

Impact of integrated genetic information on diagnosis and prognostication for myeloproliferative neoplasms in the next-generation sequencing era

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Abstract

Background: Since next-generation sequencing has been widely used in clinical laboratory, diagnosis and risk stratification of hematologic malignancies are greatly dependent on genetic aberrations.

Methods: In this study, we analyzed the genomic landscape of 200 patients with myeloproliferative neoplasms (MPNs) using targeted panel sequencing covering including 86 genes. Conventional BM karyotyping was also performed to determine chromosomal aberration. We analyzed relationships between genetic profiles and clinical outcomes including acute transformation, bone marrow fibrosis and death.

Results: Mutations in *JAK2*, *CALR*, and *MPL* were detected in 76.4% of MPNs. The proportion of patients with clonal genetic markers increased up to 86.4% when all detectable genetic aberrations were included. Significant co-occurring genetic aberrations potentially associated with phenotype and/or disease progression, including those in *JAK2/SF3B1* ($P=0.017$) and *TP53/del(13q)*, *del(5q)*, *-7/del(7q)* and complex karyotypes ($P=0.038$, $P<0.001$, $P<0.001$ and $P<0.001$, respectively) were detected. We also identified genetic aberrations associated with patient outcomes: *TP53* and *-7/del(7q)* for inferior survival (HR, 95% CI: 64.2, 3.8-1096.5, $P=0.0041$, HR, 95% CI: 14.0, 1.5-132.7, $P=0.0219$), *RUNX1*, *TP53* and *IDH1/2* for leukemic transformation (HR, 95% CI: 68.1, 3.6-1300.4, $P=0.005$, HR, 95% CI: 16.3, 1.2-222.7, $P=0.0364$, HR, 95% CI: 32.5, 2.8-371.1, $P=0.0051$), *SF2B1*, *IDH1/2*, *ASXL1* and *del(20q)* for fibrotic progression (HR, 95% CI: 31.5, 4.1-243.3, $P=0.0009$, HR, 95% CI: 21.2, 3.4-132.2, $P=0.0011$, HR, 95% CI: 4.3, 1.1-16.4, $P=0.0358$, HR, 95% CI: 44.5, 6.1-323.0, $P=0.0002$). We compared risk stratification systems and found that mutation-enhanced prognostic scoring systems could identify lower risk polycythemia vera and essential thrombocythemia and higher risk primary myelofibrosis ($P<0.001$, $P<0.001$ and $P<0.001$, respectively). Furthermore, the new risk stratification systems showed better predictive capacity of patient outcome.

Conclusions: These results collectively indicate that integrated genetic information can enhance diagnosis and prognostication in patients with myeloproliferative neoplasms.

Background

Myeloproliferative neoplasms (MPNs) are characterized by clonal proliferation of hematopoietic cells that are fully differentiated and functional. MPNs are mainly classified into polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) according to disease manifestations. Some rare disease categories such as chronic neutrophilic leukemia (CNL) and chronic eosinophilic leukemia are also considered MPNs, in addition to unclassified MPNs. Mutations in three well-known driver genes, *JAK2*, *CALR*, and *MPL*, are used to diagnose PV, ET, and PMF. Recently, *CSF3R* was identified as a driver gene in CNL[1, 2]. *JAK2* mutations have been detected in 90% to 95% of PV patients and 50% to 60% of ET and PMF patients. *CALR* mutations are the second most common mutation in MPNs, and are found in 20% to 25% of ET and 25% to 30% of PMF patients. *MPL* mutations are found in 3% of ET patients and

5% of PMF patients[3]. Patients without mutations in any of these driver genes can be diagnosed with ET or PMF when their clinical and hematologic features meet specific criteria, including clonal mutations in any of the following genes: *ASXL1*, *EZH2*, *TET2*, *IDH1/2*, *SRSF2*, and *SF3B1* [1]. Knowledge of the mutational landscape not only facilitates objective diagnosis of MPN, but can also potentially be used as a prognostic factor. This area of research has benefited from application of next-generation sequencing (NGS) technology in clinical laboratories as well as research laboratories. Important gene mutations associated with patient outcomes, such as mutations in *TP53* and *ASXL1*, have been identified using NGS approaches.[4-8]

Risk stratification of MPNs is based on clinical and hematologic features. Advanced age, leukocytosis, and venous thrombosis are considered risk factors for survival in PV [9] and ET [10]. PMF-specific risk models such as the International Prognostic Scoring System (IPSS) [11], Dynamic IPSS (DIPSS) [12], and DIPSS-plus, which includes cytogenetic abnormalities in addition to DIPSS, have been developed [13]. Results of recent studies indicate that gene mutations are significant prognostic factors in MPN and mutation-enhanced prognostic systems for MPNs have been introduced [14-16]. These systems can be used to determine risk for survival as well as disease progression, including leukemic transformation and fibrotic progression[17, 18]. Moreover, integration of genetic information with clinical variables can enable prediction of patient outcomes and allow development of personalized therapeutic plans based on consideration of causal biologic mechanisms [19-21]. It is challenging at least in the present moment because data are limited to make consensus across the ethnics and counties under different clinical environments.

In this study, we analyzed genetic aberrations using cytogenetic and molecular genetic methods, including NGS, to reveal their impact on the diagnosis of MPNs in current era. We also tried to evaluate their contributions for risk stratification for survival as well as disease progression

Methods

Patients and samples

Among patients diagnosed with Philadelphia-negative MPN and treated at Seoul St. Mary's Hematology Hospital, a total of 200 patients who requested an NGS study or available DNA samples for NGS analysis were included. MPN diagnosis and their subtypes were strictly re-evaluated and classified based on the 2017 WHO classifications.[22] Demographics and clinical and hematologic features, including outcome data, were reviewed retrospectively from medical records. Most DNA samples were from bone marrow (BM) aspirates taken at the time of diagnosis, but some were collected at the time of symptom aggregation during observation or the course of treatment. This study was carried out in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Seoul St. Mary's Hospital (KC20RISI0371). This retrospective study involves only minimal risk to the participants, so the IRB waived the requirement to obtain informed consent from patients.

Molecular analysis

DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). DNA concentration and purity were assessed using an ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). NGS was performed using a customized myeloid panel, the “SM panel”, as described in our previous report [23]. Briefly, the SM panel contains 87 genes frequently mutated in patients with MPN (Supplemental Table 1). Target capturing sequencing was performed using a customized target kit (Agilent Technologies, 3039061) according to the manufacturer’s instructions. DNA libraries were constructed according to the protocol provided by the manufacturer and sequencing was performed on an Illumina HiSeq4000 platform.

Sequenced reads were mapped to the human reference genome (hg19, Genome Reference Consortium, Feb 2009) using Burrows-Wheeler aligner. To call variants, we used VarScan v.2.3.9 with mpileup2snp and mpileup2indel. SnpEff v.4.2 was used to select variants located in coding sequences and predict their functional consequences. Annotated variants were further classified into four tiers according to the standards and guidelines of the Association for Molecular Pathology (AMP)[24]. All variants with a minor allele frequency >0.01 were filtered out based on the Exome Aggregation Consortium (ExAC, <http://exac.broadinstitute.org/>) and genome aggregation database (gnomAD, <https://gnomad.broadinstitute.org/>), as well as the ethnic-specific Korean Variant Archive (KOVA, <http://kobic.re.kr/kova/>). Variants of synonymous, intronic, or non-coding regions were further excluded. Among the remaining variants, variants with more than 20 reads and 5% variant allele frequencies (VAF) were considered to be mutations. Canonical mutations in *JAK2*, *CALR*, and *MPL* less than 5% VAF were considered to be present with a low allele burden. All mutations were manually verified using the Integrative Genomic Viewer.

Cytogenetic study

A total of 169 BM karyotypes were available. Conventional BM karyotyping was performed on G-banded metaphase chromosomes using routine techniques. Karyotypes were interpreted according to ISCN 2016 [25]. The number of cytogenetic events was considered the sum of chromosome gains, losses, partial gains, partial losses, and rearrangements.

Statistical analyses

Group comparisons according to phenotype were performed using the Kruskal-Wallis test and Chi-square test. Mann-Whitney test and Fisher’s exact test were also used for post-hoc analysis. The significance of co-occurrences of mutations and chromosomal abnormalities was determined using Fisher’s exact test.

The Chi-square test was used to assess whether the distributions of risk scores from two systems were systematically different. We defined overall survival (OS) as time from diagnosis to death from any cause, and event-free survival (EFS) as time from diagnosis to leukemic transformation, myelofibrosis progression, or death. Medical record tracking was done until February 2020. The Kaplan-Meier Log-rank test was used to estimate OS and EFS in each groups. Cox proportional-hazards regression was used for

univariate and multivariate analyses. Statistical analyses were performed using MedCalc version 19.1.7 (MedCalc software, Mariakerke, Belgium).

Results

Genetic landscape of MPNs

Patients' clinical and hematologic characteristics are summarized in Table 1. According to disease category of WHO classification, ET was the largest group (n = 70, 35.0%), followed by PMF (n = 66, 33.0%), PV (n = 55, 27.5%), and other MPNs (n = 9, 4.5%) consisting of chronic neutrophilic leukemia (CNL, n = 4, 2.0%) and MPN-unclassifiable (n = 5, 2.5%). Age at diagnosis differed significantly according to disease category. ET patients (43.2±15.3 years) were younger than PV (50.3±12.3 years), PMF (52.5±13.6 years), and other MPN (61.4±14.2 years) patients ($P = 0.0034$, $P = 0.0002$ and $P = 0.0024$, respectively). Clinical and genetic variables according to disease category are described in Supplemental Figure. 1.

Genetic landscapes for all patients are shown in Figure 1A. Mutations were identified in 85.5% of patients (n = 171). The mean number of mutations was 1.3±1.0, and ET patients harbored significantly fewer mutations than PMF patients (1.1±0.9 vs. 1.6±1.1, $P = 0.024$). *JAK2* mutations (n = 98) were the most common followed by *CALR*, *ASXL1*, *TET2*, and *MPL* mutations (n = 43, 24, 17, and 5, respectively). Abnormal karyotypes were identified in 22.5% of patients (38/169), and 4.7% (8/169) of individuals had a complex karyotype with three or more cytogenetic abnormalities. The proportion of abnormal karyotypes in PMF patients (37.5%, 21/56) was higher than that in PV (18.6%, 8/43) or ET (9.8%, 6/61) patients ($P = 0.047$, $P = 0.0004$, respectively). -5/del(5q) (n = 10) was the most common cytogenetic abnormality, followed by del(20q) (n = 9), del(13q) (n = 9), and -7/del(7q) (n = 6) (Figure 1B).

We also analyzed the co-occurrence of mutations and abnormal karyotypes. Multiple pairs of co-occurring mutations were found, including *JAK2* with *SF3B1*, *ASXL1* with *SRSF2*, and *DNMT3A* with *IDH1/2* ($P = 0.017$, $P = 0.01$, and $P = 0.012$, respectively). *TP53* mutations were significantly associated with del(13q), -5/del(5q), -7/del(7q), and complex karyotypes ($P = 0.038$, $P < 0.001$, $P < 0.001$ and $P < 0.001$, respectively) (Figure 1C) [26]. We reviewed Prussian blue iron staining of available BM samples with *JAK2/SF3B1* co-mutations and found ring sideroblasts in all three samples.

Impact of genetic aberrations on diagnosis

JAK2 (n = 98, 49.0%), *CALR* (n = 43, 21.5%), and *MPL* (n = 5, 2.5%) mutations were detected in 76.4% of PV, ET, and PMF patients (n = 146). *JAK2* mutations were detected in 49 PV patients (89.1%) while *JAK2*, *CALR*, and *MPL* mutations were detected in 26 (37.1%), 22 (31.4%), and 3 (4.3%) ET patients, respectively. *JAK2*, *CALR*, and *MPL* mutations were detected in 23 (34.8%), 21 (31.8%), and 2 (3.0%) PMF patients, respectively. Among 39 triple-negative ET and PMF patients, 8 (2 ET and 6 PMF) patients had any of myeloid neoplasm-associated mutations in *ASXL1*, *EZH2*, *TET2*, *IDH1/2*, *SRSF2*, or *SF3B1*. *CSF3R* T618I mutation was detected in 2 of 4 CNL patients. Thus, clonal genetic markers for diagnosis according the

WHO classification were present in 80.6% (n = 154) of three MPN as 89.1% of PV patients, 75.7% of ET patients, 78.8% of PMF patients, and 50% of CNL patients, representing 80.6% (n = 154) of all MPN patients.

We considered all gene mutations, including those not mentioned above, in addition to an abnormal karyotype, as clonal genetic markers. Genetic aberrations were detected in 12 patients including mutations of *U2AF1*, *DNMT3A*, *RUNX1*, *TP53*, *ZRSR2*, *KDM2B*, *NRAS*, and *KIT* genes as well as karyotype abnormalities such as del(20q) and -5/del(5q). The proportion of MPN patients with clonal genetic markers increased up to 86.4% (n = 165). When broken down according to MPN type, 90.9% of PV patients, 77.1% of ET patients, and 92.4% of PMF patients had clonal genetic markers (Table 2).

Prognostic impact of genetic aberrations

The mean overall survival of the study population was 400.5 months with a 95% confidence interval (CI) of 367.0-434.1 months. Fourteen deaths were recorded in the whole series (3 ET, 7 PMF and 4 other MPN). Clinical outcome in terms of shortest OS was significantly poorer in other MPN groups (36.8 months, 95% CI, 21.9–51.6) than the PV group (325.0 months, 95% CI, 325.0–325.0), ET group (296.4 months, 95% CI, 272.0–320.7), or PMF group (318.8 months, 95% CI, 218.8–418.7) (Figure 2A). Univariate analysis showed that mutations in *TP53*, -5/del(5q), -7/del(7q), del(20q), del(13q), number of abnormal karyotypes, and a complex karyotype were associated with poor outcomes. Diagnosis of PMF and presence of bone marrow fibrosis were also associated with poor outcomes. Hemoglobin level and *JAK2* mutation were associated with a favorable outcome (Figure 2B). Next, we investigated the impact of clinical and hematologic variables and genetic aberrations on disease progression, including leukemic transformation and fibrotic progression. Nine patients experienced leukemic transformation (2 PV, 3 ET and 4 PMF), and there was no difference in frequency between the three disease groups. *IDH1/2*, *RUNX1*, and *TP53* mutations, -5/del(5q), -7/del(7q), number of abnormal karyotypes, and a complex karyotype were significant risk factors for leukemic transformation (Figure 2C). Fibrotic progression occurred in 24 (8 PV and 16 ET) patients. *ASXL1*, *IDH1/2*, and *SF3B1* mutations, number of mutations, and del(20q) were associated with fibrotic progression (Figure 2D). Details of the prognostic impact of each parameter are summarized in Supplemental Table 2.

In addition, we performed multivariate analysis. *TP53* mutation, -7/del(7q), and diagnosis of PMF were adverse survival factors. PB blasts counts in addition to *RUNX1*, *IDH1/2*, and *TP53* mutations were identified as risk factors for leukemic transformation. *ASXL1*, *SF3B1*, and *IDH1/2* mutations, number of mutations, and del(20q) were defined as risk factors for fibrotic progression (Table 3). We also investigated the prognostic impact of genetic aberrations in each disease category. Despite limited numbers of patients in each disease category and duration, genetic aberrations identified as risk factors by multivariate analysis had statistical significance in log rank analysis (Supplemental Table 3).

Impact of genetic aberrations on risk stratification

To evaluate the impact of genetic aberrations on risk stratification, we compared the risk of each patient group before and after applying genetic aberrations. Although there were statistically significant relationships between risk groups (contingence coefficient 0.514 - 0.554), we found some interesting changes. Thirty-seven of 55 PV patients had more than one risk factor including advanced age, leukocytosis, and a vascular event [9]. When adjusting for MIPSS-PV, which included *SRSF2* mutation, 20 patients (54.1%) were reclassified into the low risk group. In ET, 28 patients had more than one risk factor including advanced age, leukocytosis, and a vascular event [10]. When applying MIPSS-ET, which included *SF3B1*, *SRSF2*, *TP53*, and *U2AF1* mutations, the majority of patients (67.9%, 19/28) were reclassified as being low risk (Table 4). Among patients with PMF, 25 were classified as DIPSS low risk, 16 as intermediate risk, 21 as intermediate-2, and four as high risk (n = 4). It is notable that 10 and 6 patients in the low risk group were reclassified into intermediate and high risk groups based on MIPSS70[14] and MIPSS70+[27], respectively. MIPSS70 includes *ASXL1*, *EZH2*, *SRSF2*, and *IDH1/2* mutations, while MIPSS70+ includes *ASXL1*, *EZH2*, *SRSF2*, and *IDH1/2* mutations and cytogenetic risk categories. Three patients in intermediate-1 risk group were reclassified into high and very high risk groups by MIPSS70 and MIPSS70+, respectively (Table 5). It meant that the newly developed risk stratification systems including genetic aberrations discriminated more patients with low risk in PV and ET. On the other hand, the new systems selected PMF patient with higher risk among those with low or intermediate-1 risk.

Additionally, we analyzed the prediction capacity of mutation-enhanced prognostic scoring systems. In ET, MIPSS-ET revealed significant prediction of OS ($P = 0.003$) while the previous risk factors did not ($P = 0.922$). In PMF, MIPSS70+ ($P = 0.003$) and MIPSS70 ($P = 0.006$) had a lower P value for prediction of OS than DIPSS ($P = 0.024$). MIPSS70+ ($P = 0.002$) and MIPSS70 ($P = 0.005$) predicted EFS significantly better than DIPSS ($P = 0.201$) (Supplementary Table 4).

Discussion

Continuous advances in genetic technology have resulted in great changes in clinical practice. As a consequence of the advance, current diagnoses and classifications of hematologic malignancies are largely based on genetic aberrations. Diagnosis of MPNs is made primarily based on clinical and hematologic features supported by mutations in *JAK2*, *CALR*, and/or *MPL* genes. In the absence of mutations in any of these three genes, other myeloid neoplasm-associated mutations are recognized as another clonal markers for ET and PMF.[1] In the current study, we identified the driver mutations in 76.4% of MPN patients, with the highest frequency in PV patients (89.1%) followed by ET (72.8%) and PMF (69.7%) patients. There are no clear definitions of myeloid neoplasm-associated mutations other than those in seven genes in the WHO classification. We found that gene mutations other than the three driver genes and cytogenetic abnormalities accounted for a considerable proportion of cases. When applying 7 myeloid neoplasm associated genes proposed by WHO classification, 8 patients became to possess clonal genetic markers. When including all detectable genetic aberrations, 12 patients were added to the list of patients with clonal genetic markers, and the proportion of patients with clonal genetic markers increased by 10.0%. The added mutations and karyotype abnormalities were commonly detected in

myeloid neoplasms or other malignancies such as *U2AF1*, *DNMT3A*, *RUNX1*, *TP53*, del(20q) and -5/del(5q). Therefore, it is necessary to define specific gene mutations and karyotype abnormalities and to clarify their role in the diagnosis of MPN.

Overall genetic landscapes were similar to those reported in previous studies.[28, 29] We found significant co-occurring mutations. *JAK2/SF3B1* co-mutations, which are known to be associated with myelodysplastic/myeoproliferative neoplasms with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T), were identified in 6 patients (2 ET and 4 PMF)[30, 31]. It is notable that ring sideroblasts were detected in all available BM samples with *JAK2/SF3B1* co-mutations. Even though the proportion of RS was far less than 5%, it was evident that the *SF3B1* mutation affected the formation of RS. Further studies should determine an appropriate diagnostic strategy for this disease, which has a broad spectrum of phenotypes. *TP53* mutation, co-occurring with del(13q), -5/del(5q), -7/del(7q), and a complex karyotype, was a significant adverse survival factor. Studies have shown that *TP53* mutations occur at a low frequency in chronic-phase MPNs and increase significantly according to disease progression. *TP53* mutations were positively correlated with poor survival outcomes, including early death [4, 5, 21], and -7/del(7q) was associated with poor survival in PMF patients [32]. Because all patients with *TP53* mutations had a complex karyotype, we postulated that presence of *TP53* mutation resulted from accumulation of genetic aberrations through disease progression.

In addition, we identified genetic aberrations associated with disease progression. Results from this and previous studies showed that *IDH1/2* mutations are associated with risk of leukemic transformation. *IDH1/2* mutations occurred frequently in the blast phase of MPN compared to the chronic phase, suggesting a pathogenetic contribution to leukemic transformation [33]. This is worth noting because IDH inhibitors may offer a therapeutic advantage in high-risk MPN patients [34]. We also found that *RUNX1* mutations were predictive of leukemic transformation. This finding is consistent with previous studies that demonstrated that *RUNX1* mutations had adverse impacts on leukemia-free survival in PV and ET patients [16, 17] and that *RUNX1*-mutated PMF patients had inferior overall survival and leukemia-free survival [35]. Another important gene was *ASXL 1*; mutations in this gene are typically associated with fibrotic progression. *ASXL 1* was the most frequently mutated gene with the exception of the three driver genes. Previous studies showed that *ASXL 1* mutations were enriched for in patients with PMF [29], and *ASXL 1* mutations are considered to be high-risk mutations along with mutations in epigenetic regulators such as *SRSF2* and *EZH2* in patients with MPNs [8, 17, 28, 36]. *TET2* is an epigenetic regulator frequently mutated in all MPN subtypes. Although previous studies have demonstrated that *TET2* mutations increase the self-renewal capacity of hematopoietic stem cells [37] and can lead to disease initiation and progression [6], the prognostic value of *TET2* mutations is controversial [7, 38]. Our study revealed that *TET2* mutations were frequently detected in MPNs with little prognostic impact. Del(20q) was another significant factor associated with fibrosis progression. It is notable that most instances of del(20q) were identified in PMF patients or patients with PV or ET with fibrosis progression during follow-up.

Clinicians should stratify each patient's risk on the basis of updated information. This is even more important in the current era due to the increased availability of therapeutic options. Recent updates

included genetic abnormalities into risk stratification for MPNs; *SRSF2* for PV, *SF3B1*, *SRSF2*, *TP53* and *U2AF1* for ET, and *ASXL1*, *EZH2*, *SRSF2*, *IDH1/2* and karyotype for PMF. It was expected that risk groups were correlated before and after applying genetic abnormalities, however, there were changes worth paying attention. In PV and ET, mutation-enhanced risk stratification system appeared to select low risk patients among patients with clinical and laboratory risk variables. By contrast, in PMF, mutation and additional karyotype-enhanced risk stratification systems identified higher risk patients among low or intermediate-1 risk groups, as supported by a previous finding that demonstrated the significance of *ASXL1* and *SRSF2* mutations for treatment decision-making in low or intermediate-1 risk patients with PMF [39].

Due to the restricted number of patients in each disease category and relatively short follow-up period, further studies should be performed in a larger cohort considering racial heterogeneity. However, this study highlights the usefulness the diagnostic and prognostic potential of targeted sequencing in the NGS era. In addition, the results of our contemporary study can be used as valuable source to tailor therapeutic plans. Integrated genetic information not only facilitates diagnosis and prognostic expectations but also our understanding of the presentation and progression of MPNs.

Conclusions

Genetic landscape demonstrated that 86.4% of MPN patients harbored any clonal markers including 76.4% of MPN patients with triple driver mutations. Some genetic aberrations such as *TP53*, *IDH1/2*, *SF3B1*, *RUNX1*, *-7/del(7q)* and *del(20q)* were related to overall survival and/or disease progression, and mutation-enhanced scoring systems performed better than phenotype-based risk stratification. These findings support that integrated genetic information has a significant impact on the diagnosis and prognostication of MPN patients.

Declarations

Ethics approval and consent to participate

This study was carried out in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Seoul St. Mary's Hospital (KC20RISI0371). This retrospective study involves only minimal risk to the participants, so the IRB waived the requirement to obtain informed consent from patients.

Consent for publication

All authors agreed to publish.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

M.S.K. and Y.G.K. designed research; J.M.L. and H.W.L. performed research and analyzed and interpreted data; K.S.E. and S.E.L performed validation and proofreading; J.M.L., M.S.K. and Y.G.K. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Tables

Table 1. Overall characteristics of patients in this study

Variables	Total, N=200	PV, N=55	ET, N=70	PMF, N=66	Other MPN ^a , N=9	P
Age at diagnosis, mean±SD	49.0±14.7	50.3±12.3	43.2±15.3	52.5±13.6	61.4±14.2	<0.001
Sex, male (%)	43.0 (86/200)	52.7 (29/55)	35.7 (25/70)	39.4 (26/66)	66.7 (6/9)	0.109
Follow-up months, mean±SD	64.7±69.4	76.4±67.7	78.5±73.8	46.0±64.8	23.6±20.2	<0.001
Blood cell count						
White blood cells (10 ⁹ /l), mean±SD	12.9±13.7	12.9±7.4	9.5±5.5	11.8±11.9	47.8±37.3	<0.001
Hemoglobin (g/l), mean±SD	129.3±33.6	164.7±31.3	126.2±20.6	107.7±21.3	95.3±18.7	<0.001
Platelet (10 ⁹ /l), mean±SD	560.5±484.8	542.2±324.9	803.7±608.7	367.5±340.3	197.2±147.5	<0.001
Bone marrow fibrosis, %	46.0 (92/200)	16.4 (9/55)	21.4 (15/70)	100.0 (66/66)	22.2 (2/9)	0.064
Splenomegaly, %	31.5 (63/200)	27.3 (15/55)	11.4 (8/70)	54.5 (36/66)	44.4 (4/9)	0.364
Vascular event ^b , %	20.0 (40/200)	36.4 (20/55)	20.0 (14/70)	9.1 (6/66)	0 (0/9)	0.275
Abnormal karyotype ^c , %	22.5 (38/169)	18.6 (8/43)	9.8 (6/61)	37.5 (21/56)	33.3 (3/9)	0.003
Complex karyotype ^c , %	4.7 (8/169)	4.7 (2/43)	3.3 (2/61)	7.1 (4/56)	0 (0/9)	0.692
Number of mutations, mean±SD	1.3±1.0	1.4±0.9	1.1±0.9	1.6±1.1	0.9±0.9	0.024

PV polycythemia vera, ET essential thrombocythemia, PMF primary myelofibrosis, MPN myeloproliferative neoplasm, CNL chronic neutrophilic leukemia, SD standard deviation.

Bold indicates significant values with $P < 0.05$

Group comparisons were performed using the Kruskal-Wallis and Chi-square test for continuous and categorical variables, respectively.

^aOther MPN includes CNL (n = 4) and MPN-unclassifiable (n=5)

^bVascular event includes thrombosis history at diagnosis and related microcirculation symptoms such as headaches, lightheadedness, atypical chest pain, transient visual disturbances, and acral paresthesias

^cTotal of 169 BM karyotypes were available.

Table 2. Proportion of patients possessing clonal genetic marker after applying sequential criteria for genetic aberrations.

	PV	ET	PMF	3 MPNs ^a	Other MPN ^b
Case number	55	70	66	191	9
Triple mutations ^c	49 (89.1%)	51 (72.9%)	46 (69.7%)	146 (76.4%)	2 (22.2%)
Any of seven mutations ^d	0 (0%)	2 (2.9%)	6 (9.1%)	8 (4.2%)	3 (33.3%)
Other mutations ^e and/or abnormal karyotype	1 (1.8%)	1 (1.4%)	9 (13.6%)	11 (5.8%)	4 (22.4%)
Any clonal genetic marker ^f	50 (90.9%)	54 (77.1%)	61 (92.4%)	165 (86.4%)	7 (77.8%)
All negative	5 (9.1%)	16 (22.9%)	5 (7.6%)	26 (13.6%)	2 (22.2%)

PV polycythemia vera, ET essential thrombocythemia, PMF primary myelofibrosis, MPN myeloproliferative neoplasm, CNL chronic neutrophilic leukemia.

^aPV, ET and PMF, ^bCNL (n = 4) and MPN-unclassifiable (n=5), ^cJAK2, CALR and MPL for 3 MPNs and CSF3R for CNL, ^dany of mutations including ASXL1, EZH2, TET2, IDH1/2, SRSF2, SF3B1 among triple negative patients, ^eany mutation other than the above-mentioned mutations, ^fany mutation and/or abnormal karyotype.

Table 3. Multivariate analysis of clinical and genetic factors for overall survival, leukemic transformation, and fibrosis progression

Variables	Overall survival			Leukemic transformation			Fibrotic progression		
	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI
Diagnosis ^a PB blasts (%)	0.0087	78.2	3.0-2027.3	0.0486	1.3	1.0-1.6			
No. mutation							0.0352	2.0	1.1-4.0
ASXL1							0.0358	4.3	1.1-16.4
RUNX1				0.005	68.1	3.6-1300.4			
SF3B1							0.0009	31.5	4.1-243.3
TP53	0.0041	64.2	3.8-1096.5	0.0364	16.3	1.2-222.7			
IDH1/2				0.0051	32.5	2.8-371.1	0.0011	21.2	3.4-132.2
-7/del(7q)	0.0219	14.0	1.5-132.7						
Del(20q)							0.0002	44.5	6.1-323.0

HR hazard ratio, CI confidence interval, PB peripheral blood, No.number.

Variables assigned to be significant prognostic factors in univariate analysis were included in the multivariate analysis. The fibrosis progression was analyzed among patients with polycythemia vera and essential

thrombocytopenia, excluding primary myelofibrosis.

^aDiagnosis of primary myelofibrosis.

Table 4. Comparison of risk groups in patients with PV and ET.

	Low	Intermediate	High	<i>P</i> (contingency coefficient)
PV	18	23	14	<0.001 (0.514)
MIPSS PV				
Low	18	14	6	
Intermediate	0	9	5	
High	0	0	3	
ET	42	24	4	<0.001 (0.554)
MIPSS ET				
Low	41	19	0	
Intermediate	1	3	2	
High	0	2	2	

MIPSS Mutation-enhanced International Prognostic Scoring System, *PV* polycythemia vera, *ET* essential thrombocythemia.

Risk factors for PV: advanced age (≥ 67 years, 5 points ; 57-66 years, 2 points), leukocytosis (≥ 15x10⁹/l, 1 point), and presence of a vascular event (1 point); low risk (no risk factors), intermediate risk (1-2 points), high (≥ 3 points).

Risk factors for ET: advanced age (≥ 60 years, 2 points), leukocytosis (≥ 11x10⁹/l, 1 point), and presence of vascular event (1 point); low risk (no risk factors), intermediate risk (1-2 points), high (3-4 points).

Table 5. Comparison of risk groups in PMF patients.

DIPSS	Low	Intermediate-1	Intermediate-2	High	<i>P</i> (Contingency coefficient)
MIPSS70					<0.001 (0.620)
Low	15	1	0	0	
Intermediate	8	12	11	0	
High	2	3	10	4	
MIPSS70+					<0.001 (0.603)
Very low	5	1	0	0	
Low	10	5	2	0	
Intermediate	4	4	3	0	
High	2	3	8	1	
Very High	0	0	6	2	

DIPSS Dynamic International Prognostic Scoring System, *MIPSS70* Mutation-enhanced International prognostic scoring system, *MIPSS70+* Karyotype-enhanced MIPSS70.

Figures

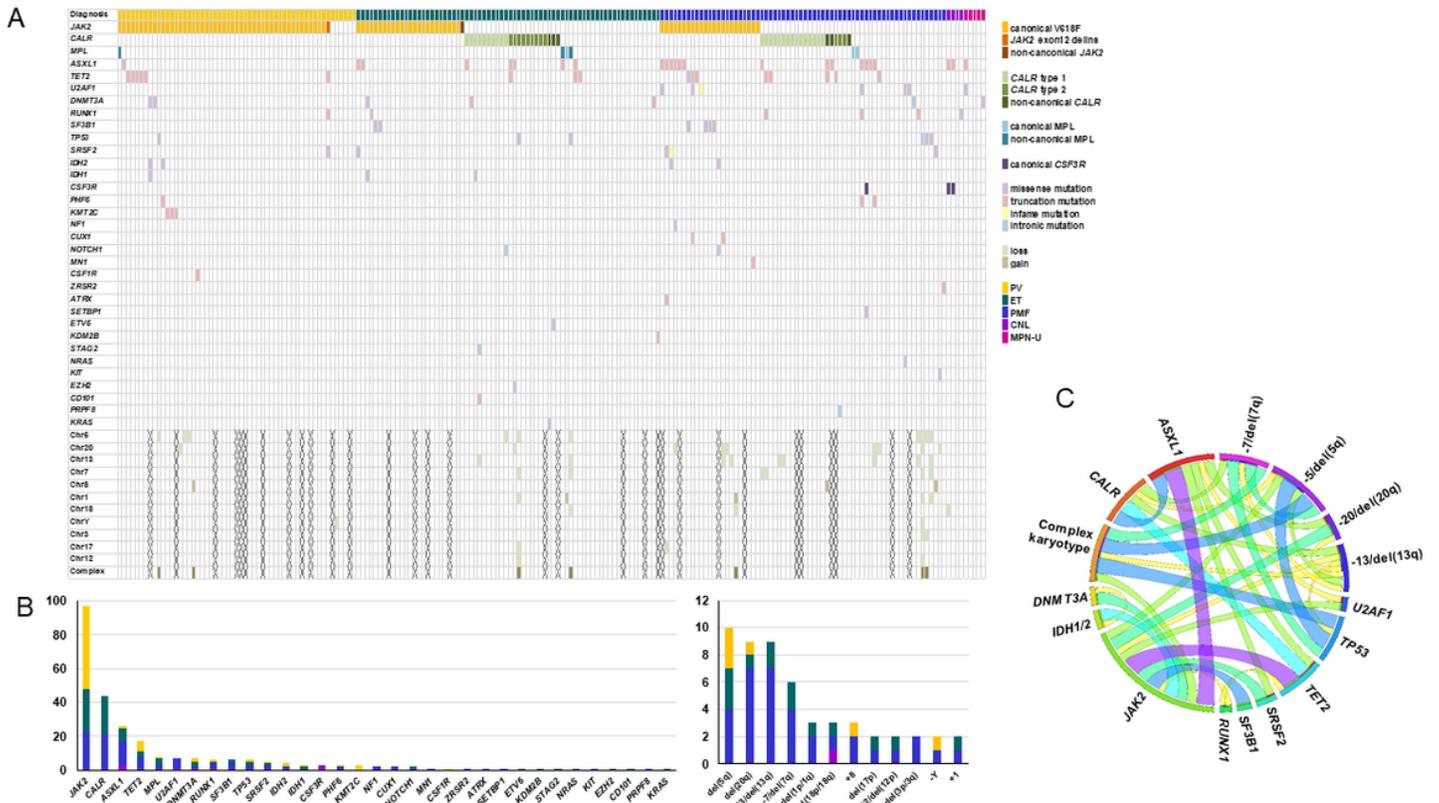


Figure 1

Genomic landscapes of 200 MPN patients (A) The mosaic plot and (B) bar-charts of mutations and chromosomal abnormalities (C) The circos plot indicating recurrent co-occurrences of genetic aberrations by ribbon widths. PV Polycythemia vera, ET essential thrombocythemia, PMF primary myelofibrosis, CNL chronic neutrophilic leukemia, MPN-U, MPN-unclassifiable, MPN myeloproliferative neoplasm

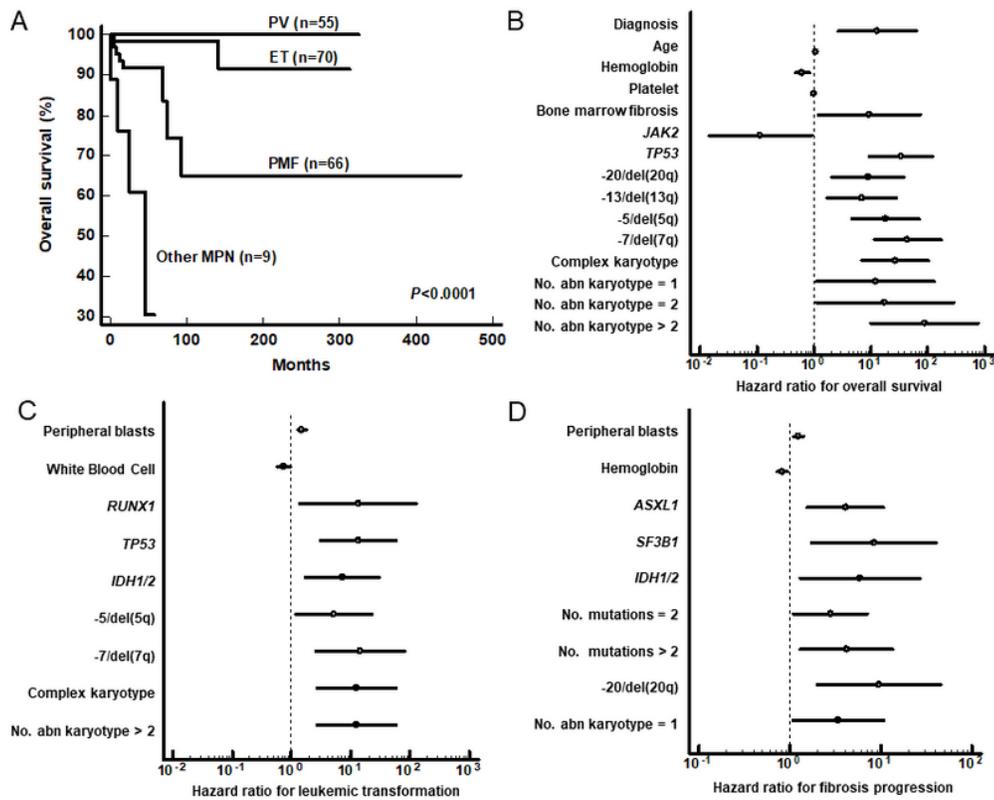


Figure 2

(A) Overall survival curves of the patients according to disease category. (B) Hazard ratios of significant factors for overall survival and (C) leukemic transformation in PV, ET, PMF and (D) fibrosis progression in PV and ET patients. PV Polycythemia vera, ET essential thrombocythemia, PMF primary myelofibrosis, MPN myeloproliferative neoplasm, No number, abn abnormal.

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