from disease heterogeneity within, and clinical overlap between, subtypes. A genomic classification has the virtue of identifying patients with shared causative disease biology, is stable over time, and does not rely on blood count thresholds for the assignment of particular disease labels.

Of 8 MPN subgroups identified, the *TP53*-mutated group were genomically unstable and had poor outcomes – this same subgroup, with similar clinical implications, has been identified in AML and other hematological malignancies[32,34]. Likewise, the subgroup of MPNs with mutations in genes regulating chromatin and RNA splicing is mirrored in both MDS[35] and AML[32]. In MPNs, these patients typically have myelofibrosis, although some have ET or PV, and have a relatively poor prognosis. Similar poor outcomes for chromatin/spliceosome subgroups are seen in MDS and AML. This raises the intriguing possibility that these driver mutations define a myeloid cancer of older patients that transcends traditional diagnostic categories.

Our model accurately identifies a minority of chronic phase MPN patients for whom there is substantial risk of disease progression. These patients should be the cohort targeted in clinical trials of novel therapeutic agents since they are the most likely to benefit and the trials will be more efficient if higher-risk patients are preferentially enrolled. Our model can also accurately identify the majority of chronic phase MPN patients who seemingly have a benign outlook at diagnosis. For these patients, experimental therapy would be unnecessary, and a conservative management strategy based on cytoreduction and reduction of vascular risk will suffice to give long-term, event-free survival. MPNs do continue to evolve, however, and it would be an interesting extension of this study 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. Integration of clinical data with diagnostic genome profiling will provide prognostic predictions personally tailored to individual patients. In MPNs, this 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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Figure Legends

Figure 1. Genomic landscape of myeloproliferative neoplasms. (A) Frequency of recurrently mutated genes and chromosomal abnormalities in the cohort. Mutations are stratified according to type, namely missense, nonsense, affecting a splice site or other (eg stop-gain/loss etc). Insertions and deletions are categorised by whether they resulted in a shift in the codon reading frame, by either 1 or 2 base pairs, or were in-frame. Chromosomal gains include whole-chromosome gains (trisomy) and sub-chromosomal amplifications. Chromosomal losses include whole-chromosome deletions (monosomy) and sub-chromosomal deletions. *Loss of heterozygosity (LOH) was predominantly copy number neutral, but in some cases, chromosome losses could not be excluded. **(B)** Site within the gene and protein consequence of *PPM1D* mutations are illustrated. PP2C, Protein phosphatase 2C domain **(C)** Clonal structures of two *PPM1D*-mutated patients determined by genotyping of hematopoietic colonies (BFU-E) derived from peripheral blood mononuclear cells. Each circle represents a clone; non-*PPM1D* mutated (black); *PPM1D*-mutated (yellow). The earliest detectable clone is represented at the top of each diagram, with subsequent subclones shown below. Somatic mutations acquired in each sub-clone are indicated beside respective nodes, and represent those that are acquired in addition to mutations present in earlier subclones. ET, Essential thrombocythemia; PV, Polycythemia vera **(D)** Site within the gene and protein consequence of non-canonical mutations of *JAK2* and *MPL* are illustrated. V617F and exon 12 mutations in *JAK2*, and W515 mutations in *MPL* are not shown. Colored shapes represent the characteristics of the patient carrying the specific mutation (shape, MPN subtype; color, phenotypic driver). Mutations highlighted in red are likely to be relevant to disease pathology and where previous studies have demonstrated somatic acquisition, familial inheritance or functional consequences for the specific variants.

Figure 2. Factors affecting disease classification at presentation and timing of somatic mutations. Histogram showing the frequency of driver mutations and/or chromosomal changes (gains, losses, or LOH) identified in **(A)** the different molecular subgroups of MPN (excluding 24 patients with >1 detectable phenotypic driver mutation), and **(B)** according to patient age at diagnosis. ET, Essential thrombocythemia; PV, Polycythemia vera; MF, Myelofibrosis. **(C-D)** Forest plots showing the associations between genetic or demographic features and presentation with ET versus PV in *JAK2*^{V617F}-mutated patients **(C),** and presentation in chronic phase versus MF across *JAK2*-, *CALR*-, or *MPL*-mutated patients **(D).** Significant associations

from univariate analyses after correction for multiple hypothesis testing are shown. p-values are derived from logistic regression modelling, identifying independent associations. **(E)** Of 671 patients that harbored more than one somatic mutation, the order of mutation acquisition of at least one pair of mutations was determined in 271 patients (40%). These ordered pairings were used to determine the relative probabilities of occurring first or second for a given pairing using Bradley-Terry modelling, providing 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, compared to *JAK2*, *PPM1D* mutations have a log odds of 1.45, and therefore are $e^{1.25}$=4.3 times more likely to occur secondary to *JAK2*. 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.

Figure 3. **Genomic sub-groups in MPN and phenotypic characteristics.** Using a Bayesian clustering algorithm (Dirichlet process), patients could be classified into 6 distinct subgroups based on the presence or absence of mutations and chromosomal abnormalities. Remaining patients either had no detectable genomic changes or had clonal markers that were not defining for one of the 6 groups. The flowchart shows the logic allowing patients to be classified into the total of 8 groups. Proportions of patients with essential thrombocytosis (ET), polycythemia vera (PV), myelofibrosis (primary or secondary MF) or other MPN diagnoses are shown, as well as rates of overall survival and myelofibrotic or leukemic transformation for patients within the individual sub-groups. [^^]Patients that have more than one mutation across *JAK2*, *CALR* or 20q-, and *MPL* can belong to more than one classification. [$] at least a 10% clone, consider other diagnoses in such patients depending on the nature of the genetic aberration.

Figure 4. Modelling outcome in patients. (A) Model predictions versus actual event free survival in patients. Comparisons of the actual event-free survival (EFS) versus the predicted EFS derived from multistate random-effects Cox proportional hazards modelling for patients in chronic phase (CP) and myelofibrosis (MF) patients, for both the training and external validation cohorts, are shown. Each cohort was split into equally sized subgroups of patients, and each of these subgroups is represented by a data point plotted according to the observed and predicted EFS, overall showing good correlation between predicted and actual outcomes for both training and external validation cohorts at several different timepoints (brown, 5 year EFS; blue 10 year EFS; red, 20 year EFS). **(B) Transition states during a patient's disease and the factors contributing to the risk of each transition.** Patients may present in either chronic phase (CP, comprising patients with PV, ET or MPNu) or myelofibrosis (MF), as represented by the two

central red rounded rectangles. The patient may subsequently remain alive in these disease states, alternatively, the patient can transition to one of four states: (i) Death in CP, (ii) Death in MF, (iii) MF transformation of CP, and (iv) Acute myeloid leukemia (AML) transformation of either CP or MF. Individual models were created for each of these 4 disease-state transitions and combined into a single multistate model allowing for the prediction of probability of being each disease state occurring at any time-point in the future (up to 25yrs post diagnosis) being calculated on an individual patient basis. Pie charts show those variables that contribute the most to the predicted risk for each of the 4 transitions. These demonstrate the impact on disease transitions of both rare variables with a strong effect and common variables with a milder effect. Variables with a hazard ratio of >2.0 are written in blue letters, and those variables with hazard ratio <0.5 are written in orange letters. The number of patients presenting in CP and MF are shown in brackets alongside the numbers that transitioned to other states. Of note, patients may transition more than once during their clinical course, for example, from CP to MF, and then to AML. *Risk of AML transformation was highest for patients with MF.

Figure 5. Personalised predictions of patient outcome. Each of the tiles represents the personalised predicted outcome of an individual patient. Two tiles **(A)** and **(B)** have been enlarged for illustrative purposes. The top left panel (A) depicts the predicted outcomes of a 79 year old female patient who presented with ET with hemoglobin (Hb) 104g/l, white cell count (WCC) 8.4x10^9/l and platelet count (Plt) of 2300x10^9/l, mutations in *CALR*, *SRSF2*, *IDH2* and 18q loss of heterozygosity (LOH). For such a patient presenting in chronic phase (CP, comprising PV or ET), the model incorporates all clinical, demographic, laboratory and genomic parameters to predict the overall probabilities over time of (i) being alive in CP (grey), (ii) suffering death in CP (light blue), (iii) being alive in post-CP MF (brown), (iv) suffering death in post-CP MF (turquoise), (v) transforming to AML from CP (pink), or (vi) transforming to AML from post-CP MF (magenta). The varying probabilities of each of these transitions can be judged from the vertical axis and their respective Kaplan-Meiers over a 25 year time period shown along the horizontal axis. The labelled black curve shows the predicted Kaplan-Meier curve of overall survival. The patient in (A) transformed to myelofibrosis (MF) and died within 5yrs and this actual outcome is shown along the bottom of the plot where the length of the horizontal black line depicts the duration of follow-up, and the cause of death (if occurred during follow up) by the shading of the circle at the end of the black line.

For a patient presenting in MF, as shown in panel (B), the same model predicts the probabilities of (i) being alive in MF (brown), (ii) suffering death in MF (turquoise) or transforming to AML (magenta) over 25yrs. This tile shows the predicted and actual outcome of a 57 year old male patient diagnosed with MF with Hb 125g/l, WCC $27x10^9$/l and Plt 119 $x10^9$/l, mutated *TET2*, *ASXL1*, *CBL* and *BCOR* along with chr7q- and 11q-. This patient died in MF within 2yrs as shown along the bottom of the plot.

All patients diagnosed in chronic phase (CP, namely ET or PV) or MF, with either a disease event (death or disease progression) or >10 year follow-up (>5yrs for MF patients), were ranked by their overall predicted event free survival (EFS). The predicted and actual outcomes for 36 individual patients in CP and MF evenly spaced across this ranking are shown in panels **(C)** and **(D)** demonstrating discrimination between patients in terms of event free survival and cause of death across the cohort.

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