Leukemia
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LETTER

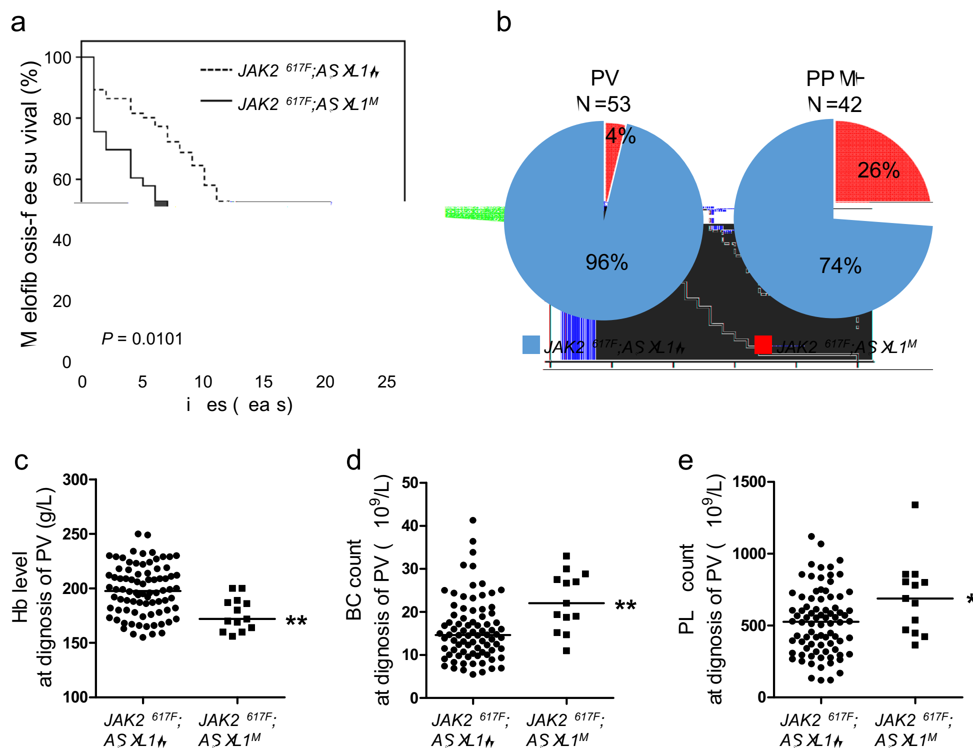
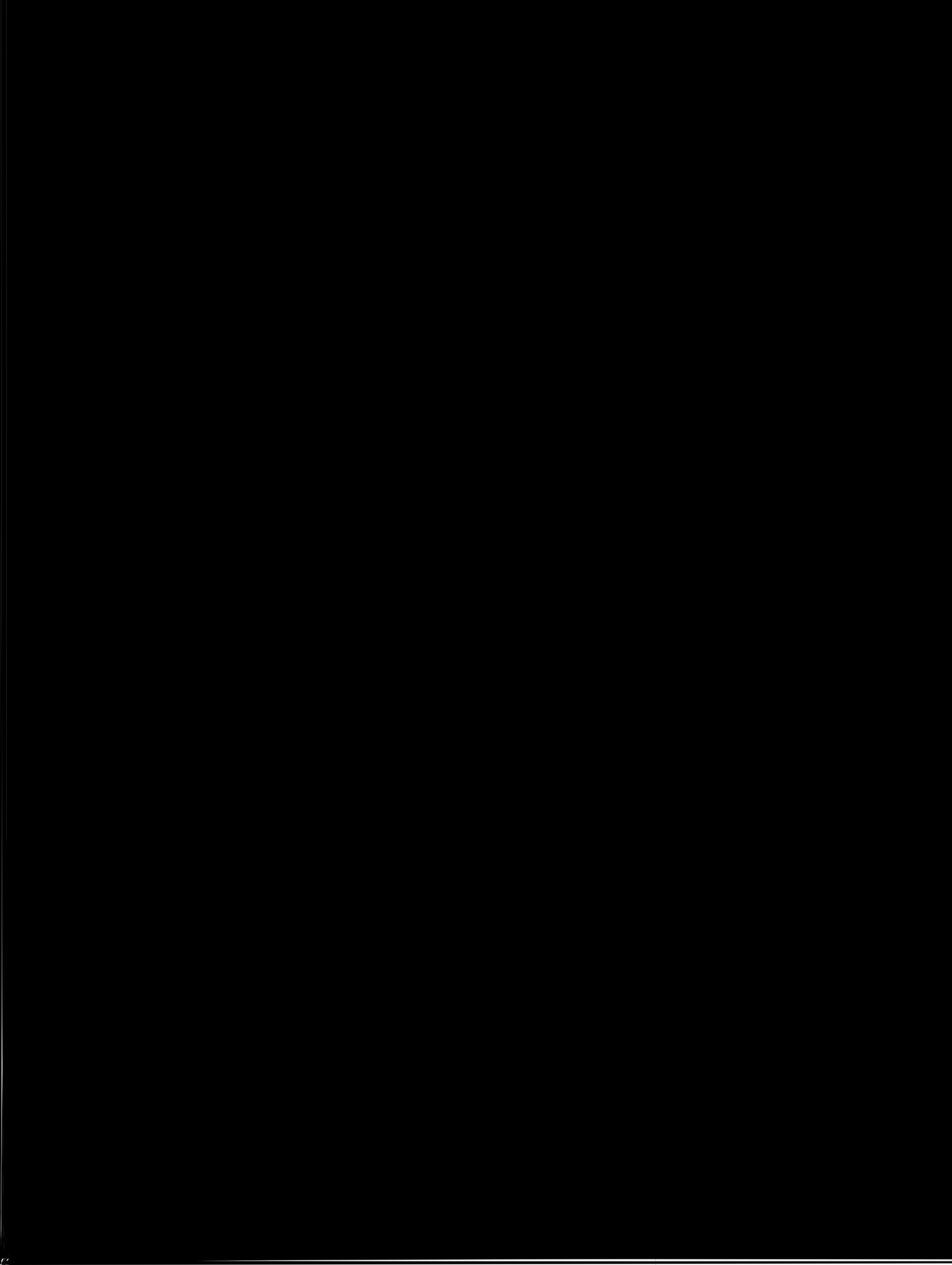


Fig. 1 *ASXL1* alteration cooperates with *JAK2*V617F to accelerate MPN progression in PV patients. **a** Myelofibrosis-free survival analysis of *JAK2*V617F PV patients according to the *ASXL1* mutational status from a Cox regression model. $ASXL1^{WT}$: $ASXL1^{wild\text{-}type}$ ($n = 82$). $ASXL1^{MUT}$: $ASXL1^{mutated}$ ($n = 13$). Log-rank test was used for survival statistics. **b** Among the 95 PV patients with *JAK2*V617F mutations, the proportion of *ASXL1* mutations is higher in PPMF (26%) than in PV patients without MF (4%). **c–e** PB counts of Hb, WBC, and PLT in *JAK2*V617F PV patients with WT *ASXL1* or mutated *ASXL1*. $*p < 0.05$; $**p < 0.01$

analyses including necropsy, histology, and flow cytometry on peripheral blood (PB), bone marrow (BM), and spleen. Fibrosis was assessed at each specified time points. We found that both $JAK2^{V617F}$;$Asxl1^{+/-}$ and $JAK2^{V617F}$ mice developed progressive MPN, including PV, ET, and MF (Supplementary Figure S1B). Notably, 5 (26%) of the 19 $JAK2^{V617F}$;$Asxl1^{+/-}$ mice developed MF at 2–6 months of age, which was much earlier than $JAK2^{V617F}$ mice (1 (6%) of the 18 mouse develop MF at 6 months of age) (Fig. 2a). Additionally, 3 (12%) of the 26 $JAK2^{V617F}$;$Asxl1^{+/-}$ mice, but not the littermate controls, progressed/transformed to secondary acute myeloid leukemia (Fig. 2a, Supplementary Figure S1B–C).

The average numbers of WBC, neutrophil, and PLT were significantly higher in the PB of $JAK2^{V617F}$;$Asxl1^{+/-}$ mice compared with the wild-type (WT) group at the age of 2–3 months. In contrast, there was no significant difference in the WBC and neutrophil counts in the $JAK2^{V617F}$ mice at the age of 2–3 months, and from 4–6 months of age, the $JAK2^{V617F}$ mice started to exhibit higher counts of WBC and neutrophil (Fig. 2b–d). The Hb levels in $JAK2^{V617F}$;$Asxl1^{+/-}$ mice with MF were the lowest among all the groups of mice at the age of 8–10 months (Supplementary Figure S2A). The $JAK2^{V617F}$;$Asxl1^{+/-}$ mice exhibited splenomegaly (Supplementary Figure S2B). The histologic analysis of the femur sections from $JAK2^{V617F}$;$Asxl1^{+/-}$ mice displayed megakaryocytic hyperplasia (Fig. 2e). Spleen sections from $JAK2^{V617F}$;$Asxl1^{+/-}$ mice showed a disrupted splenic architecture and prominent megakaryocytes and myeloid precursors (Fig. 2e). Reticulin staining of femur sections revealed extensive fibrosis in the BM of $JAK2^{V617F}$;$Asxl1^{+/-}$ mice at the age of 3 months (Fig. 2e). Consistently, flow cytometric analysis demonstrated that $JAK2^{V617F}$;$Asxl1^{+/-}$ mice had a significant expansion of erythroid precursors ($Ter119^{+}CD71^{+}$) in the BM and spleen compared with the $JAK2^{V617F}$ and WT groups (Supplementary Figure S2C). The PB smear of $JAK2^{V617F}$;$Asxl1^{+/-}$ mice contained more bluish red blood cells compared with WT PB smear, suggesting an impaired red blood cell differentiation (Supplementary Figure S2D). Interestingly, the frequency of $CD41^{+}CD61^{+}$ megakaryocytic precursors was significantly increased in the BM of $JAK2^{V617F}$;$Asxl1^{+/-}$ mice by flow cytometric analysis compared with WT and $JAK2^{V617F}$ mice (Supplementary Figure S2E). These data demonstrate that concurrent haploinsufficiency of *Asxl1* and *JAK2*V617F enhances megakaryopoiesis and increases erythroid precursors in the BM and spleen, which may accelerate MF development in vivo.

To determine the effect of *Asxl1* alteration on $JAK2^{V617F}$ HSC/HPCs, we performed flow cytometric analyses and found that the frequencies of short-term (ST)- HSC and megakaryocyte/erythroid progenitor (MEP) were significantly increased in the BM of $JAK2^{V617F}$;$Asxl1^{+/-}$ mice compared with those in WT mice, while the frequency of multipotent hematopoietic progenitors (MPP) was significantly decreased in the BM of $JAK2^{V617F}$;$Asxl1^{+/-}$ mice compared with that in WT mice (Supplementary Figure S3A–B). Colony-forming unit (CFU) assays



revealed the total colony number and replating capacity in the BM cells of *JAK2*^{V617F};*Asxl1*$^{+/-}$ mice was significantly higher compared with those in the WT group of mice (Supplementary Figure S3C–D). Furthermore, we observed the frequencies of burst-forming unit-erythroid (BFU-E), CFU-erythroid (CFU-E), and CFU-megakaryocyte (CFU-MK) colonies were significantly increased in response to a series of doses of erythropoietin (EPO) in the spleen and BM cells from *JAK2*^{V617F};*Asxl1*$^{+/-}$ mice (Fig. 2f, Supplementary Figure S3E). Notably, EPO-independent BFU-E and CFU-E formation, a hallmark feature of PV [15], were also higher in the spleen and BM cells from *JAK2*^{V617F};*Asxl1*$^{+/-}$ mice compared with other groups of mice (Fig. 2f, Supplementary Figure S3E). Thus *Asxl1* alteration cooperates with *JAK2*V617F mutation leading to biased lineage skewing, favoring erythroid and megakaryocytic differentiation.

We have reported that leukemic transformation in MDS/MPN can occur in the aged *Asxl1*$^{+/-}$ mice (>16 month old) [14]. In the current study, we found that three *JAK2*^{V617F};*Asxl1*$^{+/-}$ mice developed myeloid leukemia at the age of 6–8 months, which is much earlier than that found in *Asxl1*$^{+/-}$ mice. Two of the *JAK2*^{V617F};*Asxl1*$^{+/-}$ mice also had intestinal myeloid sarcoma, which were verified by histology and flow cytometric analysis (Supplementary Figure S4A–C). The moribund leukemic *JAK2*^{V617F};*Asxl1*$^{+/-}$ mice had blast cells in PB, >20% blast cells in BM, and splenomegaly (Supplementary Figure S4D–G). The histologic analyses of femur sections revealed an increase of megakaryocytes and a decrease in erythroid islands of these *JAK2*^{V617F};*Asxl1*$^{+/-}$ mice but not in any other groups of mice. The spleen sections of *JAK2*^{V617F};*Asxl1*$^{+/-}$ mice showed a disrupted architecture with an increased proportion of myeloid cells. Reticulin staining showed extensive BM fibrosis in *JAK2*^{V617F};*Asxl1*$^{+/-}$ mice (Supplementary Figure S4H). These data indicate that *Asxl1* alteration cooperates with *JAK2*V617F mutation to accelerate myeloid leukemic transformation.

In summary, PV patients with co-mutations of *ASXL1* and *JAK2*V617F had a poor MF-free survival. Likewise, *Asxl1* loss accelerates MF in *JAK2*V617F-driven MPN in mice. *JAK2*^{V617F};*Asxl1*$^{+/-}$ mice induces megakaryocytic hyperplasia and can transform to myeloid leukemia. Future studies using the *Asxl1* and *JAK2*^{V617F} co-mutated mice to further investigate the cooperative effect between *ASXL1* mutant and *JAK2*V617F in the progression of myeloid malignancies are warranted.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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