blood advances

TO THE EDITOR:

Hematopoietic fitness of *JAK2*^{V617F} myeloproliferative neoplasms is linked to clinical outcome

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Classical myeloproliferative neoplasms (MPNs) are chronic, phenotypically diverse malignancies[1] associated with significant morbidity, shortened survival,[2] and limited treatment options.[3] Development of life-prolonging, potentially curative drugs in MPNs has been more challenging than expected for neoplasms harboring highly recurrent driver mutations that activate targetable tyrosine kinases. Decades of monitoring may be required for analysis of the most important clinical outcomes in MPNs: thrombosis, progression, and mortality. This has prompted use of more convenient clinical trial endpoints with unclear connection to these long-term events. Short-term biomarkers linking MPN biology to event risk may help identify and develop disease-modifying agents with greatest potential to improve event-free survival (EFS). Unfortunately, no such biomarkers are presently available.

MPNs are initiated by acquisition of a driver mutation in a single hematopoietic stem cell (HSC) decades prior to clinically evident MPN.[4,5] Driver mutations in *JAK2*, *CALR*, or *MPL*[6] augment JAK/STAT cytokine signaling pathways and offer MPN stem and progenitor cells (MPN-SPCs), and their progeny, a competitive advantage to supplant their normal cellular counterparts and manifest disease. Given the centrality of driver mutations to MPN biology, *JAK2*^{V617F} mutation allele frequency (MAF) is an appealing biomarker because this mutation is present in the majority of MPNs and is easily measured in whole blood (WB). Nevertheless, this approach has been disappointing because WB MAF does not accurately distinguish clinical phenotypes or predict outcomes.[7-9] Although WB MAF $>$ 50% can identify patients with polycythemia vera (PV) at higher risk for venous thrombotic events,[10] fewer than a quarter of these patients experienced a venous thrombotic event within 20 years of measurement. Similarly, among 240 patients with available WB MAF in our large single-center PV cohort,[11,12] EFS was indistinguishable by WB MAF for 75% of patients (supplemental Figure 1A, quartiles Q1-Q3). Changes in WB MAF did not predict EFS (supplemental Figure 1B), suggesting that WB MAF was inadequate as a monitoring biomarker.

To manifest MPN phenotypes, MPN-SPCs and/or their progeny must be able to outcompete their normal counterparts. Over time, a survival advantage of mutant over normal HSCs increases MAF within the HSC compartment. Mutant gene products may also provide a proliferative and differentiation advantage to HSC progeny along certain trajectories. The competitive potential to pass on alleles/traits to progeny is referred to as "fitness" in evolutionary biology. Fitness integrates the intricate phenotypes that enrich for the selected alleles without necessarily defining the mechanism underlying success. Analogously, MAF within well-defined hematopoietic populations reports hematopoietic fitness of mutant cells. Herein, we tested the link between *JAK2*^{V617F}-driven MPN fitness and clinical phenotypes and outcomes.

We directly measured MPN fitness by quantifying *JAK2*^{V617F} MAF in WB and 11 well-defined and strictly validated immunophenotypic hematopoietic stem and progenitor cells and mature leukocytes purified from routinely collected peripheral blood (PB) specimens (Figure 1A; supplemental Figure 2; supplemental methods). MAF was quantified by droplet digital polymerase chain reaction using DNA extracted from purified cells. Between August 2017 and August 2021, 173 PB specimens from 107 patients with *JAK2*^{V617F} MPNs were collected and analyzed (supplemental Table 1). Unsupervised,

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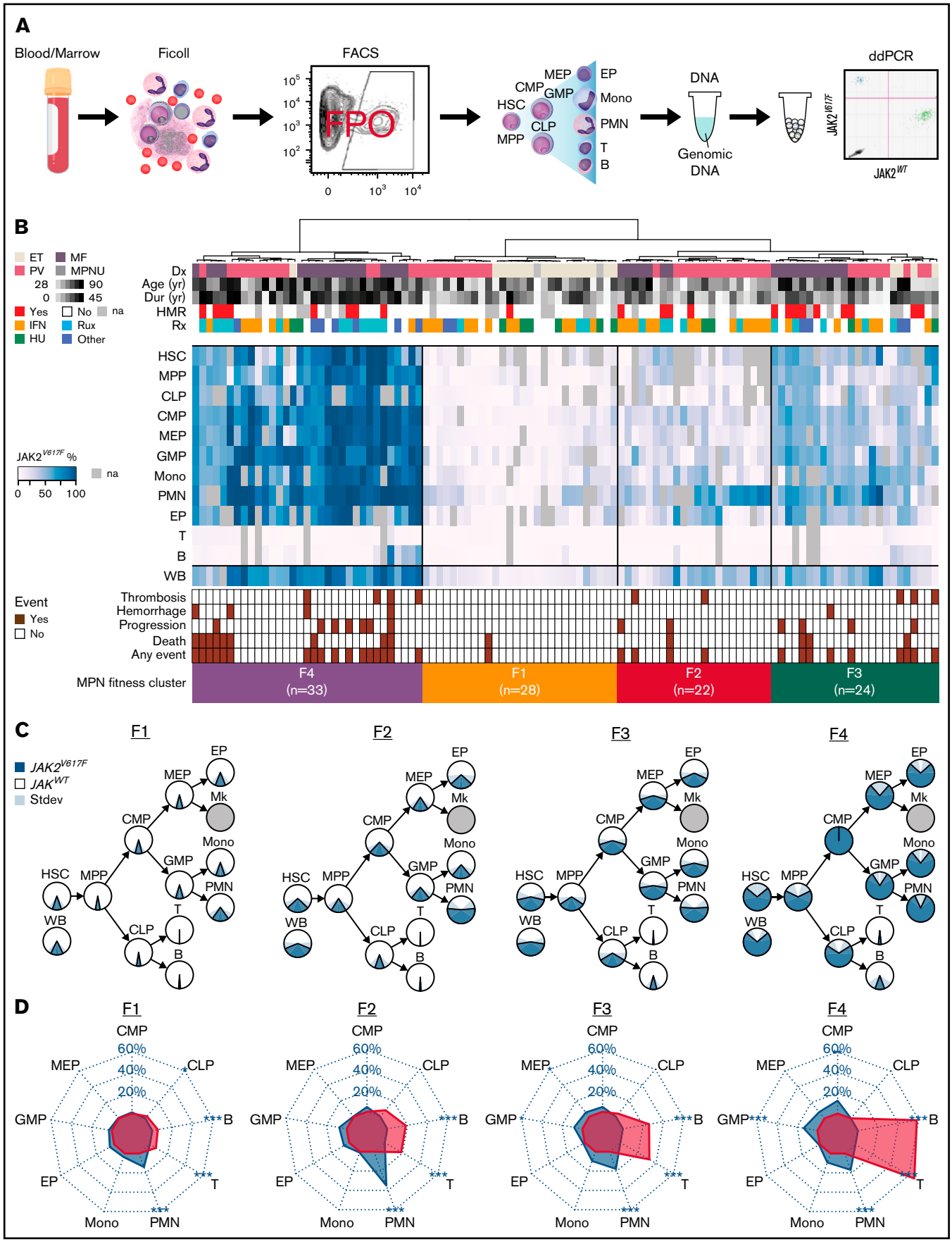
A
Blood/Marrow
Ficoll
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FPO
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CMP
GMP
Mono
HSC
CLP
PMN
MPP
T
B
DNA
Genomic DNA
ddPCR
JAK2V617F
JAK2WT
B
ET
MF
PV
MPNU
28
90
0
45
Yes
No
na
IFN
Rux
HU
Other
Dx
Age (yr)
Dur (yr)
HMR
Rx
HSC
MPP
CLP
CMP
MEP
GMP
Mono
PMN
EP
T
B
WB
JAK2V617F %
na
0
50
100
Event
Yes
No
Thrombosis
Hemorrhage
Progression
Death
Any event
MPN fitness cluster
F4 (n=33)
F1 (n=28)
F2 (n=22)
F3 (n=24)
C
F1
F2
F3
F4
JAK2V617F
JAKWT
Stdev
HSC
MPP
WB
CMP
MEP
EP
Mk
GMP
Mono
PMN
CLP
T
B
D
F1
F2
F3
F4
CMP
60%
40%
20%
MEP
CLP
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Figure 1.

hierarchical clustering of *JAK2*^{V617F} MAF within the 11 populations identified 4 major MPN fitness levels: F1, F2, F3, and F4 (Figure 1B). The pattern of *JAK2*^{V617F} propagation through hematopoiesis was both lineage- and differentiation-stage specific and varied across fitness levels (Figure 1C-D). MPN fitness was lowest in F1 and highest in F4. MAF increased progressively in hematopoietic stem and progenitor cells from F1 to F4 and was further augmented during granulopoiesis, indicating that *JAK2*^{V617F}-driven fitness manifests both in HSCs and during myeloid differentiation.

Risks of disease progression, morbidity, and death are variable in MPNs. Making an accurate clinical diagnosis of MPN, ET, PV, or MF, is important because it immediately stratifies risk and informs treatment. However, because of phenotypic mimicry, clinicopathologic heterogeneity, and the dynamic nature of MPNs, precision biomarkers are needed to predict and monitor individualized risk. Because MPN fitness underlies core biology of MPN pathogenesis, we hypothesized that it should be linked to known and potentially unknown sources of clinical heterogeneity and risk. Although clinical features alone could not segregate fitness levels (supplemental Figure 3), those with MF, older age, longer disease duration, and higher WB MAF were enriched in F3/F4. However, several patients with ET and PV unexpectedly clustered within these higher fitness levels, and some patients with PV or MF clustered in lower fitness levels (F1/F2) (Figure 1B). Although MPN treatment did not strictly correlate with fitness, patients treated with interferon-α were more common in F1/F2 than F3/F4 (50% vs 25%; $P = .0002$). These data suggest that MPN fitness level was linked to known sources of clinical heterogeneity.

The EFS differed significantly across the 4 fitness levels ($P = .0001$; Figure 2A) with F1 having the longest EFS and F4 having the shortest (100% vs 66%, respectively, at 12 months, and 96% vs 28%, respectively, at 36 months). On the other hand, WB MAF quartiles were only modestly prognostic for EFS (Figure 2A), similar to findings from our clinical PV cohort (supplemental Figure 1A). To directly compare prognostic biomarker value of MPN fitness relative to WB MAF, we performed survival receiver-operator characteristic (ROC) analysis. Twelve-month ROC area under the curve (AUC) was significantly higher for MPN fitness level than for WB MAF quartiles (0.8 vs 0.67, $P = .003$) (supplemental Figure 4A), indicating MPN fitness is an excellent prognostic measure for EFS, whereas WB MAF performs modestly.

Multivariable analysis confirmed that the association between MPN fitness level and EFS was independent of age, sex, diagnosis, MPN duration, MPN treatment, and WB MAF (supplemental Table 2). In fact, aside from age, none of the clinical variables was independently predictive of event risk in the multivariable analysis. This suggests that MPN fitness captures salient aspects of these clinical variables as they relate to MPN heterogeneity and EFS.

Clinical variables, such as age, sex, diagnosis, and duration of disease, are not useful as monitoring biomarkers because they are invariant or unmodifiable by therapy and cannot predict individualized risk. We monitored MPN fitness longitudinally in patients to assess whether measured fitness changes correlated with clinically important events. We used principal component analysis to reduce dimensionality for statistical modeling. The first 3 component vectors (PC1 to 3) explained 87% of the variance between samples. Sample location within the 3-dimensional space of the first 3 principal components clustered according to fitness levels (Figure 2B), and Cox proportional-hazards modeling indicated that location within this space was associated with event risk.

Serial assessment of sample position within this space was used to predict relative risk of events ("MPN fitness risk") for 30 patients with a total of 96 PB specimens (supplemental Table 3). Two patients who had an event before collection of a serial sample were excluded from EFS analysis. Changes in MPN fitness risk were highly associated with EFS ($P = .0026$; Figure 2C). No events occurred in patients with decreased MPN fitness risk (36-month EFS of 100%), whereas events occurred in 7 of 14 patients with increased MPN fitness risk (36-month EFS of 36%). Change in WB MAF was not linked to EFS ($P = 1$; Figure 2C; supplemental Figure 1B) and did not correlate with change in MPN fitness risk (supplemental Figures 5-8). Survival ROC analysis shows that MPN fitness risk is highly predictive for EFS (12- and 36-month AUC of 0.81 and 0.90, respectively), whereas WB MAF has no value as a monitoring biomarker (12- and 36-month AUC of 0.43 and 0.49, respectively) (supplemental Figure 4B). Serial monitoring of MPN fitness risk can be done using PB samples collected at the same time as other clinical parameters and correlated with clinical response during treatment (supplemental Table 3; supplemental Figures 6-8).

Partial or complete response, as assessed by ELN/IWG-MRT criteria for ET, PV, and MF (supplemental methods), was achieved in 79% of patients with decreased MPN fitness risk and in 19% of patients with increased MPN fitness risk (Fisher's exact test; $P < .001$; Figure 2D). Change in WB MAF was not predictive of response (Figure 2D). Thus, MPN fitness risk outperforms WB MAF as a monitoring biomarker and may be useful as a short-term surrogate endpoint. Our study was not powered to draw firm conclusions related to individual MPN therapies, but we did observe a reduction in MPN fitness risk with interferon treatment (7 of 9; 78%) compared with patients receiving other therapies (7 of 21; 33%) ($P = .042$). Validation studies, ideally clinical trials in a less heterogenous group, are required to establish the effect of available and investigational therapies on MPN fitness.

Figure 1 (continued) MPN fitness levels are associated with clinical features and outcome. (A) Schematic of the strategy used to purify and assess 11 hematopoietic populations isolated from PB: HSC, multipotent progenitor (MPP), common lymphoid progenitor (CLP), common myeloid progenitor (CMP), megakaryocyte-erythroid progenitor (MEP), granulocyte-macrophage progenitor (GMP), erythroid precursor (EP), monocyte (Mono), neutrophil (PMN), T lymphocyte (T), and B lymphocyte (B). ddPCR, droplet digital polymerase chain reaction; FACS, fluorescence-activated cell sorter. (B) Heatmap of unsupervised, hierarchical, principal component clustering of 11-population *JAK2*^{V617F} MAFs for 107 patients with MPN. Four major fitness clusters (F1, F2, F3, F4) are highlighted with relevant clinical information indicated under the dendrogram, including diagnosis (Dx), age, duration of MPN (Dur), high-molecular risk mutation status (HMR), and treatment (Rx). Clinical outcome events are shown under the heatmap. (C) Composite fitness patterns of F1, F2, F3, F4 patient groups are presented within a hematopoiesis hierarchy with mean MAF shown for each population as a blue wedge and ± standard deviation (Stdev) indicated in translucent blue shading. (D) Radar plots showing the difference in mean MAF between immature stem and progenitors (HSC+MPP) and each of 9 progenitors and differentiated cells. *$P < .05$; **$P < .01$; ***$P < .001$.

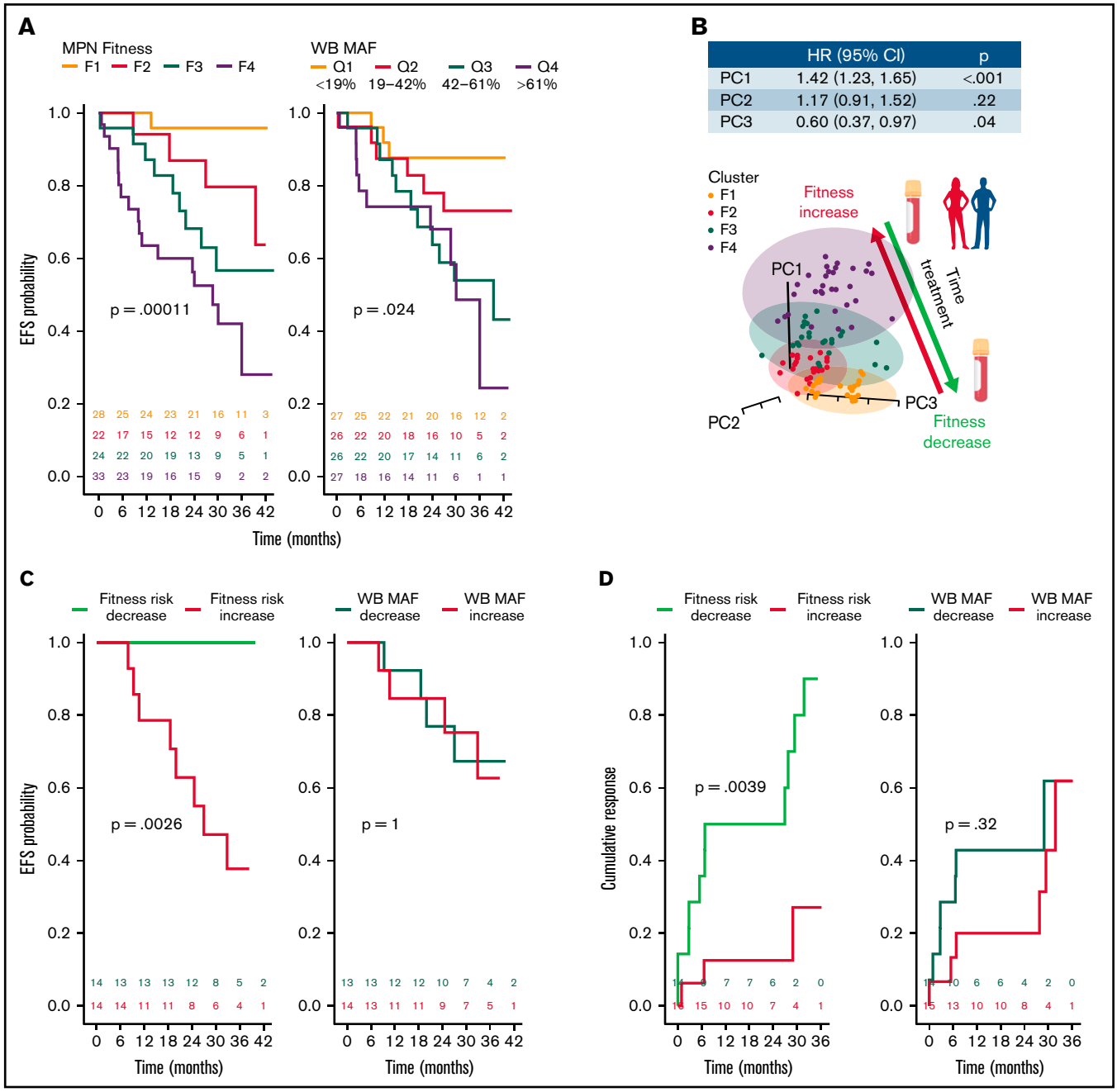

Figure 2. MPN fitness levels inform EFS, and change in MPN fitness risk is linked to EFS and clinical response. (A) EFS stratified by 4 MPN fitness levels (left) and quartiles of WB MAF (right). (B) Cox proportional hazards model associating PC1 to 3, shown in a 3-dimensional space, with risk of events to allow prediction of MPN fitness risk from serial samples. (C) EFS KM stratified by change in MPN fitness risk indicating statistically significant difference (left), and change in WB MAF, indicating no difference (right). (D) Cumulative incidence of partial or complete response stratified by change in MPN fitness risk indicating statistically significant difference (left), and change in WB MAF, indicating no difference (right). CI, confidence interval; HR, hazard ratio.

Biomarkers are needed to sensitively and robustly monitor risk of clinically important MPN outcomes, such as progression, thrombosis, and death. Without validated monitoring biomarkers, we are left with crude clinical measures that fall short as treatment decision-making tools. MPN biology is complex and influenced by a wide range of cell intrinsic[6,13,14] and extrinsic[15] microenvironmental factors. Our study offers a feasible approach to monitor the biology central to MPN propagation and disease progression. It provides a framework to interpret studies deciphering the complex mechanisms underlying MPN fitness and promises individualized prediction of MPN morbidity and response to treatment. As a surrogate endpoint, MPN fitness offers a path to identify the most promising therapeutic approaches to reduce the competitive advantage of MPN-SPCs and thereby modify disease outcomes important to patients and clinicians.

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Contribution: G.A.-Z. designed the study, examined and consented patients, provided blood specimens, performed the experiments, collected data, analyzed the data, and wrote the manuscript; S.D.G. performed experiments and reviewed the manuscript; D.C. performed experiments and reviewed the manuscript; T.C. performed experiments; K.E. consented patients, collected blood specimens, and collected data; E.T. collected data; E.K.R. and R.T.S. examined and consented patients, provided blood specimens, and reviewed the manuscript; and J.M.S. conceived and designed the study, examined and consented patients, provided blood specimens, analyzed the data, and wrote the manuscript.

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