Comments to the Author

One common challenge of NGS is to differentiate acquired somatic mutations from germline pathogenic variants. This may be done by mutation detection in germline control samples (e.g., skin fibroblasts, saliva). Also high and stable VAF (e.g., 40–50%) at follow-up may be indicative for germline alteration. The doubt about the possibility of a germline mutation of MPL must be solved.

Author response: We greatly agree with this comment and suggestions, we detected a MPL mutation with 50.11% VAF in this patient, we highly suspected a possibility of germline mutation at first time. We try to prove it with germline control samples, unfortunately we can not obtain related samples due to loss of follow-up, so we can not prove this possibility of a germline mutation of MPL, but this not affect intriguing of this case, since co-mutated CALR and MPL in MPN is rare regardless of somatic or germline origin.

The description of the case deserves more information.One would expect the report of serum LDH, and cholesterol; one expect classification in term of prognostic score (IPSS). On expect a more detailed description of bone marrow biospy, in particular presence of clusters of megakaryocytes, description of nuclear anomalies of megakaryocytes, and myeloid cellularity.

Author response:We have added more information on LDH, cholesterol, IPSS scores, and bone marrow biopsies to the case：LDH 570IU/L, total cholesterol 2.6mmol/L, IPSS score 3, high risk group. We have added figure 2 to show nuclear anomalies of megakaryocytes including abnormal nuclear-cytoplasmic ratios, malformed nucleus, abnormal chromatin clumping with hyperchromatic nucleus and extensive lobulation of nucleus. We also have added figure 3 to show the megakaryocytic proliferation, distributed in clustersthe and the diffuse and dense increase in reticulin fibres.

The reason was not clear how ruxolitinib was administered. Part of Introduction should be spent on the information on CALR, MPL and PIK3R1. How would the authors speculate the biological significance of the mutations of CALR, MPL and PIK3R1. Did the authors conclude that ruxolitinib failed? If so, was the mutations related with the treatment failure? If ruxolibinit improved the clinical cause of patient, did the mutations affect the effects of the agent?

Author response: We greatly appreciate the reviewer comment. There are some points I would like to clarify:

1) We have added information on biological significance CALR, MPL and PIK3R1 in our discussion section: Mutations in CALR and MPL are drivers in the pathogenesis of myeloproliferative neoplasms, mutant CALR can bind with MPL to activate JAK-STAT signaling pathway. The PIK3R1 gene belongs to Akt signaling pathway, plays a direct role in regulates cell survival, growth, differentiation；

2) Ruxolitinib is a JAK/STAT inhibitor, activation of the JAK/STAT pathway is an important pathogenic mechanism in myelofibrosis, so ruxolitinib can be used to treat myelofibrosis. However, this patient did not benefit from ruxolitinib treatment. After six months of ruxolitinib treatment, the patient's spleen was enlarged and anemia worsened. Whether mutations in CALR/MPL/PIK3R1 related with the treatment outcome is still unknown, we also did not retrieve

relevant research paper, we can only say this patient cannot benefit from ruxolitinib;
3) Back to our study, we found a rare MPN patients carry CALR/MPL/PIK3R1 mutation, who has poor prognosis and cannot benefit from current clinical treatment including ruxolitinib, this finding consistent with previously reported.

The results were intriguing, but the presented clinical data were immature. Results of blood examination were absent. CT was performed, but the images were absent. The authors said that the patient had splenomegaly. It would be better to show the spleen with the CT. Bone marrow biopsy was performed. The photos of bone marrow should be presented.

Author response: Blood examination results had been showed in the case: leukocyte count was 2.9×10^9/L, platelet count was 126×10^9/L, hemoglobin was 87 g/L, and a smear revealed immature granulocytes of 4%. We have added figure 1 to show the splenomegaly. We also have added figure 2 and figure 3 to show nuclear anomalies of megakaryocytes and the megakaryocytic proliferation and distributed in clustersthe.