A phase I/IIa trial of PXS-5505, a novel pan-lysyl oxidase inhibitor, in advanced myelofibrosis

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Abstract

Myelofibrosis is a progressive disease characterized by accumulation of extracellular matrix in the bone marrow (BM), which impairs normal hematopoiesis. While Janus kinase inhibitors reduce spleen volume and provide symptomatic improvement, they do not resolve BM fibrosis and may cause or exacerbate anemia and thrombocytopenia. An antifibrotic therapy capable of normalizing the BM microenvironment and function remains a significant gap in the current treatment landscape. Myelofibrosis is associated with elevated expression of lysyl oxidases, enzymes responsible for maturation of the most abundant extracellular matrix proteins: collagen and elastin. PXS-5505 is a novel pan-lysyl oxidase inhibitor designed to exert an antifibrotic effect by preventing the cross-linking of collagen and elastin. PXS5505-MF-101 is a multicenter phase I/IIa study of PXS-5505 in myelofibrosis patients which included a dose escalation phase (DEP) and a cohort expansion phase (CEP). Primary objectives of the study were to determine the safety and tolerability of PXS-5505 and to define dosing for future studies. The DEP demonstrated that the highest dose tested, 200 mg BID, was safe and achieved robust systemic reduction of lysyl oxidase activity; this dose was, therefore, selected for the CEP. Twenty-four patients (median age 72 years) with relapsed or refractory myelofibrosis were recruited into the CEP and 54% (13/24) completed 24 weeks of treatment. PXS-5505 was well tolerated and reached steady-state concentrations by day 28. Over the 24-week treatment period preliminary indications of clinical efficacy, including a reduction in BM collagen, were evident. Overall, these data support continued investigation of PXS-5505. (ClinicalTrials.gov identifier: NCT04676529).

Introduction

Myelofibrosis (MF) is a chronic myeloproliferative neoplasm characterized by the replacement of normal, hematopoietic bone marrow (BM) tissue with fibrous scar-like tissue rich in extracellular matrix (ECM) proteins.[1] Over time this structural change contributes to ineffective hematopoiesis, progressive splenomegaly and leukemic transformation. Despite improvements in therapy options for patients with MF with the approval of multiple Janus kinase (JAK) inhibitors, these medications do not prevent disease progression and thus better therapies are needed for MF patients.[2]

The formation and removal of BM connective tissue fibers are typically in dynamic equilibrium.[3] However, in disease states such as MF, these processes become upregulated and unbalanced, a problem that is not addressed by currently approved treatments. Fibrosis develops with accumulation of excessively cross-linked ECM proteins, particularly

collagen and elastin. Lysyl oxidases (lysyl oxidase [LOX] and lysyl oxidase-like 1-4 [LOXL1-4]) are a family of five secreted enzymes responsible for catalyzing this cross-link formation.[4] When the rate of cross-link formation overwhelms endogenous degradation mechanisms, normal BM is replaced by a fibro-collagenous matrix and normal hematopoietic activity ceases.

Assessment of lysyl oxidase expression in human myeloproliferative neoplasms revealed elevated levels of four of the five isoforms (LOXL4 was not studied).[5] Pharmacological inhibition of the enzymatic activity of lysyl oxidases represents a promising therapeutic approach for MF, by re-establishing homeostasis between cross-link formation and degradation.[6] Normalization of the BM microenvironment should ensue, allowing hematopoiesis to resume. This hypothesis has been confirmed pre-clinically, with pan-LOX inhibition proving effective in two relevant models of MF.[7] PXS-5505 is small molecule inhibitor designed to bind to the enzymatic pocket of lysyl oxidases, blocking the formation of collagen cross-links and, ultimately, re-establishing the equilibrium between the formation and degradation of BM tissue.[8] By abolishing excessive cross-linking, the mechanical stress on BM cells is released, thus potentially reducing clonal selection (Figure 1). In tandem, PXS-5505 may inhibit activation of the proto-oncogene platelet-derived growth factor receptor (PDGFR),[4,9] the major regulator in BM fibrosis and hematopoietic cell development. In the long term, excessive matrix will be reduced and hematopoiesis normalized. The preclinical efficacy of PXS-5505 has been demonstrated in various antifibrotic[10] and anti-cancer[8,11] models.

In humans, PXS-5505 has been administered to healthy male volunteers as single (up to 300 mg) and repeated (up to 200 mg) daily doses for 14 days.[12] PXS-5505 is orally bioavailable, safe and well tolerated and possesses excellent pharmacokinetic and pharmacodynamic properties.[13] The interplay between pharmacokinetic and pharmacodynamic properties is consistent with preclinical data and supportive of twice daily (BID) dosing to ensure sustained and strong target engagement.

We conducted a multicenter, phase I/IIa trial to determine the safety and tolerability of PXS-5505 in patients with primary, post-polycythemia vera (PV) or post-essential thrombocythemia (ET) MF who had relapsed on, were refractory to or were ineligible for JAK inhibitor treatment (Clinicaltrials.gov identifier: NCT04676529): the trial comprised a dose escalation phase (DEP), a cohort expansion phase (CEP) and an add-on phase, in which PXS-5505 is added to a stable dose of ruxolitinib for up to 12 months. Herein we report the safety, pharmacokinetics and efficacy outcomes from the first two phases of the trial.

Methods

Study design

The DEP followed a 3+3 design with a starting dose of 100 mg BID escalating to 150 and 200 mg BID with a treatment duration of 28 days. Intra-patient dose escalation was allowed and patients participating in the DEP phase were able to continue on the CEP. In the CEP, 24 patients were treated

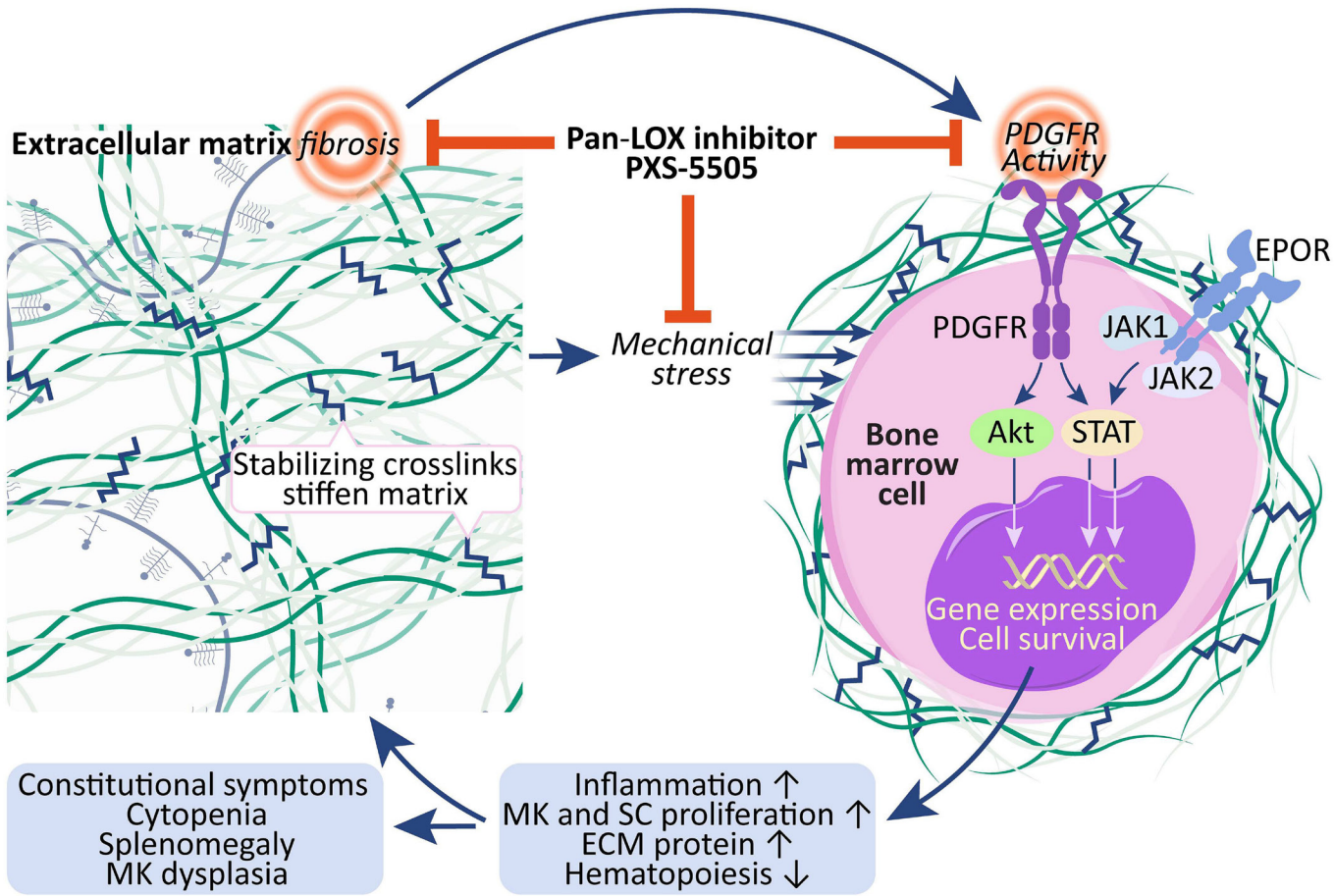

Figure 1. Mode of action of PXS-5505 in myelofibrosis. Oxidation of platelet-derived growth factor receptor by lysyl oxidases in a graphical representation of work by Lucero *et al.*[29] LOX: lysyl oxidases; PDGFR: platelet-derived growth factor receptor; EPOR: erythropoietin receptor; JAK: Janus kinase; Akt: protein kinase B; STAT: signal transducer and activator of transcription; MK: megakaryocyte; SC: stem cell; ECM: extracellular matrix.

at the dose determined appropriate based on safety, pharmacokinetic and pharmacodynamic results from the DEP, for a period of up to 6 months with on study assessments at weeks 0, 1, 4, 12, 18 and 24. Dosing and administration are further described in the *Online Supplement (Section 3)*.

Patients

Eligible patients were ≥18 years old with primary MF or post-ET/PV MF per the World Health Organization (WHO) 2016 criteria with at least grade 2 BM fibrosis.[14,15] Other criteria included ineligibility for stem cell transplantation, intermediate-2 or high-risk disease according to the Dynamic International Prognostic Scoring System (DIPSS), and symptomatic disease, defined as a score of at least 1 in at least two items of the Myelofibrosis Symptom Assessment Form (MFSAF) v4.0.[16] DEP and CEP patients were not currently on ruxolitinib or fedratinib and had been discontinued for at least 2 weeks prior to the first dose of study drug due to any of the following criteria: platelet count ≤50x10^9/L, intolerance, refractory or relapsed on treatment. The full criteria are provided in *Online Supplementary Tables S9, S10*.

Outcomes

The primary objective of the trial was to determine the safety and tolerability of PXS-5505. Other key objectives included characterization of pharmacokinetic and pharmacodynamic parameters to determine the most appropriate therapeutic dose. Efficacy assessments included symptom assessment (MFSAF v4.0 7-day recall), BM biopsy and spleen volume. Plasma samples for pharmacokinetic and pharmacodynamic analysis during the DEP were obtained at weeks 0, 1 and 4 of the dosing cycle. Blood samples were collected at t=0 (pre-dose), 1 hour (±5 mins), and 4 hours (±15 mins) post-dose for assessment of pre-dose=C_{min} and C_{1hr}=C_{max} as well as for assessments of LOX and LOXL2 inhibition in plasma.

Statistical methods

Data are summarized; no formal statistical testing was performed. All subjects enrolled were included in the assessment of safety; pharmacokinetic and pharmacodynamic assessments were performed on patients who provided samples at each timepoint and with no major compliance or sample analysis issues; efficacy assessments focused on those who completed the assessments after 24 weeks of treatment. Continuous measures are summarized using means and standard deviations or medians and ranges; proportions are summarized with the total number of subjects included in the assessment, frequency counts and percentages. Further details on statistical methods are described in *Online Supplementary Table S11*.

Ethics statement

The trial was approved by the relevant Institutional Review Board or Independent Ethics Committee at each site and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all study participants and/or their legal guardians.

Results

Patients' characteristics

From March 2021 until November 2023, a total of 27 patients were enrolled in the PXS5505-MF-101 (MF-101) study at 14 sites in the USA, Australia, South Korea and Taiwan (DEP N=5; CEP N=24; 2 patients participated in DEP and CEP) (Figure 2). In the combined cohort of patients, the median age was 72 and 59% were male. The predominant MF subtype was post-ET MF (44%) followed by primary MF (37%) and post-PV MF (19%). The baseline characteristics of patients are summarized in Table 1.

Lysyl oxidases as biomarkers of myelofibrosis

Lysyl oxidases are upregulated in the BM of MF patients.[5] LOX and LOXL2 are liquid biomarkers[17,18] and we therefore studied LOX and LOXL2 plasma concentrations in all enrolled patients at the onset of dosing and compared them to those in matched healthy controls. In line with published data, LOX (Figure 3A) and LOXL2 (Figure 3B) plasma concentrations were significantly elevated in MF patients compared to controls, suggesting both enzymes are important biomarkers of disease. Furthermore, the plasma enzymatic activities of LOX (Figure 3C) and LOXL2 (Figure 3D) were significantly increased in patients, supporting the important role of lysyl oxidase enzymatic activities in MF.[6]

Dose escalation phase

Five female patients (median age 71 years) were enrolled (Table 1). At each of the three dose levels (100, 150 and 200 mg BID) studied, three patients were treated. Dose escalation progressed from 100 to 150 to 200 mg BID with no dose-limiting toxicities observed. One patient was enrolled in all dose regimens tested and one patient participated in both the 150 and 200 mg BID dose levels (Figure 2).

There were no suspected unexpected serious adverse reactions or dose limiting toxicities and the majority (86%, 19/22) of adverse events were grade 1-2. A 77-year-old female with a past medical history of hypertension, hypercholesterolemia, osteoarthritis and subdural hematoma terminated early from the 100 mg BID cohort following transformation of her disease to acute myeloid leukemia (23 days after treatment initiation) which was reported as a serious adverse event considered by the investigator to be definitely not treatment-related. This patient died 17 days later. There were no other adverse events leading to treatment discontinuation and all other patients completed dosing without interruption. There were two hematologic events; a grade 4 platelet count decrease on 100 mg BID, and a grade 3 anemia on 150 mg BID, both considered not related to treatment; no changes were made to treatment

as a result of these events. There was one adverse event considered by the investigator to be treatment-related, a grade 1 event of peripheral edema occurring at the 150 mg BID dose level (which did not require dose adjustment or interruption). The patient continued to 200 mg BID in the DEP and into the CEP. A summary of adverse events occurring in more than one patient, split by those occurring in the DEP and CEP, is shown in Table 2.

During the DEP, PXS-5505 plasma concentrations were measured at days 0, 7 and 28 (pre-dose, 1 hour and 4 hours post-dose). PXS-5505 plasma concentrations peaked at 1 hour after drug intake at the 150 and 200 mg dose levels, whereas maximum levels were reached at 4 hours after drug intake at the lowest dose level of 100 mg BID (Figure 4A). Plasma peak concentrations increased in a dose-proportional manner, with a maximum concentration of 2,530

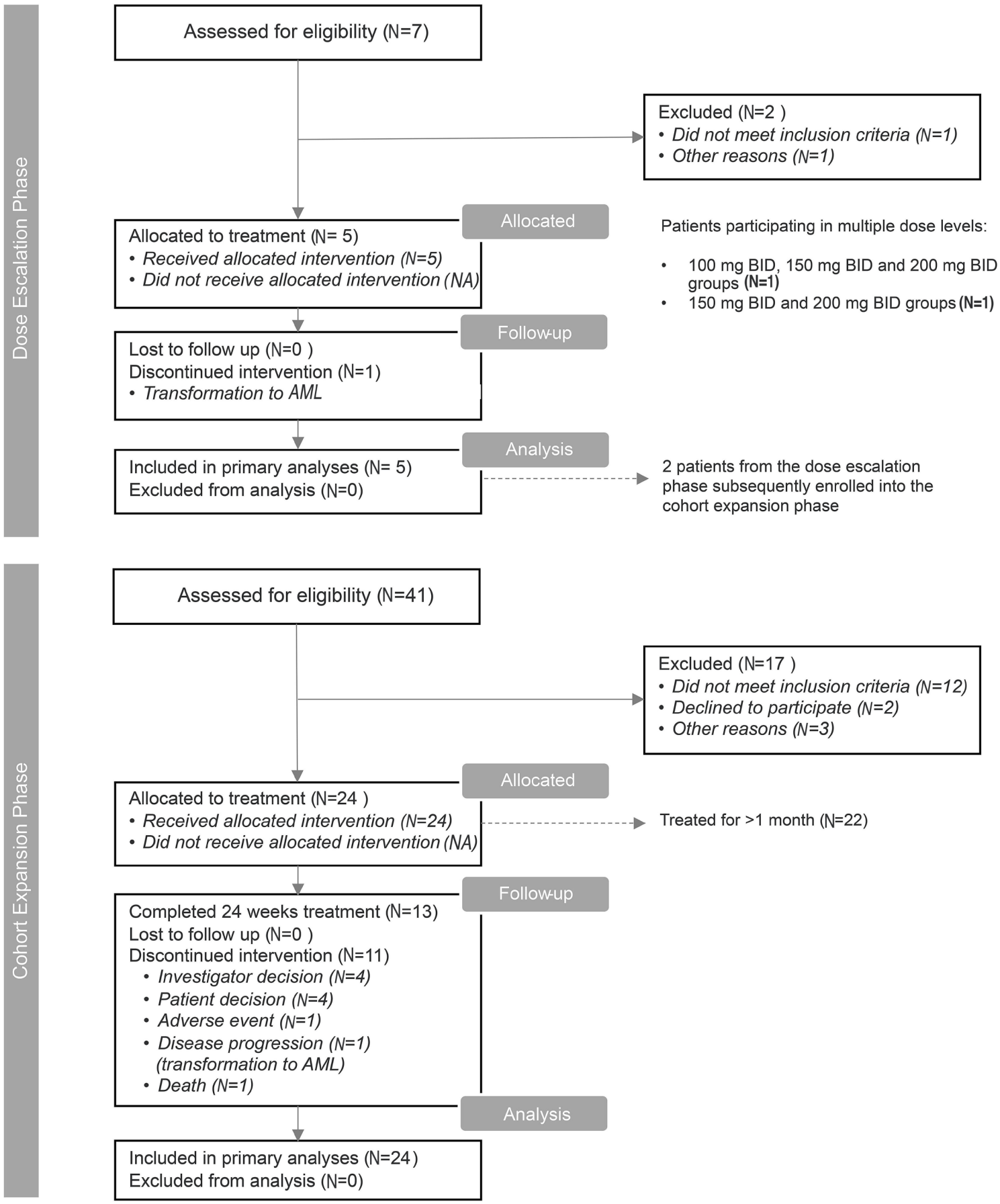

Figure 2. Patients' disposition in the dose escalation and cohort expansion phases. NA: not applicable; AML: acute myeloid leukemia; BID: twice daily.

ng/mL (equivalent to 9.0 μM) observed at the 200 mg BID dose level (1 hour post-dose on day 7). Importantly, the peak plasma concentrations were well below the threshold for drug-drug interactions.[8]

The activities of LOX (Figure 4B) and LOXL2 (Figure 4C) were measured in plasma to confirm target engagement. The rapid increase in plasma concentrations of PXS-5505 resulted in a strong reduction of systemic (plasma) lysyl oxidase activities. The inhibition at 1 and 4 hours occurred at all dose levels but significantly increased from 100 mg BID to both 150 and 200 mg BID. At the 200 mg BID level, the mean trough LOX or LOXL2 inhibition in plasma measured at the pre-dose timepoint on day 7 and day 28 was greater than 90% in both cases.

The 200 mg BID dose achieved the strongest inhibition of LOX and LOXL2 of all doses tested and, importantly, variability (with regards to the level of target engagement) between patients was low (compared to 100 and 150 mg BID). After a review of all safety and pharmacokinetic data by the Safety Monitoring Committee, 200 mg BID was, therefore, selected as the dose for the CEP.

Cohort expansion phase

Twenty-four patients were enrolled including two continuing from the DEP (Figure 2). The majority of patients were male (67%); their median age was 72 years. Ten patients had primary MF, nine patients had post-ET MF and five had post-PV MF, with 88% of patients having intermediate-2-risk disease. The median disease duration at baseline was 3.1 years. The majority (92%) had received prior JAK inhibitor treatment, with a median treatment duration of 23 months. Ten patients discontinued JAK inhibitor treatment within 4 weeks of PXS-5505 dosing (the minimum duration permitted between cessation of the JAK inhibitor and study drug dosing was 14 days). At baseline, the median hemoglobin was 89 g/L, the median platelet count was 130×10^9/L and the majority of patients (75%) had grade 3 BM fibrosis.

Twenty-two patients completed ≥1-month PXS-5505 treatment and 13 patients completed 24 weeks. Total drug exposure was 431 weeks, with a median duration of 169 days (range, 16 to 191). Eleven patients withdrew prior to completion of 24 weeks of treatment, four due to investigators' decision, four due to patients' decision, one due to an adverse event (thrombocytosis), one due to transformation to acute myeloid leukemia and one due to death (following a serious adverse event of febrile neutropenia) (Figure 2). Adverse events occurring in more than one patient across the DEP and CEP are shown in Table 2. Eighty-nine treatment-emergent adverse events were recorded in the CEP, most of which were mild. The majority (92%, 82/89) of events were considered by the investigator to be not related to treatment.

There was one fatal adverse event on treatment. A 75-year-old male with history of ischemic heart disease, hypertension, paroxysmal atrial fibrillation, peripheral sensory neuropathy and grade 4 decreased neutrophil count at baseline had two events of grade 3 febrile neutropenia which recovered after two short hospital admissions. The last admission for febrile neutropenia resulted in multi-organ failure and death. All three events of febrile neutropenia were considered by the investigator to be unrelated to treatment. There were two fatal post-treatment discontinuation/completion events (sepsis, acute myocardial infarction), both considered by the investigator to be unrelated to treatment. Eight patients had a total of 22 serious adverse events, with only one (pneumonia) considered to be possibly related to treatment.

Cytopenias were infrequent; anemia occurred in two patients (1 grade 3 considered not related and 1 grade 4 considered possibly related to treatment) and one patient had a grade 3 decrease in platelet count (considered possibly related to treatment). There were five non-hematologic adverse events considered related to treatment, all grade 1 or 2, across four different system organ classes. Details

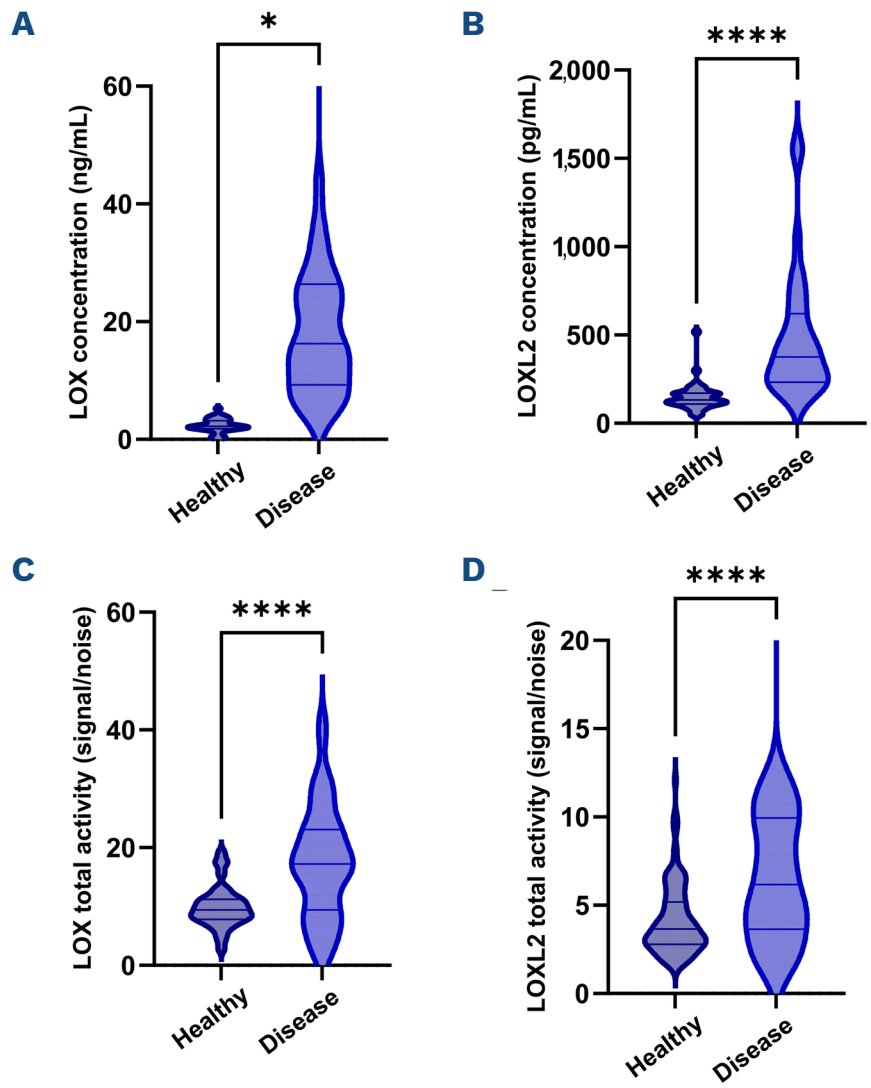

Figure 3. Elevated LOX and LOXL2 concentrations and activities in myelofibrosis patients compared to those in healthy subjects. (A) LOX and (B) LOXL2 concentrations in healthy (reasonably age-matched) controls and patients with myelofibrosis (Disease) and (C) LOX and (D) LOXL2 activity in healthy (reasonably age-matched controls) and myelofibrosis patients. Subjects assessed at baseline (i.e. pre-dose at day 0). Unpaired *t* test, **P*<0.01, *****P*<0.0001. For further details see *Online Supplementary Table S12.*

Table 1. Baseline characteristics and clinical characteristics in the dose escalation and cohort expansion phases.

Variable	DEP N=5	CEP N=24	Total[a] N=27
Median age at screening, years (min, max)	71 (60, 77)	72 (60, 86)	72 (60, 86)
Female sex, N (%)	5 (100.0)	8 (33.3)	11 (40.7)
Race, N (%)			
White	3 (60)	12 (50.0)	13 (48.1)
Asian	2 (40)	11 (45.8)	13 (48.1)
Other	0	1 (4.2)	1 (3.7)
DIPSS, N (%)			
Intermediate-2	2 (40)	21 (87.5)	21 (77.8)
High	3 (60)	3 (12.5)	6 (22.2)
MF subtype, N (%)			
Primary MF	0	10 (41.7)	10 (37)
Post-PV MF	1 (20)	5 (20.8)	5 (18.5)
Post-ET MF	4 (80)	9 (37.5)	12 (44.4)
Median disease duration at baseline, years[b] (min, max)	9.8 (0.5, 12.5)	3.1 (0.1, 12.9)	3.2 (0.1, 12.9)
Received one or more prior JAK inhibitor, N (%)	5 (100)	22 (91.7)	25 (92.6)
Median total prior JAK inhibitor treatment duration, months (min, max)[c]	8.3 (1.2, 58.7)	23.1 (2.8, 143.9)	21.6 (1.2, 143.9)
Gap from last dose of prior JAK inhibitor ≤28 days, N (%)[c]	3 (60)	10 (45.5)	13 (52.0)
Median platelets x10^9/L[d] (min, max)	20 (12, 320)	130 (4, 1975)	96 (4, 1975)
Median hemoglobin, g/L[d] (min, max)	89 (64, 104)	89 (58, 121)	89 (58, 121)
Median spleen volume, cc[d] (min, max)	1172 (1004, 2943)	1371 (252, 5647)	1172 (252, 5647)
BM fibrosis, N[e] (%)			
Grade 1,2[f]	-	4 (16.7)	-
Grade 3[g]	-	18 (75.0)	-
Could not be assessed	-	2 (8.3)	-
Median TSS (MFSAF v4.0)[d] (min, max)	14 (10, 59)	23 (7, 55)	21 (7, 59)

[a]Patients participating in both the dose escalation and cohort expansion phases under the same patient number are included only once in total column. [b]From diagnosis to first study dose. [c]For those receiving prior JAK inhibitor treatment only. [d]For patients continuing from escalation to expansion the values at baseline for the dose escalation and cohort expansion phases are different. The first value (i.e., at the beginning of the dose escalation phase) is summarized in the total column for these patients. [e]Information not collected in the dose escalation phase. [f]One patient was centrally assessed as having grade 1 bone marrow fibrosis at baseline; the inclusion criterion of at least grade 2 was based on an investigator's assessment prior to entry. [g]One patient had grade 2/3 recorded; this has been included in summaries as grade 3. DEP: dose escalation phase; CEP: cohort expansion phase; DIPSS: Dynamic International Prognostic Scoring System; MF: myelofibrosis; PV: polycythemia vera; ET: essential thrombocythemia; JAK: Janus kinase; BM: bone marrow; TSS: total symptom score; MFSAF v4.0: Myelofibrosis Symptom Assessment Form version 4.

are provided in *Online Supplementary Tables S16* and *S17*. Additional safety evaluations in the CEP included magnetic resonance imaging (MRI) of femora (at baseline and at the end of the 24-week treatment period), which was performed based on a previous report that a nonspecific lysyl oxidase inhibitor used at high doses caused additional bone formation.[19] Consistent with the disease state of MF, the majority of femoral MRI results were reported as abnormal; however, additional bone formation was not observed. There were no reports of abnormal wound healing, including in patients undergoing surgical procedures during the study period.

PXS-5505 plasma concentrations were evaluated on days 0, 28, 84 and 168 (pre-dose, 1 hour and 4 hours post-dose) (Figure 5A). PXS-5505 mean concentrations predominantly peaked at 1 hour post-dose, with a mean plasma concentration at steady state (days 28, 84 and 168) of 1,517 ng/mL. As in the DEP, target engagement was confirmed through activity-time profiles, with evident, significant reductions in LOX (Figure 5B) and LOXL2 (Figure 5C) activities. Overall, the mean normalized enzymatic activities for LOX and LOXL2 (as surrogates for the lysyl oxidase family) were reduced by approximately 90% from baseline at day 28 at trough and effectively sustained throughout the 24-week study period.

Disease response

While this study was primarily designed to assess the safety and tolerability of PXS-5505 and measure pharmacokinetics and pharmacodynamics to define therapeutic dosing for future studies, some indications of disease stabilization and clinical improvement were observed.

Across the 24-week treatment period, 62% (8/13) of patients had at least a ≥20% reduction in total symptom score at some point (12 or 24 weeks or both), and two (15%) achieved ≥50% reduction at 24 weeks. The median best symptom score percentage change at any time during treatment was

Table 2. Summary of adverse events regardless of study drug relationship occurring in more than one patient (across the dose escalation and cohort expansion phases) by preferred term.

Preferred term, N (%): E	DEP, N=5		CEP, N=24		Total, N=27	
	Any grade	Grade 3/4	Any grade	Grade 3/4	Any grade	Grade 3/4
Non-hematologic adverse events						
Decreased appetite	1 (20.0): 1	-	2 (8.3): 2	-	3 (11.1): 3	-
Fatigue	-	-	3 (12.5): 3	1 (4.2): 1	3 (11.1): 3	1 (3.7): 1
Headache	1 (20.0): 1	-	1 (4.2): 4	-	2 (7.4): 5	-
Diarrhea	-	-	2 (8.3): 3	-	2 (7.4): 3	-
Wound	1 (20.0): 1	-	1 (4.2): 2	-	2 (7.4): 3	-
Arthralgia	-	-	2 (8.3): 2	-	2 (7.4): 2	-
Hyperuricemia	1 (20.0): 1	-	1 (4.2): 1	-	2 (7.4): 2	-
Insomnia	-	-	2 (8.3): 2	-	2 (7.4): 2	-
Lower RTI	-	-	2 (8.3): 2	1 (4.2): 1	2 (7.4): 2	1 (3.7): 1
Non-cardiac chest pain	-	-	2 (8.3): 2	-	2 (7.4): 2	-
Peripheral edema	1 (20.0): 1	-	1 (4.2): 1	-	2 (7.4): 2	-
Upper RTI	-	-	2 (8.3): 2	-	2 (7.4): 2	-
Urinary retention	-	-	2 (8.3): 2	-	2 (7.4): 2	-
Urinary tract infection	-	-	2 (8.3): 2	-	2 (7.4): 2	-
Vomiting	-	-	2 (8.3): 2	-	2 (7.4): 2	-
Hematologic adverse events						
Anemia*	1 (20.0): 1	1 (20.0): 1	2 (8.3): 2	2 (8.3): 2	3 (11.1): 3	3 (11.1): 3
Platelet count decrease*	1 (20.0): 1	1 (20.0): 1	1 (4.2): 1	1 (4.2): 1	2 (7.4): 2	2 (7.4): 2

*Hematologic abnormalities are based on laboratory values. N: number of patients with the event; E: number of events; DEP: dose escalation phase; CEP: cohort expansion phase; RTI: respiratory tract infection.

-26% (range, -78% to +220%) with a median best absolute change of -5 (range, -20 to +22).

Overall, hemoglobin concentration, platelet counts and white blood cell counts were stable, not only in patients who had just come off JAK inhibitor therapy but also in patients who had a longer interval between treatments (>28 days) (*Online Supplementary Figures S2-S4*).

Of the 13 patients who completed the 24 weeks of treatment, the median platelet count was 109x10^9/L (range, 14 to 515) at baseline and 108x10^9/L (range, 8 to 633) after 24 weeks of treatment. The median maximum change in platelets from baseline across 24 weeks was +37% (range, -9% to +199%); 92% (12/13) of completers had increased platelet counts at some time during treatment and 77% (10/13) of platelet counts were improved at 24 weeks relative to baseline; notably three of the four patients with grade 4 platelet levels (<25x10^9/L) at baseline completed the study with improved platelet counts (Figure 6).

The median absolute hemoglobin level change at week 24 was -4 g/L (range, -32 to +17) and eight of the 13 patients had stable or improved hemoglobin levels, including one patient who had an anemia response (hemoglobin increase >20 g/L with no transfusion) at week 18 (Figure 6).

Of the 12 patients who had spleen volume measurements performed at both baseline and week 24, the median change was an increase of 22% (range -10% to +154%). It should be noted that baseline spleen volume assessments could be performed any time in the 6 weeks prior to the first dose of PXS-5505 (7/12 patients had the baseline measurement in the CEP within 28 days of the last dose of JAK inhibitor and 2 of these on the last day of JAK inhibitor dosing).

Fibrosis grade (reticulin and collagen) was centrally assessed in the CEP only; 12 patients had baseline, week 12 and week 24 biopsies that could be graded (for 1 patient the baseline biopsy sample could not be analyzed due to minimal assessable hematopoietic tissue).[20]

For these patients the reticulin fibrosis grade remained stable over 24 weeks of therapy. Collagen fibrosis improved by one grade in 42% (5/12), worsened in 25% (3/12) and was unchanged in the remainder over the study period (Table 3).[20]

Discussion

Regulatory approval of the first JAK inhibitor, ruxolitinib, fundamentally improved standard of care for MF patients by providing symptomatic relief and ameliorating splenomegaly.[21] However, JAK inhibitors cannot restore normal hematologic function as they do not address the fibrotic BM that underpins myeloproliferative neoplasms such as MF. A therapy capable of achieving traction on the basic biology of the disease and, ultimately, improving the health of the BM microenvironment remains a significant gap in the current treatment landscape. Herein we demonstrate, in patients resistant to, intolerant of or ineligible for rux-

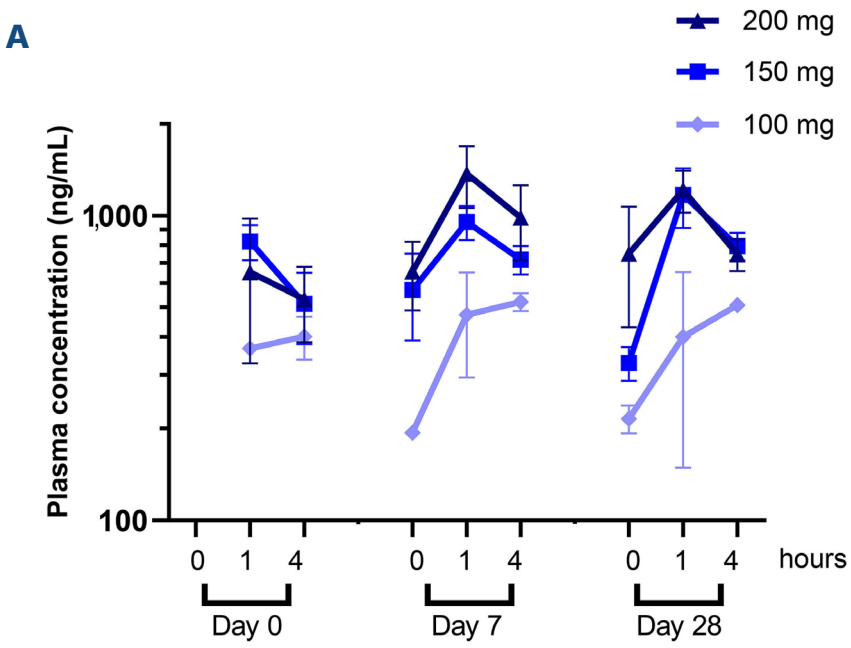

Figure 4. Pharmacokinetics and pharmacodynamics of PXS-5505 in the dose escalation phase. (A) Mean plasma concentration-time profiles (mean ± standard error of mean [SEM]) for PXS-5505 at days 0, 7 and 28 (pre-dose, 1 hour and 4 hours post-dose) after oral administration of 100, 150 or 200 mg BID for 28 days (N=3 except 100 mg BID day 28 data [N=2]). (B, C) Mean LOX (B) and LOXL2 (C) activity-time profiles depicted as activity normalized to day 0 pre-dose level (mean ± SEM) (LOX: N=3 except 100 mg BID day 28 data [N=2]; LOXL2: N=2 except 100 mg BID day 0 and 7 data [N=3]).

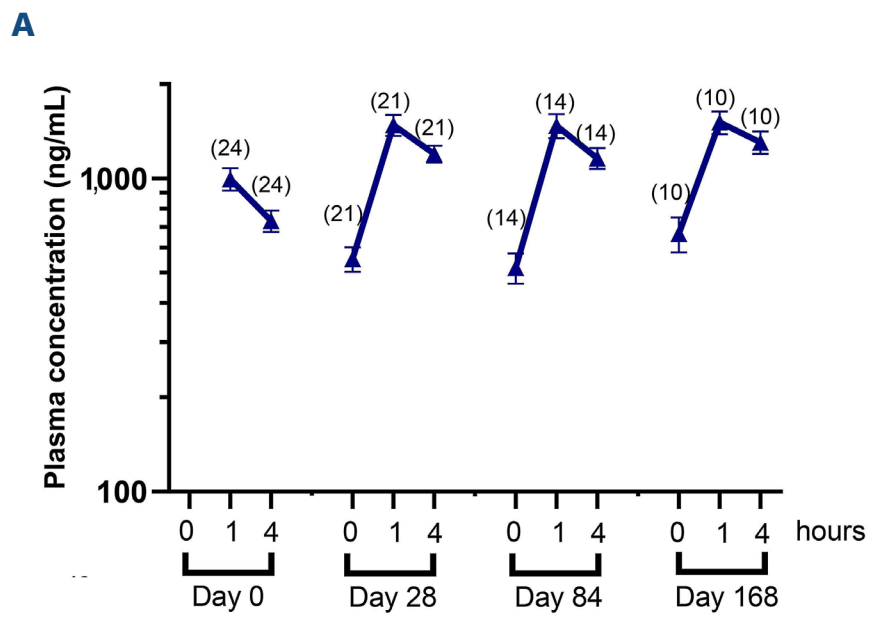

Figure 5. Pharmacokinetics and pharmacodynamics of PXS-5505 in the cohort expansion phase. (A) Mean plasma concentration-time profiles (mean ± standard error of mean [SEM]) for PXS-5505 after oral administration of 200 mg BID for 24 weeks. (B, C) Mean LOX (B) and LOXL2 (C) activity-time profiles (mean ± SEM) depicted as activity normalized to day 0 pre-dose level. Results affected by compliance issues or sample analysis issues were excluded from the analysis, refer to *Online Supplementary Table S13* for details.

olitinib treatment, the safety, pharmacokinetics and strong target engagement of a novel pan-LOX inhibitor, PXS-5505, which is associated with symptom improvement, stable hematologic parameters and an improvement in BM collagen fibrosis after 6 months of treatment.

In recent years a previously under recognized aspect of MF, namely dysregulation of the ECM and BM, has been targeted clinically with a number of antifibrotic agents. Zinpentraxin alfa (previously PRM-151) is a recombinant human form of pentraxin-2, an endogenous regulator of the inflammatory and fibrotic response to tissue injury.[22,23] In a randomized, double-blind phase II study in MF patients previously treated with or ineligible for ruxolitinib (NCT01981850), zinpentraxin alfa showed encouraging signs of clinical activity although it does not appear to have progressed further in clinical development. Interestingly, recent quantitative analysis driven by artificial intelligence has raised concerns over the subjectivity of conventional fibrosis assessment, meaning patients with myeloproliferative neoplasms other than primary or secondary MF may have been included in the study.[24]

Agents specifically directed towards reducing collagen cross-linking have also been evaluated clinically, with a LOXL2-specific antibody (simtuzumab) being the first of this class.[25] While the antibody displayed promising preclinical efficacy and was well tolerated in clinical studies, it did not consistently reduce BM fibrosis by 24 weeks in a phase II study in MF patients (NCT01369498). The limited penetration of an antibody into fibrotic BM may have contributed to its lack of effect; however, recently it became obvious that the LOXL2-specific antibody does not inhibit lysyl oxidase activity, and thus has no direct effect on collagen cross-linking or tissue stiffness.[17,26]

Testament to the perceived importance of lysyl oxidases in BM fibrosis,[27] a small molecule inhibitor of LOXL2 (GB2064, formerly PAT1251) improved BM reticulin and collagen fibrosis, suggestive of disease-modifying activity, in a phase II trial (NCT04679870).[28]

While LOXL2 is undoubtedly an enzyme of importance with respect to the cross-linking of ECM collagens and elastin, and resulting increased stiffness and mechanical stress, it is not the only member of this enzyme family associated with MF. Indeed, primary MF is known to be accompanied by elevated levels of four of the five isoforms (LOXL4 was not studied)[5] and LOX itself has been shown to be upregulated in the megakaryocytes of both mice and patients with primary MF, suggesting its role in disease progression.[6] In this study, plasma concentrations and activities of LOX and LOXL2 were shown to be significantly elevated in MF patients compared to healthy controls. LOX has also been shown to enhance platelet derived growth factor signaling by oxidizing the receptor (PDGFR).[9,29] Consequently, LOX inhibition reduces PDGFR activation of the ERK signaling pathway, as well as its proliferative effect on the megakaryocyte lineage.[9] Therefore, a broader approach, namely pan-LOX inhibition, has been evaluated in mouse models that recapitulate MF disease burden and progression. Pan-LOX inhibition attenuated BM fibrosis and megakaryocyte numbers, as well as reducing both the volume and fibrosis of spleens.[7] Pan-LOX inhibition is a complementary mech-

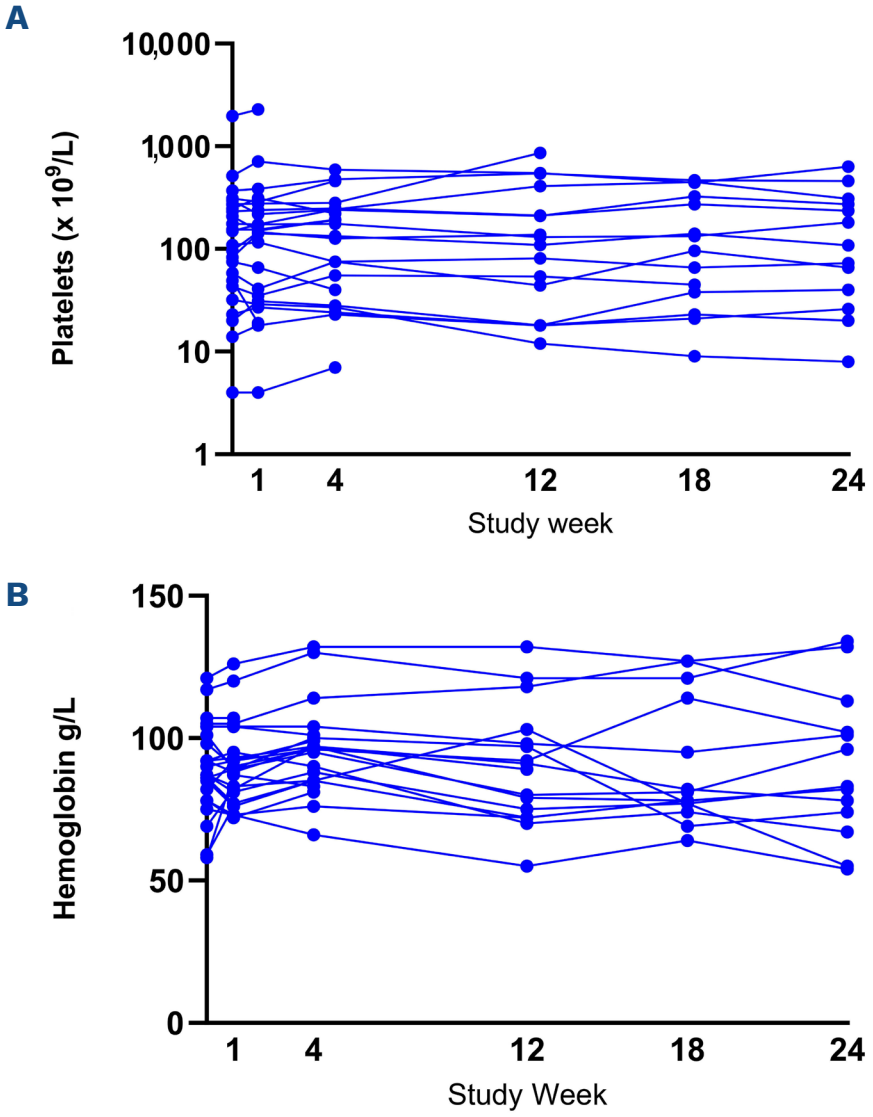

Figure 6. Platelet count and hemoglobin concentration by patient and study week in the cohort expansion phase. (A) Platelet counts and (B) hemoglobin levels for all patients (N=24) enrolled in the cohort expansion phase, by study week.

Table 3. Collagen grade over time (by patient) in the cohort expansion phase.

Subject	Baseline	12 weeks	24 weeks
1	1	1	0
2	2	2	3
3	3	2	2
4	0	1	1
5	Could not be graded	1	1
6	2	1	1
7	3	3	3
8	3	2	2
9	2	2	3
10	3	2	3
11	3	3	3
12	3	3	3
13	2	2	2

anism to both currently available therapies and those in development.

Here we established an optimal dose of 200 mg BID in patients with primary MF or post-PV/ET MF. This dose was very well tolerated and resulted in excellent LOX and LOXL2 inhibition (>90% at trough concentration).

PXS-5505 was well tolerated with a good safety profile. The CEP also provided an opportunity to monitor theoretical safety issues relating to the mode of action. β-aminopropionitrile, the compound that pioneered exploration of this target class, caused a (reversible) increase in periosteal bone following treatment for 67 days.[19] Femoral MRI performed at baseline and following 24 weeks of treatment with PXS-5505 revealed no evidence of long bone abnormalities. Given the potential impairment to wound healing with PXS-5505, patients were advised to stop taking the drug if they had to undergo a medical procedure involving tissue injury. There were no reports of abnormal wound healing, including in patients undergoing surgical procedures such as biopsies (*Online Supplementary Table S15*).

In the CEP there were preliminary indications of improvements in collagen fibrosis and symptom score along with stable or improved blood counts including in those patients with severe thrombocytopenia. The absence of any significant hematologic adverse events positively differentiates PXS-5505 from JAK inhibitors and other emerging therapies. Taken together the findings from the 24-week CEP are encouraging given the poor prognosis seen after ruxolitinib discontinuation in MF patients.[30,31]

The main limitations of this study are the open-label nature of the design and the limited treatment period which, while appropriate for determining the optimal dosing and establishing safety, are insufficient to achieve robust indicators of clinical antifibrotic response. Notably, in studies with the antifibrotic agent zinpentraxin alfa, longer treatment duration (72 weeks) was required to see improvements in hemoglobin, platelets, spleen and symptoms.[32] In the present study a reduction in spleen volume was not observed. In some patients the spleen volume increased in the first 12 weeks of treatment before tapering for the remainder of the study. This may be due to the short duration between cessation of JAK inhibitor treatment and baseline spleen volume assessment (within 28 days in 58% of patients). This is not surprising given that worsening splenomegaly as part of ruxolitinib discontinuation syndrome occurs as early as 3 weeks after cessation of ruxolitinib.[33] It is also acknowledged that limitations in the DEP were a low number of patients (N=5), and only one biological sex represented (female). In contrast, the CEP included male (67%) and female (33%) patients.

The limited options for treatment of MF offer symptomatic relief but do not adequately address BM fibrosis and their side effect profiles (particularly the development of cytopenias) often lead to treatment discontinuation and suboptimal dosing.[31]

PXS-5505 provides an opportunity for a well-tolerated therapy that improves BM health by reducing cross-links and mechanical stress and also inhibits activation of PDGFR, with the overarching aim of restoring functional hematopoiesis. By preventing new fibrosis this novel mode of action allows intrinsic mechanisms to remove existing fibrosis and normalize BM homeostatic functions. PXS-5505 is well tolerated, with a distinct mode of action suitable for patients ineligible for, relapsed after or intolerant to JAK inhibitors. Moreover, the pharmacological action and low propensity for drug-drug interactions make it an ideal candidate for use in combination with JAK inhibitors as well as other novel therapies. The next phase of this study, which is underway at the time of manuscript submission, assesses PXS-5505 treatment in MF patients already receiving a stable dose of ruxolitinib for a period of 12 months to further explore safety and efficacy within the context of longer-term use. It is expected that the use of PXS-5505 in addition to ruxolitinib may provide a complementary approach in which the reduction of ECM fibrosis and mechanical stress, as well as regulation of abnormal cell growth and division, give rise to a more durable treatment response associated with improvements in symptoms and spleen volume and normalization of blood counts over time.

Disclosures

PV has received honoraria from Incyte and Blueprint Medicines and has consulted for AbbVie, Amgen, Blueprint Medicines, Cogent Biosciences, DISC medicine, Incyte, CTI BioPharma Corp (now Sobi), Genentech, Geron, GSK, Karyopharm, Merck, MorphoSys, Novartis, Pfizer, Stemline, Servier and Takeda. A-MW has served on a Data Safety Monitoring Board for Syntara Limited. RB has received grants from Bayer, Takeda, Syntara, Pfizer, Daiichi Sankyo, CSL Behring, Roche, Amgen, Celgene, Rigel Pharmaceuticals, Ionis Pharmaceuticals, AbbVie, Sanofi, MorphoSys AG, Acerta Pharma, Janssen-Cilag, Bristol Myers Squibb, Boehringer Ingelheim and Astra Zeneca; has received honoraria from Bayer, Bristol Myers Squibb, Cardinal Health and Astra Zeneca; and has participated in Data Safety Monitoring Boards for Roche, Janssen-Cilag, CSL Behring, Pharmaxis and George Institute. H-HH has received consulting fees from Bristol Myers Squibb, Novartis and GSK and has participated in Data Safety Monitoring Boards for Bristol Myers Squibb, Novartis and GSK. LM has received support for attending meetings from GSK and has participated in Data Safety Monitoring Boards for GSK, MorphoSys, Cogent and Pharma Essentia. SYT has received honoraria from Pfizer and Otsuka. JB is an employee of Syntara Limited; has been a member of Syntara Data Safety Monitoring Boards; and holds stocks and/or stock options in Syntara Limited. BC is a medical consultant to Syntara Limited; has been a member of Syntara Data Safety Monitoring Boards; and holds stocks in Syntara Limited. AF, DH, WJ, JL, JM, KM and AZ are employees of Syntara Limited and hold stock and/or stock options in Syntara Limited.

Contributions

PV was responsible for the concept of this paper, contributed to the literature search and data collection, contributed patients, analyzed and interpreted data, and critically revised the manuscript. GH and JB were responsible for the concept of this paper, contributed to the literature search and data collection, analyzed and interpreted data, and critically revised the manuscript. AF, JL and JM were responsible for the concept of the paper, analyzed and interpreted data, and wrote the manuscript. PV, PT, A-MW, S-JW, RB, SC, S-EL, C-CC, T-YC, H-HH, J-HL, LM and SYT contributed patients and critically revised the manuscript. All authors reviewed, revised, and provided final approval of the manuscript.

Data-sharing statement

All data reported within the manuscript and further may be available upon reasonable request to the corresponding author. The data will be provided in compliance with applicable privacy laws, data protection, and requirements for consent and anonymization.

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