



Novel lysyl oxidase inhibitors attenuate hallmarks of primary myelofibrosis in mice

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Abstract

Primary myelofibrosis (PMF) is a chronic myeloproliferative neoplasm (MPN) that usually portends a poor prognosis with limited therapeutic options available. Currently, only allogeneic stem cell transplantation is curative in those who are candidates, while administration of the JAK1/2 inhibitor ruxolitinib carries a risk of worsening cytopenia. The limited therapeutic options available highlight the need for the development of novel treatments for PMF. Lysyl oxidase (LOX), an enzyme vital for collagen cross-linking and extracellular matrix stiffening, has been found to be upregulated in PMF. Herein, we evaluate two novel LOX inhibitors, PXS-LOX_1 and PXS-LOX_2, in two animal models of PMF (GATA1low and JAK2V617F-mutated mice). Specifically, PXS-LOX_1 or vehicle was given to 15- to 16-week-old GATA1low mice via intraperitoneal injection at a dose of 15 mg/kg four times a week for 9 weeks. PXS-LOX_1 was found to significantly decrease the bone marrow fibrotic burden and megakaryocyte number compared to vehicle in both male and female GATA1low mice. Given these results, PXS-LOX_1 was then tested in 15- to 17-week-old JAK2V617F-mutated mice at a dose of 30 mg/kg four times a week for 8 weeks. Again, we observed a significant decrease in bone marrow fibrotic burden. PXS-LOX_2, a LOX inhibitor with improved oral bioavailability, was next evaluated in 15- to 17-week-old JAK2V617F-mutated mice at a dose of 5 mg/kg p.o. four times a week for 8 weeks. This inhibitor also resulted in a significant decrease in bone marrow fibrosis, albeit with a more pronounced amelioration in female mice. Taking these results together, PXS-LOX_1 and PXS-LOX_2 appear to be promising new candidates for the treatment of fibrosis in PMF.

Keywords Myelofibrosis · Lysyl oxidase · Myeloproliferative neoplasm

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Introduction

Primary myelofibrosis (PMF) is a myeloproliferative neoplasm (MPN) that arises from a clonal proliferation of hematopoietic stem cells leading to progressive bone marrow (BM) fibrosis and generally a poor prognosis [1, 2]. Currently, mutation in one or more genes (*JAK2*, *MPL* and *CALR*) are present in 90% of PMF cases with 10% being “triple negative” [3, 4]. The most common mutation in PMF is the JAK2V617F hyper-activating mutation, found in 60% of PMF patients [4, 5]. Mutations in the *CALR* and *MPL* genes account for 25% and 5% of PMF cases, respectively, and both also lead to hyperactivation of the JAK/STAT signaling pathway [6–8]. Regardless of the mutation involved, PMF results in BM fibrosis and dysregulation of extracellular matrix (ECM) remodeling, which leads to progressive ECM deposition [9]. One important enzyme class involved with ECM deposition are the lysyl oxidases. Lysyl oxidases

are copper-dependent enzymes that cause oxidative deamination of lysine and hydroxylysine residues in collagen and elastin, leading to augmented cross-linking of these proteins and a stiffer matrix [10]. The lysyl oxidase family consists of five members in mammals, designated lysyl oxidase (LOX) and lysyl oxidase like-1 to lysyl oxidase like-4 (LOXL1–LOXL4). All five members have a conserved C-terminal domain that contains the active enzyme region and a more variable N-terminal pro-region. All lysyl oxidases have a signal peptide and are secreted into the extracellular environment, and their roles in different pathologies depend on which tissue and cell type each gene is expressed. Our previous studies found that one specific family member, LOX, is expressed in immature megakaryocytes (much like those in PMF) and downregulated in normally developing megakaryocytes with higher ploidy. Accordingly, LOX has been found to be upregulated in human PMF megakaryocytes and in mouse models of PMF [11–13]. Moreover, inhibition of LOX using a commercially available pan lysyl oxidase inhibitor, β -aminopropionitrile (BAPN), reduced the pathogenesis of the myelofibrotic phenotype in the GATA-1 mouse model of PMF [12]. The GATA-1low is a well-studied mouse model that has a very similar phenotype to human PMF including BM fibrosis and splenomegaly [12, 14, 15]. LOX has also been shown to affect platelet derived growth factor (PDGF) signaling by oxidizing the PDGF receptor and enhancing downstream signaling [16].

β -Aminopropionitrile (BAPN) is a molecule that inhibits all lysyl oxidase family members. It is, however, also recognized as a substrate by other amine oxidases and, therefore, might have several off-target activities. Through careful design, haloallylamine-based inhibitors have been developed that specifically target lysyl oxidases [17]. Two such inhibitors, PXS-LOX_1 and PXS-LOX_2 [18, 19] have been evaluated for their ability to reduce fibrosis in mouse models of PMF, GATA-1low and JAK2V617F [11, 12]. Recent studies showed that GATA-1 is downregulated in PMF megakaryocytes in humans and mouse models and the loss of GATA-1 drives the dysmegakaryopoiesis seen in PMF [20, 21]. The effectiveness of such inhibitors encourages further development of therapeutic approaches to target the ECM in PMF.

Methods

Mice

GATA-1low mice breeding pairs were kindly donated by Dr. John Crispino (Northwestern University) and have a bone marrow fibrotic phenotype that has been previously described [22]. Vav1-hJAK2^{V617F} (JAK2V617F) mice were gift from Dr. Zhizhuang Joe Zhao (University of Oklahoma).

This mouse line has hallmarks of PMF, including expansion of the megakaryocyte lineage and a fibrotic bone marrow [23]. Expansion of the mouse colonies was performed at Boston University School of Medicine, and all studies involving mice were approved by the Boston University Animal Care and Use Committee. Animal housing conditions and treatment protocols were approved by the Institutional Animal Care and Use Committee of Boston University School of Medicine.

Drug administration into mice

PXS-LOX_1 was mixed with pure olive oil at a concentration of 5 mg/mL. GATA-1low mice received intraperitoneal injections of either PXS-LOX_1 ($n=8$, five males and three females) or vehicle (olive oil, $n=9$, five males and four females) at a dose of 15 mg/kg four times a week for nine weeks and were then sacrifice for analysis. Spleen and femurs were harvested for analysis at sacrifice. At the beginning of experiments (drug administration), mice of 15- to 16-weeks old were used. At this mouse age we expect initiation of fibrosis [23], also based on our earlier study [24]. This dosage of PXS-LOX_1 was chosen as it provided inhibition of LOX activity.

PXS-LOX_2 has improved oral bioavailability and was given orally. PXS-LOX-2 was dissolved in phosphate-buffered saline (PBS) at a concentration of 1 mg/mL, aliquoted in single-use volumes and frozen at -20°C . At the day of the treatment, aliquots were thawed at room temperature and administered by oral gavage. Animals were treated at the dose of 5 mg/kg, once a day four times a week for a total of 8 weeks. Treatment groups consisted of JAK2V617F/PXS-LOX_2 ($n=7$, four males and three females), JAK2V617F/vehicle (PBS, $n=6$, four males and two females), C57BL6J/PXS-LOX_2 ($n=11$, five males and six females), C57BL6J/vehicle (PBS, $n=10$, four males and six females). Animals were at 15–17 weeks of age at the start of the treatment. Peripheral blood counts were analyzed monthly, including at pre-treatment, and weight was measured bi-weekly, including at pre-treatment. At the end of the treatment, plasma was obtained from EDTA-anticoagulated peripheral blood collected by cardiac puncture, aliquoted, and frozen at -80°C . Abdominal artery and ear flaps were collected and frozen immediately on liquid nitrogen and stored at -80°C . Spleen was harvested, weighted and separated into frozen samples for hydroxyproline analysis and fixed samples for histology. Femurs were fixed for histological analysis.

Blood cell counts

Blood was collected via retro-orbital plexus sampling before the start of injections and on the day of sacrifice and analyzed using Hemavet multispecies hematology analyzer

(Drew Scientific, Dallas, TX, catalog no. HV950FS) for complete blood count (CBC).

Histology and quantitation of bone marrow fibrotic burden

Femurs and spleens were taken at the end of treatment and fixed in 10% (v/v) phosphate-buffered formalin. Femurs were decalcified with 0.5 M EDTA pH 7.4 at 4 °C for 1 week, paraffin-embedded and 5 µm sections were prepared. Slides were then stained with either hematoxylin–eosin or modified Gomori stain for reticulin fibers [25], or used for immunohistochemistry (IHC) with anti-CD31 to both recognize blood vessels and megakaryocytes [26]. For bone marrow quantitative measure of fibrosis, 20 digital color images per stained and mounted bone section were captured along the diaphysis longitudinal axis following a rectangular pattern. This imaging scanning approach has been validated by determining the total reticulin fibers occupancy, and then comparing it with the value obtained from the whole image of the same BM section. This approach is published in Fig. 3 of our recent paper [25]. Briefly, by blind coding image identities to allow for threshold input, and using shape filtering to further eliminate background, we were able to quantitate reticulin fibrosis. Color images were batch-analyzed using ImageJ software, aided by our two newly added macros, as we previously described in [25]. Reticulin fibrosis in spleen sections was quantitated by the same approach. For megakaryocyte number in femur and spleens, 20 images per mouse of randomly chosen hematoxylin–eosin stained slides (H&E) were acquired at 20×. megakaryocyte number was confirmed by counting CD3-positive non-vascular cells.

Immunohistochemistry

Immunostaining was carried out in our university Immunohistochemistry (IHC) core. Briefly, IHC was performed on slides of paraffin-embedded, 5-µm-thick sections by a standard protocol on an IntelliPATH Automated Slide Staining System from Biocare Medical. Slides were heated for 15 min at 60 °C, followed by deparaffinization and rehydration through grading of ethanol to distilled water. Antigen-retrieval was performed using Rodent Decloaker reagent at 95 °C for 20 min, and cooled down at 85 °C for 10 min. Slides were incubated with Biocare Peroxidase 1 solution for 10 min at room temperature (RT), washed with Tris-buffered saline (TBS, blocked with Biocare Rodent Block M for 30 min and washed. Rabbit anti-CD31 primary antibody was incubated for 2 h at RT at a 1:200 dilution). Incubation in Biocare Rabbit on Rodent HRP polymer (Biocare cat no. RMR622) was then performed for 30 min followed by washing in TBS. 3,3'-diaminobenzidine (DAB) was diluted in DAB substrate buffer and

applied to slide for 5 min followed by washing in distilled-H₂O. A light hematoxylin stain was applied followed by dehydration, air drying and mounting with EcoMount with a coverslip.

Statistical analysis

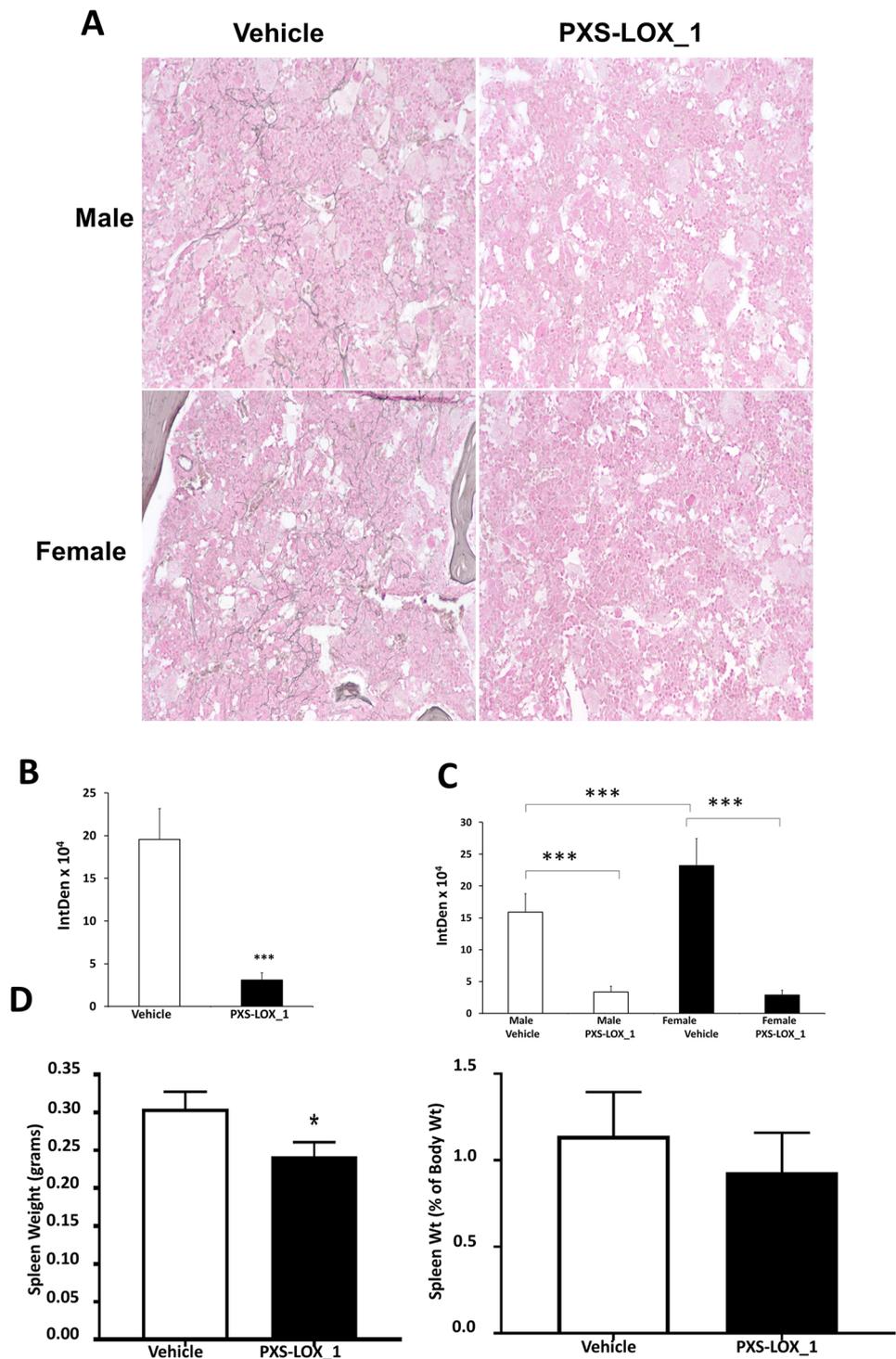
Statistical analysis was performed using GraphPad Prism. To find out if the means between two populations are significantly different, ANOVA test was run (F statistics value compared to F critical). Student's *t* test and ANOVA single factor with an α of at least 0.05 were considered significant.

Results

Properties of LOX inhibitors PXS-LOX_1 and PXS-LOX_2

The archetypal small molecule LOX inhibitor, β -aminopropionitrile (BAPN) is also known to act as a substrate for other amine oxidases, including semicarbazide-sensitive amine oxidase and diamine oxidase. This substrate activity leads to the generation of reactive species (aldehydes and hydrogen peroxide) which can cause damage in various organs and limits the therapeutic utility of BAPN for PMF. Herein, we sought to examine two novel haloallylamine-based LOX inhibitors, PXS-LOX_1 and PXS-LOX_2 [19]. While these compounds are mechanism-based inhibitors with a similar potency to BAPN (Supplemental Table 1) they have been designed to reduce off-target activity. Evidence for mechanism-based inhibition is provided by classical tests for irreversibility and time-dependent enzyme inactivation. The test for irreversibility involves measurement of the recovery of activity after 100-fold dilution from $10 \times IC_{50}$ of the inhibitor. Using LOXL1 as a surrogate for LOX (owing to similar pharmacologies), PXS-LOX_1 and PXS-LOX_2 maintained > 90% inhibition ($n = 3$) even at concentrations $< 0.1 \times IC_{50}$. PXS-LOX_1 is a fast clearing compound in mice. As shown in Supplemental Fig. 1, a single iv injection causes rapid increase in the plasma, which then quickly declines with $t_{1/2}$ of 12 min. When given orally at 10 mg/kg, PXS-LOX_1 concentration peaks at 15 min after oral gavage, and then quickly decays. The oral bioavailability calculated from AUC_{0-inf} is 17%, which is not sufficient for a block of lysyl oxidase enzymes. Therefore, PXS-LOX_1 was dissolved in olive oil and injected ip. This resulted in a high initial concentration that lasted for several hours. In contrast to the low oral bioavailability of PXS-LOX_1, PXS-LOX_2 has a substantially improved oral bioavailability (Supplemental Fig. 1).

Fig. 1 The fibrotic burden is reduced in PXS-LOX_1-treated GATA-1low mice. **a** GATA-1 low mouse femur sections were subjected to reticulin staining (shown is a representative image). **b** Image analysis was performed as described in “Histology and quantitation of bone marrow fibrotic burden” section. Reticulin fibrosis in both male and female mice that were treated with PXS-LOX_1 showed a reduction in fibrotic burden by 87.8% when compared to untreated mice. **c** In vehicle-treated GATA1low mice females showed 31.6% higher level of reticulin fibrosis than males. Treatment with PXS-LOX_1 reduced fibrosis by 79% and 88% in male and female mice, respectively. **d** Spleens were collected from mice at sacrifice and weighed. Data are averages \pm SD for nine vehicle-treated (five male, four female) and eight PXS-LOX_1-treated (five male, three female) mice, with 20 randomly chosen images analyzed for fibrosis per mouse. All compared values showed $p < 0.05$, and F statistic $> F$ critical, thus rejecting the null hypothesis by ANOVA single factor analysis. * $p < 0.05$, *** $p < 0.001$



LOX inhibitor PXS-LOX_1 attenuated bone marrow fibrosis in GATA-1low mice

Both male and female GATA1low mice tolerated the PXS-LOX_1 regimen at the concentration and duration used (see “Methods”). Upon sacrifice, LOX activity was measured and the reduction in activity in treated versus untreated animals

was confirmed (Supplemental Fig. 2). There was no difference in the weekly weights between the vehicle- and PXS-LOX_1-treated groups in either sex (Supplemental Fig. 3). Blood cell count was similar in the experimental groups, except for a small but statistically significant reduction in platelet count at the end of the injection period (Supplemental Fig. 4). Using our previously published method of blindly

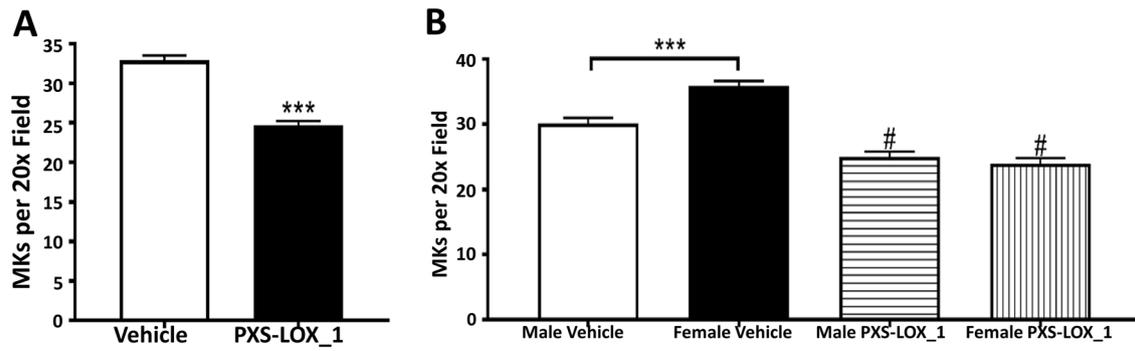


Fig. 2 Megakaryocyte number is reduced in PXS-LOX₁-treated GATA-1low mice. **a** Megakaryocyte number in GATA-1low mice treated with vehicle or PXS-LOX₁. Numbers were counted in sections parallel to those evaluated in Fig. 1 as described under methods. Data shown are combined for male and female mice. **b** Megakaryo-

cyte number in female vs male GATA-1low mice treated with vehicle or PXS-LOX₁. Data are averages \pm SD for $n=9$ GATA-1low vehicle (five male, four female) and 8 GATA-1low PXS-LOX₁ treated (five male, three female) mice. *** $p < 0.001$, # $p < 0.001$ vs respective sex vehicle

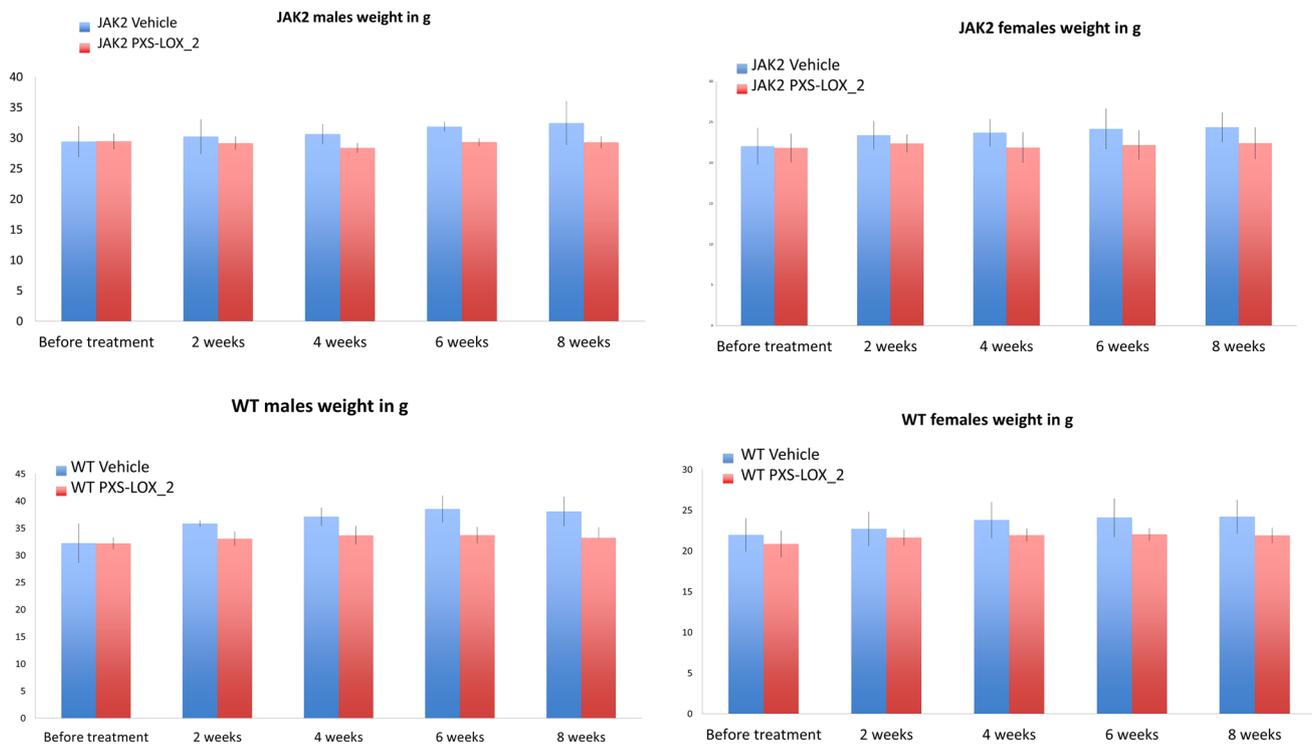


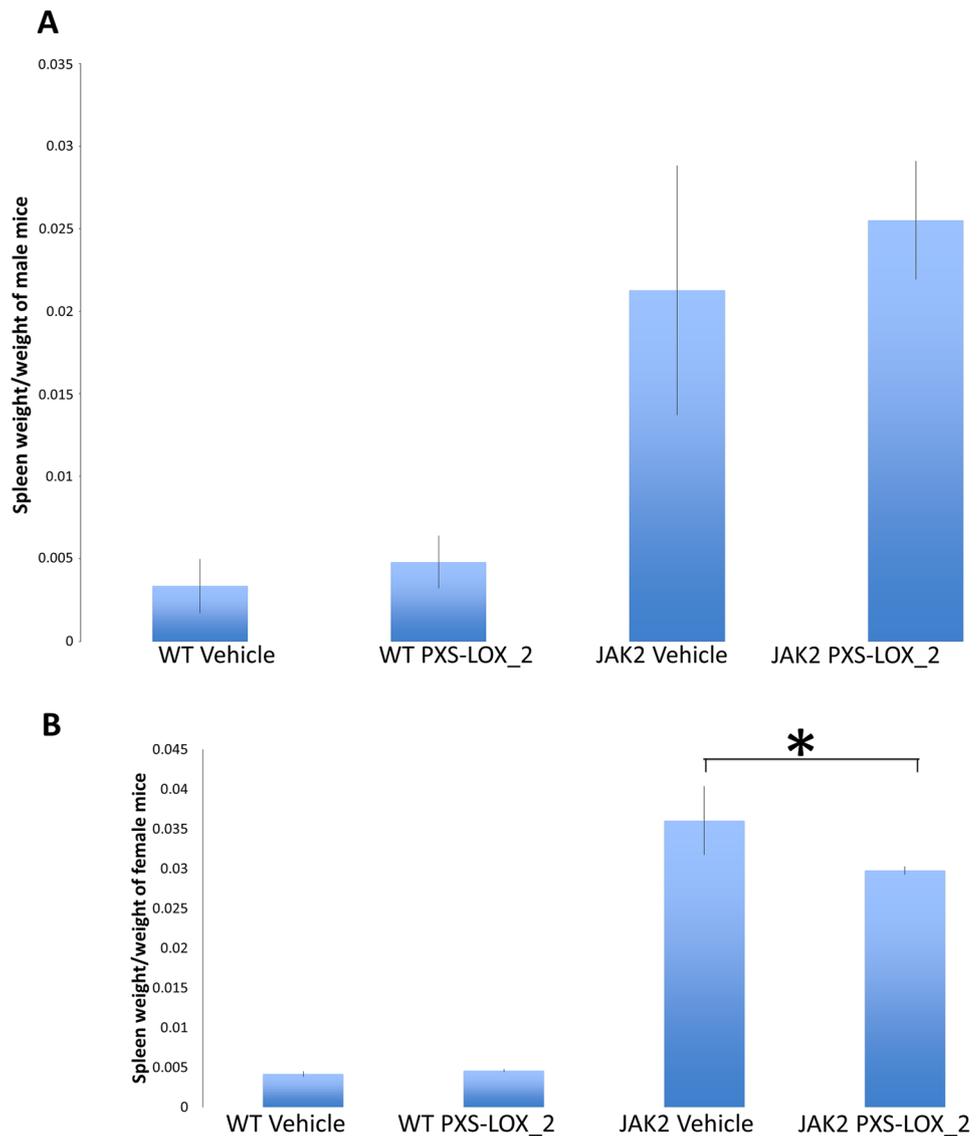
Fig. 3 Weights of PXS-LOX₂- or vehicle-treated WT or JAK2V617F (JAK2) mice. Male and female JAK2 and wild type (WT) matching vehicle- or PXS-LOX₂-treated mice were weighted every 2 weeks for a total of 8 weeks. Data are averages \pm SD, for four

vehicle- and five PXS-LOX₂-treated male WT mice, and four vehicle- and four PXS-LOX₂-treated male JAK2 mice; six vehicle- and six PXS-LOX₂-treated female WT mice, and five vehicle- and four PXS-LOX₂-treated female JAK2 mice

quantitating of bone marrow (BM) fibrosis within several BM grids [25] we looked at the effect of PXS-LOX₁ on BM fibrosis. BM fibrosis was significantly reduced in GATA-1low PXS-LOX₁-injected mice compared to vehicle in both males and females (Fig. 1). Interestingly, female

vehicle-treated mice had significantly greater BM fibrosis compared to male vehicle-treated mice, however, there was no difference between female and male PXS-LOX₁-injected mice (Fig. 1a–c). Splenomegaly is a hallmark of PMF and contributes to the debilitating symptoms of this

Fig. 4 Spleen weight is reduced in PXS-LOX₂-treated female JAK2V617F (JAK2) mice. Splens were collected from male (a) and female (b) mice at sacrifice and weighed. Female PXS-LOX₂-treated JAK2 mice had significantly decreased spleen weight compared to vehicle-treated mice. Data are averages \pm SD, for four vehicle- and five PXS-LOX₂-treated male WT mice, and four vehicle- and four PXS-LOX₂-treated male JAK2 mice; six vehicle- and six PXS-LOX₂-treated female WT mice, and five vehicle- and four PXS-LOX₂-treated female JAK2 mice; * $p < 0.05$ for PXS-LOX₂-treated female mice compared to vehicle-treated female mice



pathology. PXS-LOX₁-injected GATA1^{low} mice appeared to have a modest but significant decrease in spleen size compared to vehicle-injected mice, which appeared as a trend when normalized to body weight (Fig. 1d). Fibrosis was also reduced in the spleen of the drug-treated mice (Supplemental Fig. 5).

PXS-LOX₁ reduced the number of megakaryocytes in bone marrow of GATA1^{low} mice

Our previous studies showed that LOX boosts megakaryocyte expansion via enhancement of PDGF signaling [12, 16]. We counted megakaryocytes in H&E stained BM slides and confirmed this by staining for CD31. Mice injected with PXS-LOX₁ showed a significantly reduced numbers of megakaryocytes in the BM compared to vehicle-injected mice (Fig. 2). Interestingly, and congruent to the BM fibrosis

burden described above, female vehicle-treated mice had a significantly greater number of BM megakaryocytes compared to male vehicle-treated mice. There was no difference between males and females in the PXS-LOX₁-treated group (Fig. 2 and Supplemental Fig. 6).

LOX inhibitor PXS-LOX₂ administration

Encouraged by the results of the above exploratory experiment using PXS-LOX₁, we moved to evaluate the orally bioavailable LOX inhibitor PXS-LOX₂ in a classical mouse model of PMF, the humanized mouse bearing the JAK2V617F hyper-activating mutation. Oral administration (see “Methods”) of PXS-LOX₂ had no significant impact on blood cell count (Supplemental Fig. 7). There was a small tendency for reduced body weight (in range of 10%) in both male and female JAK2V617F mice administered with the

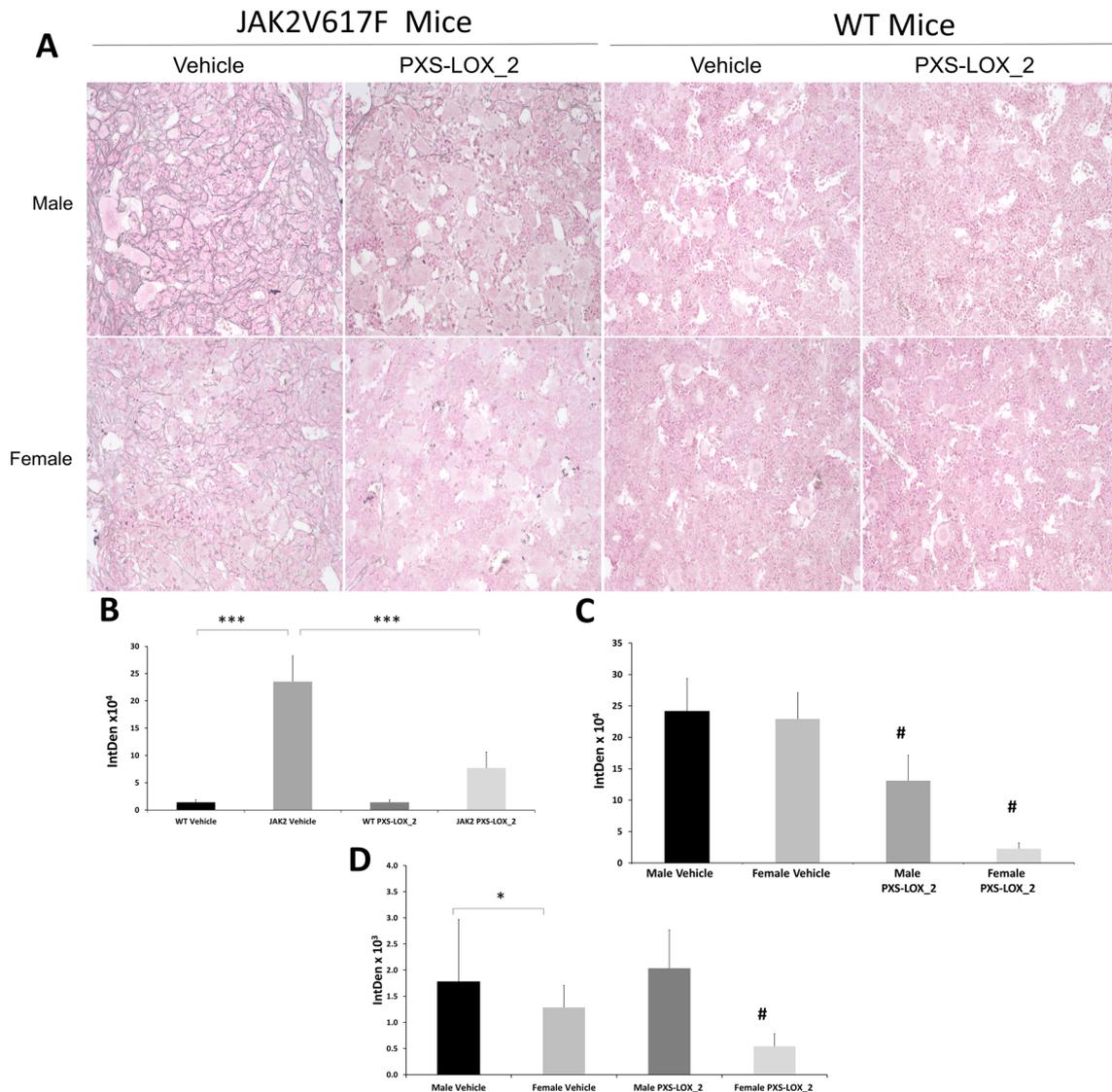


Fig. 5 The fibrotic burden is reduced in PXS-LOX₂-treated JAK2V617F mice. **a** JAK2V617F (JAK2) and WT mouse femur sections were subjected to reticulin staining and reticulin fibrosis quantitation was done as referenced in Fig. 1 legend. **b** JAK2 mice treated with PXS-LOX₂ showed a 67.3% reduction in reticulin. The background reticulin content in wild type mice was insensitive to PXS-LOX₂ treatment. **c** Controls male and female JAK2 mice showed similar level of reticulin fibrosis. LOX₂ treatment reduced reticulin fibrosis by 45.7% in male mice and 90.0% in female mice. **d** WT male controls showed 37.0% higher reticulin content than female controls.

PXS-LOX₂ treatment reduced reticulin content by 61.5% in female mice with no significant effect on male mice. Data are averages ±SD for JAK2 vehicle-treated (four male, five female), seven JAK2 PXS-LOX₂-treated (four male, three female), ten WT vehicle-treated (four male, six female) and 11 WT PXS-LOX₂-treated (five male, six female) mice, with 20 randomly chosen images analyzed per mouse. All compared values showed *p* < 0.05, and F statistic > F critical, thus rejecting the null hypothesis by ANOVA single factor analysis. ****p* < 0.001, #*p* < 0.001 vs respective sex vehicle

drug compared to vehicle (Fig. 3). The reduction in LOX activity in treated versus untreated animals was confirmed (Supplemental Fig. 2). Spleen weight in female mice was significantly reduced in PXS-LOX₂-treated mice compared to vehicle-treated, but this was not noted in male mice (Fig. 4).

PXS-LOX₂ reduces bone marrow fibrosis and megakaryocyte number in female JAK2V617F mice

JAK2V617F and age- and sex-matched WT mouse femur sections were stained and evaluated for reticulin fibers (Fig. 5a). There was no difference between WT vehicle-treated and WT PXS-LOX₂-treated mice (Fig. 5b). Male

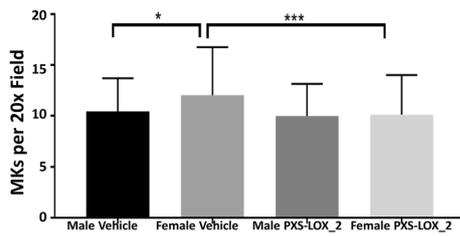
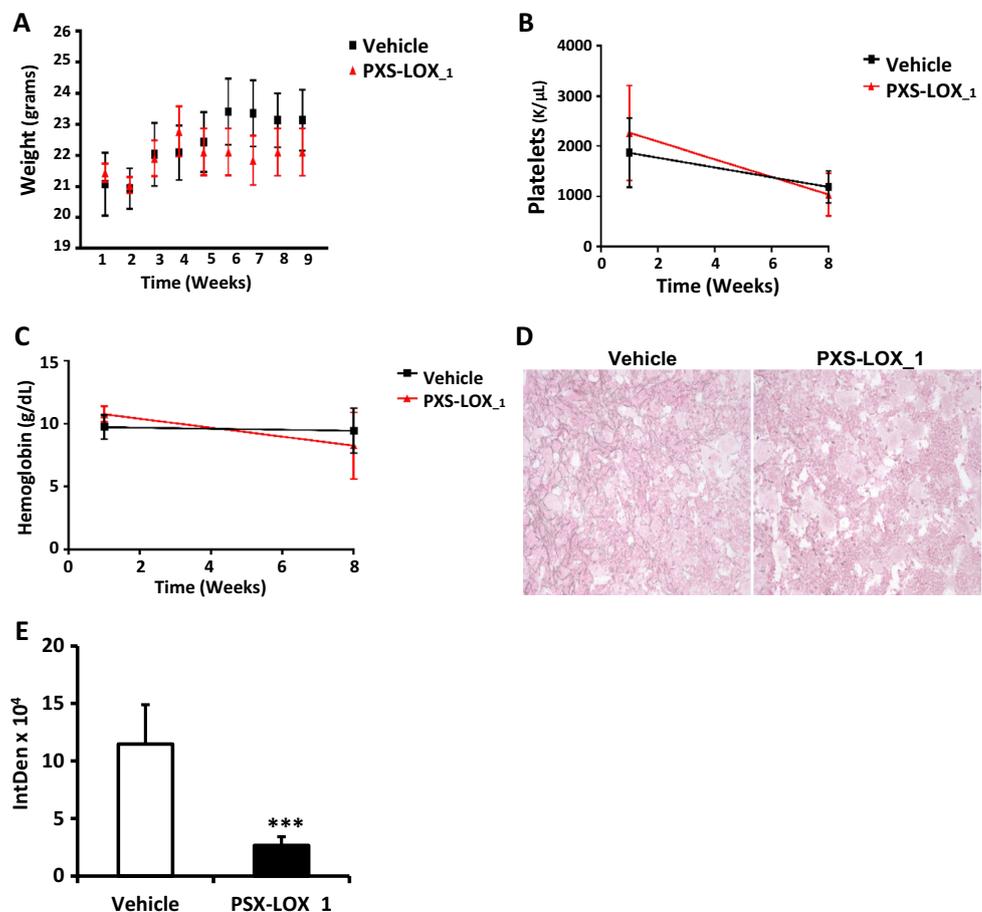


Fig. 6 Megakaryocyte number is reduced in PXS-LOX₂-treated JAK2V617F female mice. Megakaryocyte number in female vs. male JAK2 mice treated with vehicle or PXS-LOX₂. Data are averages \pm SD, $n=9$ JAK2 vehicle (four male, five female), seven JAK2 PXS-LOX₂ treated (four male, three female), ten WT vehicle (four male, six female) and 11 WT PXS-LOX₂ (five male, six female). Twenty images per mouse of randomly chosen hematoxylin–eosin stained slides (H&E) were acquired at $\times 20$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ using One-way ANOVA

vehicle-treated JAK2V617F mice had slightly more BM fibrosis compared to female vehicle-treated JAK2V617F mice (Fig. 5c). PXS-LOX₂ treatment reduced reticulin fibrosis by 45.7% in male mice and by 90.0% in female mice. The bone marrow of WT mice displays reticulin at least in two orders of magnitude lower than JAK2V617F mice (compare the scale in Fig. 5b, d). WT male controls showed 37.0% higher reticulin content than female controls.

Fig. 7 PXS-LOX₁ reduces bone marrow fibrosis in JAK2V617F male mice. **a** PXS-LOX₁ treatment had no effect on mice weights measured throughout the experiment. **b, c** Blood was collected from mice before treatment and at the time of sacrifice, and platelet counts and hemoglobin levels were determined. **d** Gomori reticulin staining of femur bone marrow from vehicle- and PXS-LOX₁-treated mice. **e** Reticulin fibrosis quantitation was done as referenced in legend of Fig. 1. PXS-LOX₁ reduced reticulin fibrosis by 76.8%. Data are averages \pm SD for four vehicle-treated and four PXS-LOX₁-treated male mice, with 20 randomly chosen images analyzed per mouse. All compared values showed $p < 0.05$, and F statistic $>$ F critical, thus rejecting the null hypothesis by ANOVA single factor analysis. *** $p < 0.001$



PXS-LOX₂ treatment reduced reticulin level by 61.5% in female mice, with no significant effect on male mice (Fig. 5d). Using methods as described in Fig. 2, megakaryocyte number was counted in bone marrow sections evaluated for fibrosis. There was only a trend for a difference between vehicle-treated and drug-treated JAK2V617F mice when both sexes are pooled (not shown). However, this is likely accounted for by the fact that JAK2V617F females displayed a reduction of megakaryocyte number when treated with PXS-LOX₂, whereas treatment did not affect megakaryocyte number in male mice (Fig. 6).

LOX inhibitor PXS-LOX₁ attenuated bone marrow fibrosis in JAK2V617F mice

While PXS-LOX₂ was more effective in inhibiting BM fibrosis in female JAK2V617F mice (Fig. 5d), PXS-LOX₁ inhibited fibrosis in both male and female GATA-1low mice (Fig. 1b). Thus, we tested the influence of PXS-LOX₁ on JAK2V617F male mice, but used it orally in a suspension (see “Methods”) to reduce the differences in the two protocols. To this end, JAK2V617F male mice received by oral gavage either PXS-LOX₁ ($n=4$) or vehicle (water, $n=4$) at a dose of 30 mg/kg four times a week for eight weeks and were killed for analysis.

Both groups of mice had similar change in body weight, platelet level and hemoglobin (Fig. 7a–c). As shown in Fig. 7d, e, the drug-treated mice showed a 76.8% reduction in fibrosis, compared to vehicle-treated mice, but there was no effect of the drug on spleen size (not shown). Taken together, the results of our studies involving two novel LOX inhibitors and two mouse models of fibrosis confirm the crucial role of this enzyme in the ECM dysregulation seen in PMF.

Discussion

Current treatment of PMF is mainly palliative and only allogeneic hematopoietic cell transplant (allo-HCT) is curative [27]. JAK1/2 inhibition is commonly used in PMF with the JAK1/2 inhibitor ruxolitinib currently being the main Food and Drug Administration (FDA) approved drug for the treatment of PMF. Ruxolitinib was shown to improve splenomegaly and constitutional symptoms in PMF patients and modestly improve survival in long-term studies. However, anemia, cytopenias and withdrawal syndrome (acute relapse of PMF symptoms, splenomegaly and septic shock-like symptoms) are troublesome [28–30]. There are currently no therapies that specifically target the ECM dysregulation seen in PMF, while this aspect of the pathology is of significant clinical burden.

In a previous study, we tested the ability of BAPN, a small molecule inhibitor of LOX, to reduce fibrosis in the GATA-1 mouse model of PMF [12]. While BAPN is a recognized inhibitor of LOX, it is also a substrate for related amine oxidases (semicarbazide-sensitive amine oxidase and diamine oxidase), and the corresponding release of reactive aldehyde(s) and hydrogen peroxide are potentially damaging to various organs. In this work, we sought to evaluate recently developed haloallylamine-based lysyl oxidase inhibitors that, importantly, are not recognized as substrates by other amine oxidase enzymes. Two inhibitors, PXS-LOX_1 and PXS-LOX_2, were tested for their ability to inhibit bone marrow fibrosis in mouse models of PMF.

PXS-LOX_1 attenuated BM fibrosis and megakaryocyte number in males and females in a similar fashion. Owing to limited oral bioavailability PXS-LOX_1 is best administered via intraperitoneal injection, which is less favorable when considering potential translational applications for oral dosing in humans. PXS-LOX_2 is orally bioavailable and clearly reduced fibrosis in JAK2V617F female mice, but was not effective in male mice. We speculate that this is related to the steep and more severe progression of fibrosis in male JAK2V617F mice, compared to female ones, which PXS-LOX_2 was not able to attenuate.

Our data suggests that specific LOX inhibitors have promise in the treatment of PMF, particularly pre-fibrotic

PMF (pre-PMF). Pre-PMF is defined by the WHO as megakaryocytic proliferation and atypia without reticulin fibrosis, as well as splenomegaly, leukocytosis, anemia and the presence of the known driver mutations [31]. In pre-PMF, there is little obvious BM fibrosis but there is megakaryocyte proliferation and atypia not seen in essential thrombocythemia (ET). Pre-PMF is also associated with a worse prognosis and overall survival than ET [32]. Currently, there is no FDA approved treatment for pre-PMF, nor any studies on treatment to delay the onset of overt PMF, therefore necessitating the development of such interventions. Our studies in mice suggest that LOX inhibition may be beneficial in this setting.

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Compliance with ethical standards

Conflicts of interests The authors declare that they have no conflict of interest. The authors affiliated with Pharmaxis synthesized and characterized the inhibitors and tested their efficacy in terms of inhibition of LOX in vivo, but were not involved in performing the experiments related to evaluation of fibrosis burden, and in interpretation of the results.

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