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LETTER

Animal models

ASXL1 alteration cooperates with *JAK2*V617F to accelerate myelofibrosis

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To the Editor:

M yeloproliferative neoplasms (MPNs) are clonal hematopoietic stem cell disorders. MPNs are characterized by aberrant hematopoietic proliferation of one or more hematopoietic cell lineages with increased risks of myelofibrosis (MF) progression and leukemic transformation [1]. MPNs are distinguished into three clinical entities: polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) [2]. The somatic mutation of *JAK2V*617F is considered to be the most notable landmark in the diagnosis of classic Philadelphia chromosome-negative MPNs and is present in >95% of PV patients and in ~50% of ET and PMF [3, 4]. High-throughput genomic analyses of MPN patients have identified the concurrent somatic mutations of epigenetic regulators such as *ASXL1*, *TET2*, *IDH*, *EZH2*, and *DNMT3A* co-occurring with *JAK2* mutation, which are involved in clonal evolution, disease progression, and/or poor survival in MPNs [5, 6]. ASXL1 (additional sex combs-like 1) is a polycomb group protein that putatively functions as a chromatin modifier. *ASXL1* mutations were identified in all types of myeloid malignancies [7–11]. In all, 34.5% of *ASXL1* mutations are found in PMF patients [11]. Despite the significant impact of *ASXL1* alteration and *JAK2*V617F on the pathogenesis of MPNs, the importance of concomitant alterations of *ASXL1* and *JAK2*V617F within distinct hematopoietic compartments and disease progression remains to be elucidated.

We assessed the frequency of *ASXL1* mutations, the clinical features, and the cumulative MF-free survival in 95 PV patients with *JAK2*V617F mutations. The diagnoses were made according to the 2016 World Health Organization criteria [12]. Among the 95 PV patients, 13 patients carrying co-mutations of *ASXL1* and *JAK2*V617F had poor MF-free survival (Fig. 1a), and the proportion of *ASXL1* mutations is higher in post-PV MF (PPMF) patients (26%) than in PV patients without MF (4%) (Fig. 1b). These patients carrying co-mutations of *ASXL1* and *JAK2*V617F had decreased level of hemoglobin (Hb), increased counts of white blood cell (WBC) and platelet (PLT), palpable splenomegaly, and clonal abnormal karyotypes when compared with PV patients with *JAK2*V617F mutation only (Fig. 1c–e, Supplementary Tables S1 and S2).

To further study the impact of *Asxl1* alteration on disease progression in *JAK2*V617F-mediated MPNs and *JAK2*V617F-mutant hematopoietic stem and progenitor cell (HSC/HPC) function, we next crossed the $JAK2^{V617F}$ mice with $Asxl1^{+/-}$ mice and assessed hematopoietic phenotypes in vivo [13, 14]. We found that $JAK2^{V617F}$;$Asxl1^{+/-}$ mice had significantly shorter mean survival rate (~60%) than $JAK2^{V617F}$ or $Asxl1^{+/-}$ mice (Supplementary Figure S1A). To classify the hematopoietic phenotypes in $JAK2^{V617F}$;$Asxl1^{+/-}$ mice, we performed a series of

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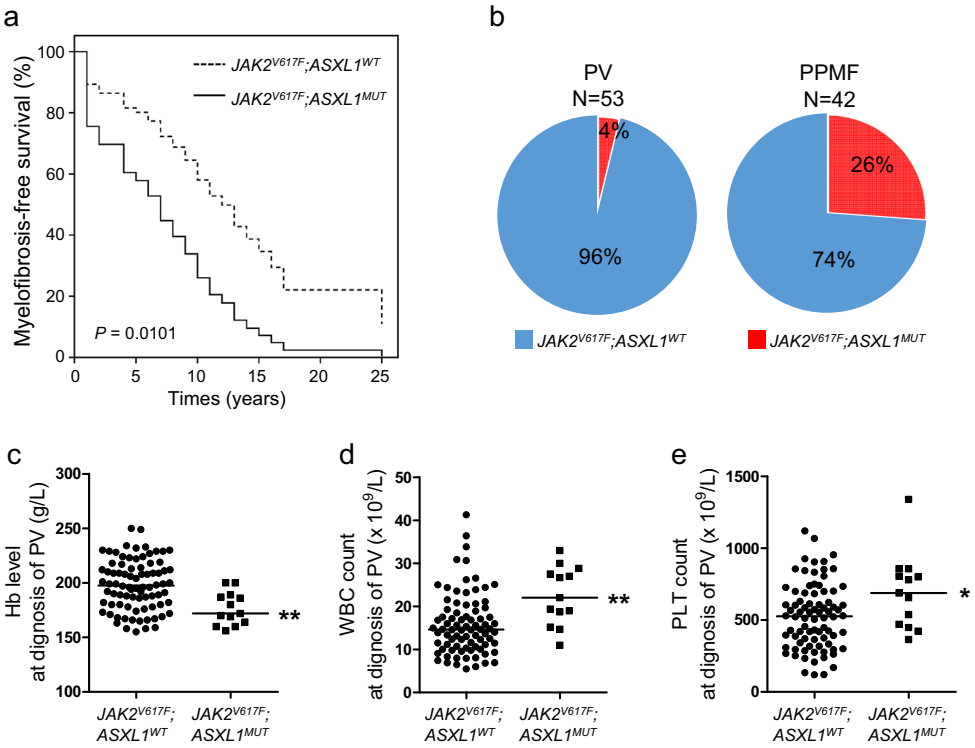

Fig. 1 *ASXL1* alteration cooperates with *JAK2*V617F to accelerate MPN progression in PV patients. **a** Myelofibrosis-free survival analysis of *JAK2*V617F PV patients according to the *ASXL1* mutational status from a Cox regression model. $ASXL1^{WT}$: $ASXL1^{wild\text{-}type}$ ($n = 82$). $ASXL1^{MUT}$: $ASXL1^{mutated}$ ($n = 13$). 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

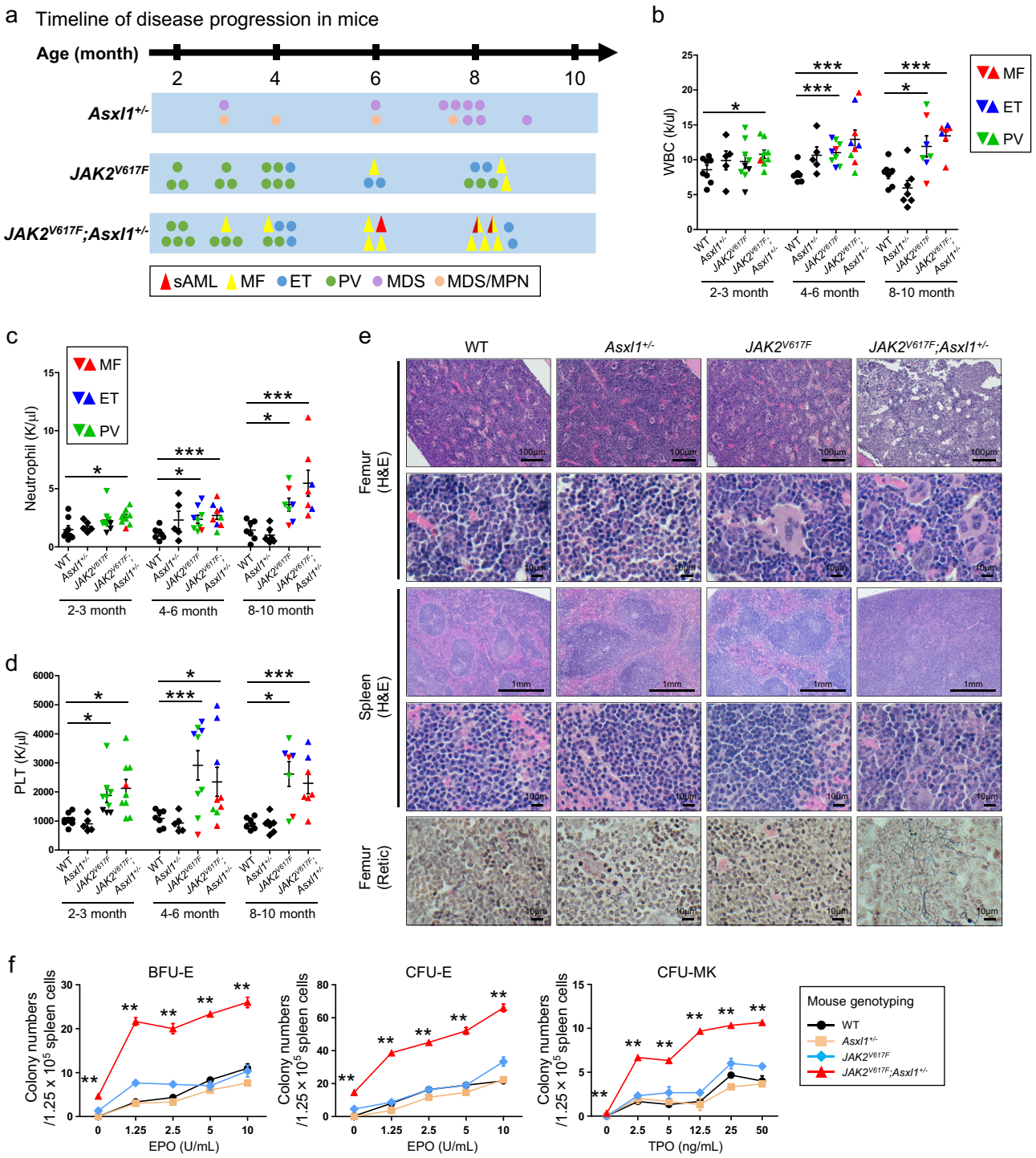

Fig. 2 *Asxl1* alteration cooperates with *JAK2*V617F to promote MF in mice. **a** Timeline of disease progression in diseased *Asxl1*$^{+/-}$,*JAK2*^{V617F}, and *JAK2*^{V617F};*Asxl1*$^{+/-}$ mice. 5 of the 19 *JAK2*^{V617F};*Asxl1*$^{+/-}$ mice developed MF at 2–6 months of age ($p = 0.003$, binomial test). sAML: secondary acute myeloid leukemia **b–d** PB counts showing the numbers of WBC, neutrophil, and PLT in WT, *Asxl1*$^{+/-}$,*JAK2*^{V617F}, and *JAK2*^{V617F};*Asxl1*$^{+/-}$ mice according to ages of mice. **e** Shown are the hematoxylin and eosin (H&E) staining of representative femur and spleen sections and reticulin staining of femur sections from representative WT, *Asxl1*$^{+/-}$,*JAK2*^{V617F}, and *JAK2*^{V617F};*Asxl1*$^{+/-}$ mice at 3 months of age. **f** Spleen cells from four groups of mice (1 month old, 3 mice per genotype) were plated in methylcellulose medium supplemented with cytokines and varying concentrations of EPO or thrombopoietin (TPO). Error bars represent mean ± standard error of the mean (s.e.m.). $*p < 0.05$; $**p < 0.01$; $***p < 0.001$

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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