

The following are our responses to the editors:

Request: Therefore you are kindly requested to change the title of your revised manuscript to avoid any future complication.

Comment: The title of the manuscript was changed as follows: **Pathogenesis and mutations of Ph-negative myeloproliferative neoplasms. An overview**

Other Revisions:

1- The whole manuscript was reedited for spelling errors. All changes were highlighted in yellow.

2- Table 1 was deleted and replaced by a new Table 1. It was added to the end of the manuscript.

3-4- We made use of all the literatures the reviewers suggested for us and made the necessary additions to the manuscript.

5- **Hindilerden Fehmi** was added as a co-author and was highlighted in yellow. For the mentioned co-author, the institution was added as **Istanbul Bakirkoy Sadi Konuk Training and Research Hospital, Hematology Clinic.**

6- The following sentences were added to the Abstract section:

The somatic mutation of SRSF2, one of the RNA splicing machinery genes, was associated with worse survival and increased leukemic transformation in PMF.

In MPNs, genetic abnormalities affecting epigenetic regulation are often expressed in patients carrying JAK2, MPL, or CALR mutations, indicating a cooperation between these two classes of mutations in MPN pathogenesis.

7- The following sentence was deleted from the Abstract section:

Further studies are needed to address importance of CALR mutations in MPN.

8- The following paragraph was added after the Introduction section:

Mutations in myeloproliferative neoplasms

95% of PV patients harbor the JAK2V617F mutation. The 5% JAK2V617F unmutated PV patients are frequently heterozygous for JAK2 exon 12 mutations.^{2,3} JAK2, MPL, and CALR mutations together provide a genetic marker for the majority of PMF and ET (91.9% and 85%, respectively).^{4,5} Several

genes including regulators of DNA methylation (TET2, DNMT3A, IDH1/2), chromatin structure (EZH2, ASXL1), MAPK signaling pathway (NRAS), regulators of transcription factors (including IKZF1, RUNX1, and Tp53) and negative regulators of signaling pathways (LNK, CBL), are also mutated in MPNs.⁶⁻¹⁹

9- The following paragraph was added to the section **JAK2 exon 12 mutations**:

Although only a minority of sporadic PV patients do not carry the JAK2V617F mutation, the proportion of familial cases of JAK2V617F-negative PV patients is definitely higher.^{28, 29} JAK2 exon 12 mutations seem to account for the majority of mutations in PV patients negative for JAK2V617F.³⁰

In the same section, the following paragraph was deleted:

The low sensitivity of the methods used for genotyping, the presence of hereditary genetic diseases related to erythrocytosis, procedures such as the use of interferon that have the potential to eliminate JAK2 mutated clones may be accounted for the aforementioned finding. The presence of additional hereditary phenomena not yet described may as well be considered responsible for disease phenotype.

10- The following paragraphs were added to the section **MPL mutations**:

-In ET, a correlation between W515L and low haemoglobin (Hgb) levels and between W515K alleles and high platelet counts were reported.³⁸ Also, bone marrow examination in W515L/K mutation-positive patients showed reduced total and erythroid bone marrow cellularity, whereas the number of megakaryocytes, megakaryocytic clusters, and small-sized megakaryocytes were all significantly increased.³⁸ These data show that MPLW515L/K mutations do not define a distinct phenotype in ET.

-In conclusion, it is not possible to explain the pathogenesis of PMF solely by the presence of these mutations. The genetic basis of PMF likely stems from the combination of several genetic and epigenetic phenomena.

11- The following were added to the section **Casitas B-cell Lymphoma mutation**:

The greatest frequency of mutations is found in chronic myelomonocytic leukemia (CMML) and juvenile myelomonocytic leukemia.^{45, 46} In AML, transforming activity of c-CBL may be related to augmented FLT3 signaling.⁴⁵

In the same section, a reference was added for the following sentence:

In a cohort of patients diagnosed with chronic phase MPN, CBL mutations were reported at a frequency of 6% among PMF patients whereas CBL mutations were not detected in a small series of PV and ET patients.

12- The following was added to the section **EZH2 mutation**:

Another study including 370 PMF patients revealed reduced leukemia-free survival (LFS) and overall survival (OS) after a median follow-up of 39 months in the presence of EZH2 mutations.⁵⁵ In that study, researchers concluded that EZH2 mutations are independently associated with shorter survival in PMF patients.⁵⁵

13- The following was added to the section **RAS mutations**:

NRAS mutations are found in 7-13% of post-MPN AML.

14- The following changes were made in the section **ASXL1 mutation**:

The sentence Contrary to the presence of detailed clinical studies assessing the impact of ASXL1 mutations on lifespan and response to therapy in AML and MDS, there have been few studies including limited number of patients as regards to ASXL1 in MPNs was rewritten as A number of studies have assessed the impact of ASXL1 mutations in MPNs.^{17, 91-95}

15- A new section entitled **SRSF2 gene mutations** was added to the manuscript.

16- The following were added to the section entitled **Somatic CALR mutations**:

In a recent study, the frequency of CALR mutations was 17.7% in ET and 14.8% in PMF. CALR mutations were not detected in PV.

Recent studies revealed that CALR-mutated ET is associated with a higher platelet count, lower hemoglobin, and lower leucocyte count compared to JAK2-mutated ET.^{132, 134} Although CALR-mutated ET patients have a reduced incidence of thrombosis, no differences in survival have been thus far been demonstrated for different mutation subgroups in ET.^{132, 134} Presence of CALR-mutation in PMF patients showed a favorable impact on survival independent of both DIPSS-plus risk and ASXL1 mutation status.^{131, 135}

Finally, 2014 WHO Clinical Molecular and Pathological (WHO-CMP) Diagnostic criteria suggested CALR mutations as a novel major criteria for ET and PMF.¹³⁶

17- The following was added to the **Conclusion** section:

-JAK2, MPL and CALR are the driver mutations in pathogenesis of Philadelphia-negative MPNs.¹³⁶

2008 European Clinical, Molecular and Pathological (ECMP) criteria and 2014 WHO-CMP Diagnostic criteria distinguished five distinct clonal MPNs: JAK2V617F mutated-ET and PV; JAK2 exon 12 PV and the JAK2 wild-type ET and MF mutated with MPL or CALR, thus leaving a small group of triple negative group of MPN.¹³⁶⁻¹⁴⁰ The diagnostic differentiation of JAK2, MPL and CALR-mutated MPNs should be made according to bone marrow morphology mainly based upon megakaryocyte morphology.¹³⁶

-Screening for CALR mutations is now also part of the diagnostic workup of suspected ET or PMF.

-Individualized diagnostic and therapeutic approaches are warranted to successfully treat this complex disease.

-In PMF, mutations in genes such as ASXL1, EZH2, IDH1/2 and SRSF2 were associated with a poorer survival and increased leukemic transformation.^{28, 93, 123, 141} In a study including 254 patients from the Mayo Clinic, triple-negative PMF (JAK2, MPL and CALR-negative) was associated with inferior OS and LFS.¹⁴² The aforementioned study also showed the prognostically detrimental effect of CALR⁻ASXL1⁺ mutation status.¹⁴² The prognostic advantage of CALR mutation, especially in the absence of a concomitant ASXL1 mutation was demonstrated in another large cohort of PMF patients (277 from Mayo Clinic and 293 from the University of Florence).¹⁴³ In that study, multivariable analysis showed CALR⁻ASXL1⁺ mutational status as the most significant risk factor for survival and was DIPSS-plus independent.¹⁴³ COMFORT-II study revealed that ruxolitinib (JAK1/JAK2 inhibitor) improved survival in PMF patients harboring high-molecular-risk (ASXL1, EZH2, IDH1/2 and SRSF2).¹⁴⁴ The high-molecular-risk mutations may guide novel therapeutic approaches for PMF patients. Given the relative rarity of epigenetic mutations in PV and ET patients, larger sequencing studies with comprehensive mutational data are greatly needed. CALR mutations are associated with reduced thrombosis in ET.^{132, 134} This finding remains to be validated in future prospective clinical trials. The current era of epigenetic research is giving rise to the testing of many novel agents as part of clinical trials. CALR mutations in ET and PMF result in a novel protein C terminus which holds the potential to be a target for development of tumor-specific therapy. Although certain mutations provide prognostic

information in PMF patients, several unresolved questions regarding the importance of these alterations as therapeutic targets still exist.