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Safety and efficacy of zinpentraxin alfa as monotherapy or in combination with ruxolitinib in myelofibrosis: stage I of a phase II trial

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Abstract

Pentraxin 2 (PTX-2; serum amyloid P component), a circulating endogenous regulator of the inflammatory response to tissue injury and fibrosis, is reduced in patients with myelofibrosis (MF). Zinpentraxin alfa (RO7490677, PRM-151) is a recombinant form of PTX-2 that has shown preclinical antifibrotic activity and no dose-limiting toxicities in phase I trials. We report results from stage 1 of a phase II trial of zinpentraxin alfa in patients with intermediate-1/2 or high-risk MF. Patients (n=27) received intravenous zinpentraxin α weekly (QW) or every 4 weeks (Q4W), as monotherapy or an additional therapy for patients on stable-dose ruxolitinib. The primary endpoint was overall response rate (ORR; investigator-assessed) adapted from International Working Group-Myeloproliferative Neoplasms Research and Treatment criteria. Secondary endpoints included modified Myeloproliferative Neoplasm-Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) change, bone marrow (BM) MF grade reduction, pharmacokinetics, and safety. ORR at week 24 was 33% (n=9/27) and varied across individual cohorts (QW: 38% [3/8]; Q4W: 14% [1/7]; QW+ruxolitinib: 33% [2/6]; Q4W+ruxolitinib: 50% [3/6]). Five of 18 evaluable patients (28%) experienced a ≥50% reduction in MPN-SAF TSS, and six of 17 evaluable patients (35%) had a ≥1 grade improvement from baseline in BM fibrosis at week 24. Most treatment-emergent adverse events (AE) were grade 1–2, most commonly fatigue. Among others, anemia and thrombocytopenia were infrequent (n=3 and n=1, respectively). Treatment-related serious AE occurred in four patients (15%). Overall, zinpentraxin alfa showed evidence of clinical activity and tolerable safety as monotherapy and in combination with ruxolitinib in this open-label, non-randomized trial (*clinicaltrials gov. Identifier: NCT01981850*).

Introduction

Myelofibrosis (MF) is a rare and often fatal hematologic neoplasm characterized by a chronic pro-inflammatory state and progressive bone marrow (BM) failure.[1-3] The pressures of chronic inflammation, such as pro-inflammatory cytokine and chemokine release, allow for the selection, clonal expansion, and evolution of mutant hematopoietic stem cells (HSC).[2,4-6] Mutant HSC proliferate in the BM, driving cytokine release, myeloid hyperproliferation, and BM fibrosis.[2,5] This chronic inflammation also results in remodeling of the BM microenvironment, perturbing the BM niche, and in turn disturbing normal hematopoiesis.[7] The resulting ineffective hematopoiesis and activation of inflammatory pathways have been linked to low hemoglobin levels, high leukocyte count, progress-

ive thrombocytopenia, and circulating blast cells, which have predicted shortened survival of patients with MF.[3,8]
Pentraxin 2 (PTX-2, also known as serum amyloid P component) is a circulating, endogenous regulator of the inflammatory response to tissue injury that is constitutively produced by the liver (Figure 1).[9-12] At the sites of tissue injury, PTX-2 binds to damaged cells and nuclear components (damage- and pathogen-associated molecular patterns) in the early stages of apoptosis, and facilitates their safe removal in a nonimmunogenic manner.[9,10,13-16] By binding to Fcγ receptors and DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin), PTX-2 promotes immunoregulatory and phagocytic polarization of monocytes and inhibits their differentiation into fibroblast-like, collagen-producing cells called fibrocytes.[9,10,12-15] Consequently, low PTX-2 levels may contribute to fibrotic diseases.[14] Supporting this hypothesis, plasma levels of PTX-2 were shown to be lower in patients with primary MF[17] and idiopathic pulmonary fibrosis (IPF) than in healthy controls.[15]
Zinpentraxin alfa is a recombinant human form of PTX-2 with a mechanism of action that has shown broad anti-fibrotic and anti-inflammatory activity in preclinical models of fibrotic disease.[17-19] Zinpentraxin alfa prevented BM fibrosis through its inhibition of primary MF BM-derived fibrocyte differentiation *in vitro*, and prevented and reversed fibrosis in animal models of MF.[17] In addition, zinpentraxin alfa significantly improved survival in a mouse model with a lethal MF-like phenotype.[17] Clinically, zinpentraxin alfa has shown no dose-limiting toxicities in phase I trials in healthy volunteers and patients with IPF.[20] Evaluation of zinpentraxin alfa in a phase II trial (*clinicaltrials gov. Identifier: NCT02550873*) in patients with IPF has demonstrated clinical activity and a generally well-tolerated safety profile.[21-23]
Currently, there are three approved drug therapies for controlling the symptoms of MF, all of which are Janus kinase (JAK) inhibitors: ruxolitinib, fedratinib, and pacritinib.[24-26] Clinically, JAK inhibitors have consistently demonstrated the ability to improve symptoms and reduce spleen size in patients with MF[25,27-31]; however, ruxolitinib is associated with suppression of hematopoiesis, and the resulting anemias often limit the duration of therapy in real-world settings.[32-34] The effect of JAK inhibition on BM fibrosis remains controversial: an analysis of BM biopsies from patients with MF in the phase III COMFORT-II study showed no improvements in histopathological abnormalities at 6 months of therapy with ruxolitinib,[35] and no consistent pattern of fibrosis grade improvement was observed with prolonged ruxolitinib therapy for up to 5 years.[33] Therefore, there remains an unmet medical need for disease-modifying therapies for patients with MF with improved tolerability, and potential targeting of not only the MF HSC but also the malignant BM niche.[7,36,37] Supplementation of ruxolitinib with a therapy that can deplete affected monocytes, and, thus reduce fibrosis and inflammation, is anticipated to be a promising approach in MF.[38]

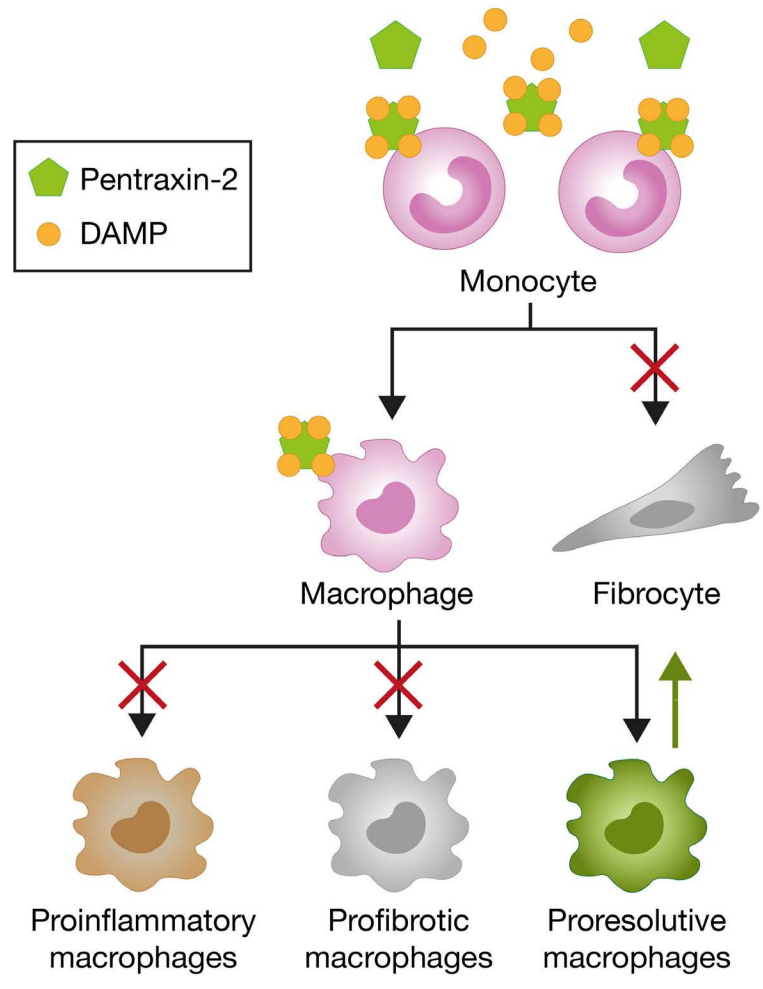

Figure 1. Zinpentraxin alfa mechanism of action. DAMP: danger-associated molecular pattern.

We conducted a phase II trial (*clinicaltrials gov. Identifier: NCT01981850*) to explore the efficacy and safety of zinpentraxin alfa in patients with MF. Here, we report the efficacy and safety findings from stage 1 of this trial, which investigated zinpentraxin alfa as monotherapy and as an additional therapy for patients on stable-dose ruxolitinib, each with two different dosing schedules, in patients with primary MF, post-essential thrombocythemia (post-EV) MF or post-polycythemia vera (post-PV) MF, with primary analysis at week 24, followed by an open-label extension (OLE) for patients with a clinical benefit from treatment.

Methods

Ethics statement

The trial was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice of the International Council for Harmonization. The protocol was approved by the relevant Institutional Review Board/Independent Ethics Committee at each site. All patients provided informed written consent.

Study design

This is an open-label, phase II trial comprising a 4-week screening period, 24-week treatment period (6×4-week

cycles), and 4-week follow-up period (*Online Supplementary Figure S1*). An OLE was available for patients without disease progression or toxicity warranting treatment discontinuation, and with clinical benefit as judged by the investigator, for as long as there was a benefit. Patients in the monotherapy cohorts received zinpentraxin alfa weekly (QW; cohort 1) or every 4 weeks (Q4W; cohort 2). Patients in the combination cohorts (on a stable dose of ruxolitinib for ≥12 weeks) continued with oral ruxolitinib twice daily at the same dose plus zinpentraxin alfa QW (cohort 3) or Q4W (cohort 4). Dosing frequency allocation was based on patients' availability for weekly visits. Zinpentraxin alfa 10 mg/kg was administered as a 60-minute intravenous infusion. Dosing and administration are further described in the *Online Supplementary Appendix*.

Patients

Key eligibility criteria included: aged ≥18 years with primary, post-ET, or post-PV MF; intermediate-1, intermediate-2, or high-risk disease per the International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) Dynamic International Prognostic Scoring System (DIPSS[39]); and locally assessed MF grade 2 or 3 BM fibrosis according to the European Consensus on Grading of Bone Marrow Fibrosis. A BM biopsy was required within 4 weeks prior to treatment initiation to establish the baseline fibrosis grade by central review.[40,41] Patients in the monotherapy arms must have received no treatment for MF for ≥2 weeks.

Outcomes

The overall response rate (ORR) at week 24 was the primary endpoint. ORR was defined as the percentage of patients with a response (assessed by the investigator) as follows (*Online Supplementary Table S1*): clinical improvement, partial remission, or complete remission per IWG-MRT criteria[42] at a post-baseline assessment of treatment response OR stable disease for three consecutive end-of-cycle response assessments (i.e., day 1 of the subsequent cycle) along with an improvement in the BM fibrosis score (determined by central review, blinded to subject, treatment, and time point) relative to baseline by ≥1 grade at any time point during the period of stable disease.

Key secondary endpoints included: modified Myeloproliferative Neoplasm-Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) change from baseline over time and proportion of patients with ≥50% relative reduction versus baseline at the beginning of each cycle (cycle 2 onward); BM MF grade reduction based on reticulin and trichrome staining[40,41] relative to baseline at week 24; pharmacokinetics (PK); and safety.

Response assessments in the OLE were performed on day 1 of every third cycle (every 12 weeks), beginning on cycle 10 day 1 (except ORR: best response at any time).

Additional endpoints and analyses (palpable spleen size, fibrocyte quantification, cytokine levels, and gene expression analyses), procedures, and statistical methods are described in the *Online Supplementary Appendix*.

Results

Patients

In total, 27 patients were enrolled at seven sites in the USA and Canada and assigned to one of the four treatment cohorts: cohort 1, n=8; cohort 2, n=7; cohort 3, n=6; cohort 4, n=6 (*Online Supplementary Figure S1*). Baseline demographics and clinical characteristics are shown in the *Online Supplementary Table S2*. The median age of patients was 67.0 (range, 51–85) years, with 66.7% of patients aged ≥65 years. The majority of patients were anemic (hemoglobin <100 g/L), around half of the patients were thrombocytopenic (platelets ≤100×10^9/L), and a considerable proportion had severe thrombocytopenia at baseline (37.0%; platelets ≤50×10^9/L). By central BM review, around a third of patients had BM fibrosis grade 2 and approximately half had grade 3 at baseline; over two-thirds of patients had intermediate-2 or high-risk MF per IWG-MRT DIPSS. Approximately half of patients had primary MF, around one-third of patients had post-PV MF, and the remaining patients had post-ET MF. Five patients were categorized as platelet transfusion–dependent at baseline (≥2 platelet transfusions in any 12 weeks prior to cycle 1 day 1, regardless of baseline platelet level). Patient disposition, including reasons for discontinuations, is shown in the *Online Supplementary Figure S2*. Overall, 20 patients completed the main trial and were evaluable at week 24. A total of 18 patients continued into the OLE; two patients did not continue based on patient decision. Median treatment duration in the OLE following completion of the main study was 24.4 months, up to a maximum of 70 months (cycle 83).

Outcomes

Efficacy

Overall, nine of 27 (33.3%; 90% confidence interval [CI]: 18.6–51.0) patients were responders during the main phase of the trial (up to week 24 of treatment); these patients included three of eight (37.5%) patients receiving zinpentraxin alfa QW in cohort 1, one of seven (14.3%) receiving zinpentraxin alfa Q4W in cohort 2, two of six (33.3%) receiving zinpentraxin alfa QW plus ruxolitinib in cohort 3 and three of six (50.0%) receiving zinpentraxin alfa Q4W plus ruxolitinib in cohort 4 (Table 1). During the OLE, the ORR increased to 59.3% of patients (n=16/27), which included clinical improvement in 33.3% of patients (n=9/27), and stable disease with BM improvement in 51.9% (n=14/27).

Five of 18 evaluable patients (27.8%) experienced a ≥50%

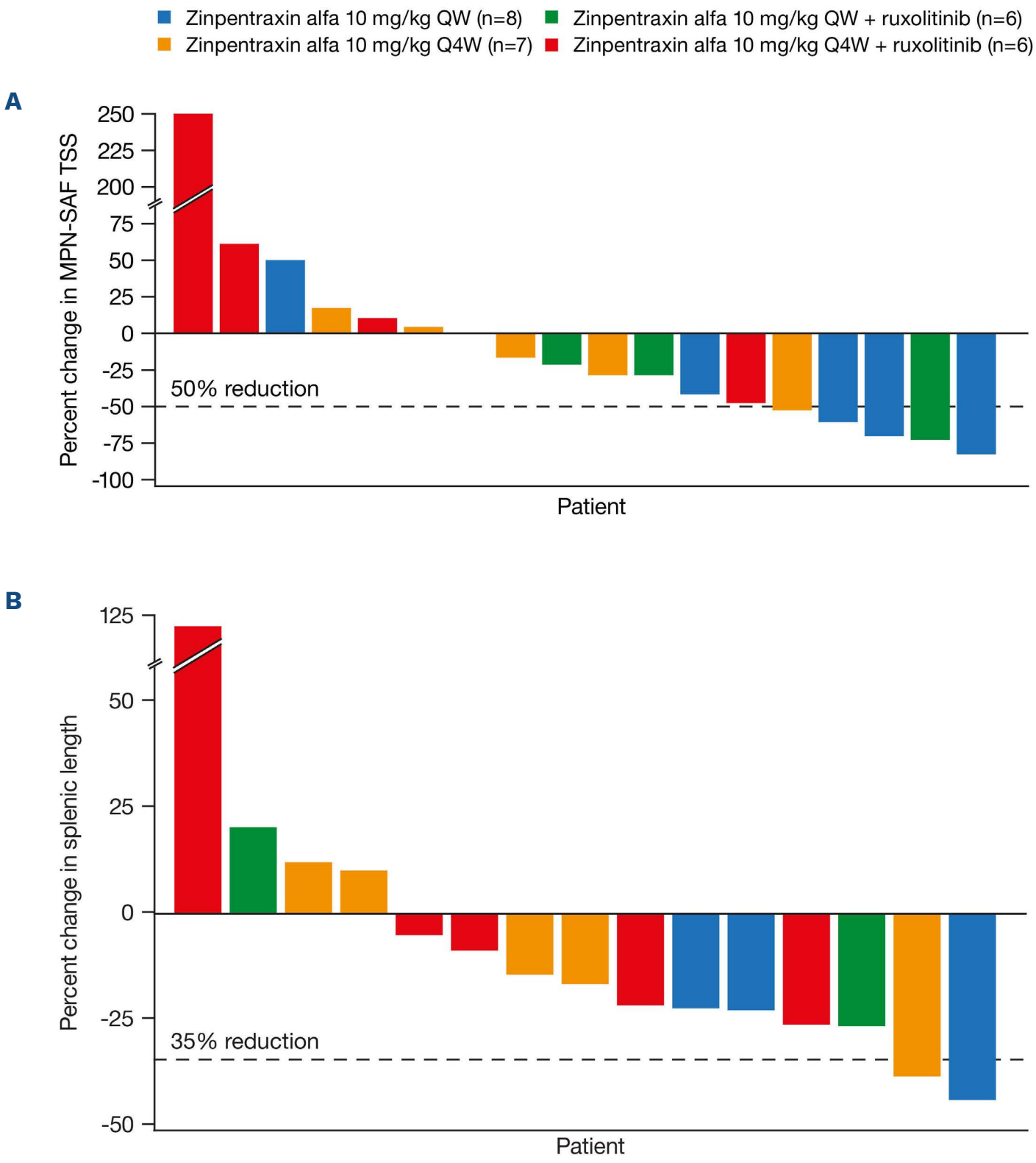

Figure 2. Percentage change from baseline in Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score and palpable spleen size at week 24. (A) Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS)[a]; (B) palpable spleen size[b]; all treated population. [a]Data shown for 18 patients in total; 7 patients withdrew from the trial before week 24 and 2 patients had missing values at week 24. [b]Data shown for 15 patients in total; 7 patients withdrew from the trial before week 24 and 5 patients had missing values at week 24. Q4W: every 4 weeks; QW: weekly.

Table 1. Overall response rate in the all-treated population at week 24 (primary endpoint).

Response, N (%)	Cohort 1: zinpentraxin alfa QW (N=8)	Cohort 2: zinpentraxin alfa Q4W (N=7)	Cohort 3: zinpentraxin alfa QW plus ruxolitinib (N=6)	Cohort 4: zinpentraxin alfa Q4W plus ruxolitinib (N=6)	Overall (N=27)
ORR [90% CI]	3 (37.5) [11.1–71.1]	1 (14.3) [0.7–52.1]	2 (33.3) [6.3–72.9]	3 (50.0) [15.3–84.7]	9 (33.3) [18.6–51.0]
Clinical improvement	0	0	1 (16.7)	0	1 (3.7)
SD + BM improvement	3 (37.5)	1 (14.3)	1 (16.7)	3 (50.0)	8 (29.6)

BM: bone marrow; CI: confidence interval; ORR: overall response rate; Q4W: every 4 weeks; QW: weekly; SD: stable disease.

reduction in MPN-SAF TSS at week 24, most of whom were in cohort 1 (n=3/8; 37.5%; Figure 2A). In total, 15 of 27 patients (55.6%) had a ≥50% reduction in MPN-SAF TSS at any time during the trial (*Online Supplementary Figure S3A*). At week 24, 35.3% (6/17) of patients with evaluable BM fibrosis grade demonstrated reduction (improvement) of ≥1 grade compared with baseline, and one of two patients with baseline fibrosis grade 1 had a one-grade increase (Table 2). Notably, one patient had complete resolution of grade 3 fibrosis; however, there was no follow-up to determine if this was sustained. Percentage change from baseline to week 24 in palpable spleen size is shown in Figure 2B. At week 24, two of 15 patients (13.3%) had a ≥35% reduction in palpable spleen size. In total, six of 21 patients (28.6%) had a ≥35% reduction in palpable spleen size at any time during the trial (*Online Supplementary Figure S3B*).

Safety

In total, 96.3% (n=26/27) of patients experienced ≥1 treatment-emergent adverse event (TEAE) in the main phase of the trial (100%, n=8 in cohort 1, n=6 in each of cohorts 3 and 4; 85.7%, n=6 for cohort 2) (Table 3). The majority of TEAE in the main phase of the trial were grade 1 or 2 in intensity, the most common being fatigue, cough, diarrhea, nausea, and oral herpes. New onset or exacerbation of anemia and thrombocytopenia were infrequently reported in all treatment cohorts (anemia, n=3 [1 patient in each of cohorts 1, 3, and 4]; thrombocytopenia, n=1 in cohort 3). Grade 3–4 TEAE were observed in three patients (11.1%; n=1 in each of cohorts 1, 2 and 3; all grade 3): anemia in two patients (7.4%; n=1 in each of cohorts 1 and 3), and abdominal pain, sialadenitis, urosepsis, hypoxia, nerve compression, sciatic nerve neuropathy, post procedural hematoma, and iron overload (3.7%, 1 patient each); grade 3 anemia (n=1), abdominal pain, and sialadenitis were considered related to zinpentraxin alfa or ruxolitinib treatment.

Serious TEAE and treatment-related serious TEAE are summarized in Table 3. Fourteen serious TEAE were observed in five patients (18.5%; n=2 in each of cohorts 1 and 3, n=1 in cohort 2) in the main trial phase. None of the individual serious TEAE were reported in more than one patient. Five serious treatment-related TEAE occurred in four patients (14.8%; n=1 in each of cohorts 1 and 2, n=2 in cohort 3): abdominal pain, sialadenitis, viral pneumonia, gastroenteritis, and respiratory syncytial virus infection (1 patient each; 1 patient experienced 2 different events). Three patients (11.1%; n=2 in cohort 1, n=1 in cohort 3) experienced a fatal TEAE during the main phase of the trial. One of these patients (cohort 1) experienced grade 5 gastroenteritis and viral pneumonia, which the investigator considered related to treatment. None of the other grade 5 events in the other two patients (cardiac arrest, organ failure, metabolic encephalopathy and renal failure in 1 patient, and pneumonia in another patient) were considered related to study treatment. An overview of adverse events during the OLE can be found in the *Online Supplementary Table S3*. Notably, one patient in the OLE continued until cycle 83.

During the main phase of the trial, infusion-related reactions (IRR) were reported in one patient (cohort 4; grade 2) after five doses of zinpentraxin alfa; no action was taken on study treatment and the event was resolved. During the OLE, two patients experienced IRR: one patient zinpentraxin alfa experienced grade 2 rash pruritic after 11 doses of zinpentraxin alfa (infusion was interrupted and restarted on the same day without reoccurrence of IRR after administration of diphenhydramine and dexamethasone) and one patient experienced a grade 1 IRR after ten doses of zinpentraxin alfa (no action was taken on study treatment and the event resolved). All three patients with an IRR tested positive for treatment-emergent anti-drug antibodies (ADA) in the main phase or the OLE of stage 1. However, there was no overall evidence of an impact of ADA status on the safety and tolerability of zinpentraxin alfa.

Pharmacokinetics

Results of the pharmacokinetics analysis are provided in the *Online Supplementary Appendix*.

Table 2. Shifts in bone marrow fibrosis grade from baseline at week 24 in the all-treated population (N=25[a]) (secondary endpoint).

Baseline grade[b]	Week 24[c], N			
	Grade 0	Grade 1	Grade 2	Grade 3
Grade 0 (N=0)	0	0	0	0
Grade 1 (N=2)	0	1	**1**	0
Grade 2 (N=4)	0	*3*	1	0
Grade 3 (N=11)	*1*	0	*2*	8

[a]A total of 25/27 patients in the study underwent a bone marrow (BM) biopsy. [b]One patient had missing BM fibrosis grade at baseline. [c]Eight patients had missing BM fibrosis grade at week 24. BM fibrosis grades according to World Health Organization criteria (as determined by central review). Patients were initially enrolled based on having grade ≥2 BM fibrosis according to local assessment (2 patients were scored as baseline grade 1 fibrosis after central review). Key: italics: reduction in grade; bold underline: increase in grade.

Hemoglobin, platelet, and transfusions

Hemoglobin (g/L) and platelet count (10^9/L) mean changes from baseline and the proportion of patients who received red blood cell and platelet transfusions during the main phase of the trial by dosing regimen/treatment group are shown in Figure 3 and the *Online Supplementary Figure S4*. The full details of this analysis are included in the *Online Supplemental Appendix*. Hemoglobin and platelet count levels fluctuated over the course of the trial and there was no obvious trend observed. Likewise, no obvious trend was observed in the proportion of patients requiring transfusions.

Biomarker analyses

BM fibrocytes were evaluated by immunostaining in eight patients from a single center, who each had completed ≥12 cycles of treatment with zinpentraxin alfa, as part of a *post hoc* analysis. Mean fibrocyte count declined from 378.0 cells/mm^3 at baseline to 115.1 cells/mm^3 at week 24, with a corresponding reduction in the proportion of fibrocytes as a percentage of total BM cells, from 11.2% to 3.0% (Figure 4). Plasma samples from five of these patients were also available for *post hoc* cytokine analysis. Changes in cytokine levels in these patients over time are shown in Figure 5 and the *Online Supplementary Figure S5*. Of 77 analyzed cytokines, downregulation in certain cytokines was observed, notably interleukin (IL)-8 (Figure 5). A correlational analysis of mean fibrocyte count and percentage of fibrocytes over time stratified by bone marrow fibrosis grade did not find any significant correlation, perhaps due to the relatively small sample size.

In an exploratory analysis, no significant changes over time with zinpentraxin alfa treatment were observed in gene expression from whole blood mRNA sequencing (*data not shown*; based on 119/124 samples from 25 patients; in total, 20,776 of 58,303 genes had sufficient expression for analysis).

Table 3. Summary of treatment-emergent adverse event in the safety population during the main phase of the trial, by cohort and overall.

TEAE, N (%)	Cohort 1: zinpentraxin alfa QW (N=8)	Cohort 2: zinpentraxin alfa Q4W (N=7)	Cohort 3: zinpentraxin alfa QW plus ruxolitinib (N=6)	Cohort 4: zinpentraxin alfa Q4W plus ruxolitinib (N=6)	Overall (N=27)
Any TEAE	8 (100)	6 (85.7)	6 (100)	6 (100)	26 (96.3)
Most common TEAE (>10% of patients[a])					
Fatigue	3 (37.5)	1 (14.3)	1 (16.7)	1 (16.7)	6 (22.2)
Cough	1 (12.5)	0	2 (33.3)	1 (16.7)	4 (14.8)
Diarrhea	2 (25.0)	0	0	2 (33.3)	4 (14.8)
Nausea	2 (25.0)	1 (14.3)	1 (16.7)	0	4 (14.8)
Oral herpes	1 (12.5)	0	2 (33.3)	1 (16.7)	4 (14.8)
ALT increased	3 (37.5)	0	0	0	3 (11.1)
Anemia	1 (12.5)	0	1 (16.7)	1 (16.7)	3 (11.1)
Epistaxis	0	0	2 (33.3)	1 (16.7)	3 (11.1)
Headache	0	0	2 (33.3)	1 (16.7)	3 (11.1)
Insomnia	1 (12.5)	0	1 (16.7)	1 (16.7)	3 (11.1)
Muscle weakness	2 (25.0)	1 (14.3)	0	0	3 (11.1)
Peripheral swelling	0	0	1 (16.7)	2 (33.3)	3 (11.1)
Pyrexia	0	0	0	3 (50.0)	3 (11.1)
URTI	0	0	1 (16.7)	2 (33.3)	3 (11.1)
Grade 3–4 TEAE	1 (12.5)	1 (14.3)	1 (16.7)	0	3 (11.1)
Grade 5 TEAE	2 (25.0)	0	1 (16.7)	0	3 (11.1)
TEAE leading to discontinuation of zinpentraxin alfa	2 (25.0)	0	0	0	2 (7.4)
Serious TEAE	2 (25.0)	1 (14.3)	2 (33.3)	0	5 (18.5)
Treatment-related serious TEAE[b]	1 (12.5)	1 (14.3)	2 (33.3)	0	4 (14.8)
Gastroenteritis	1 (12.5)	0	0	0	1 (3.7)
Pneumonia viral	1 (12.5)	0	0	0	1 (3.7)
Respiratory syncytial virus infection	0	0	1 (16.7)	0	1 (3.7)
Abdominal pain	0	1 (14.3)	0	0	1 (3.7)
Sialadenitis	0	0	1 (16.7)	0	1 (3.7)

[a]In total safety population (N=27). [b]Related to zinpentraxin alfa. ALT: alanine aminotransferase; Q4W: every 4 weeks; QW: weekly; TEAE: treatment-emergent adverse event; URTI: upper respiratory tract infection.

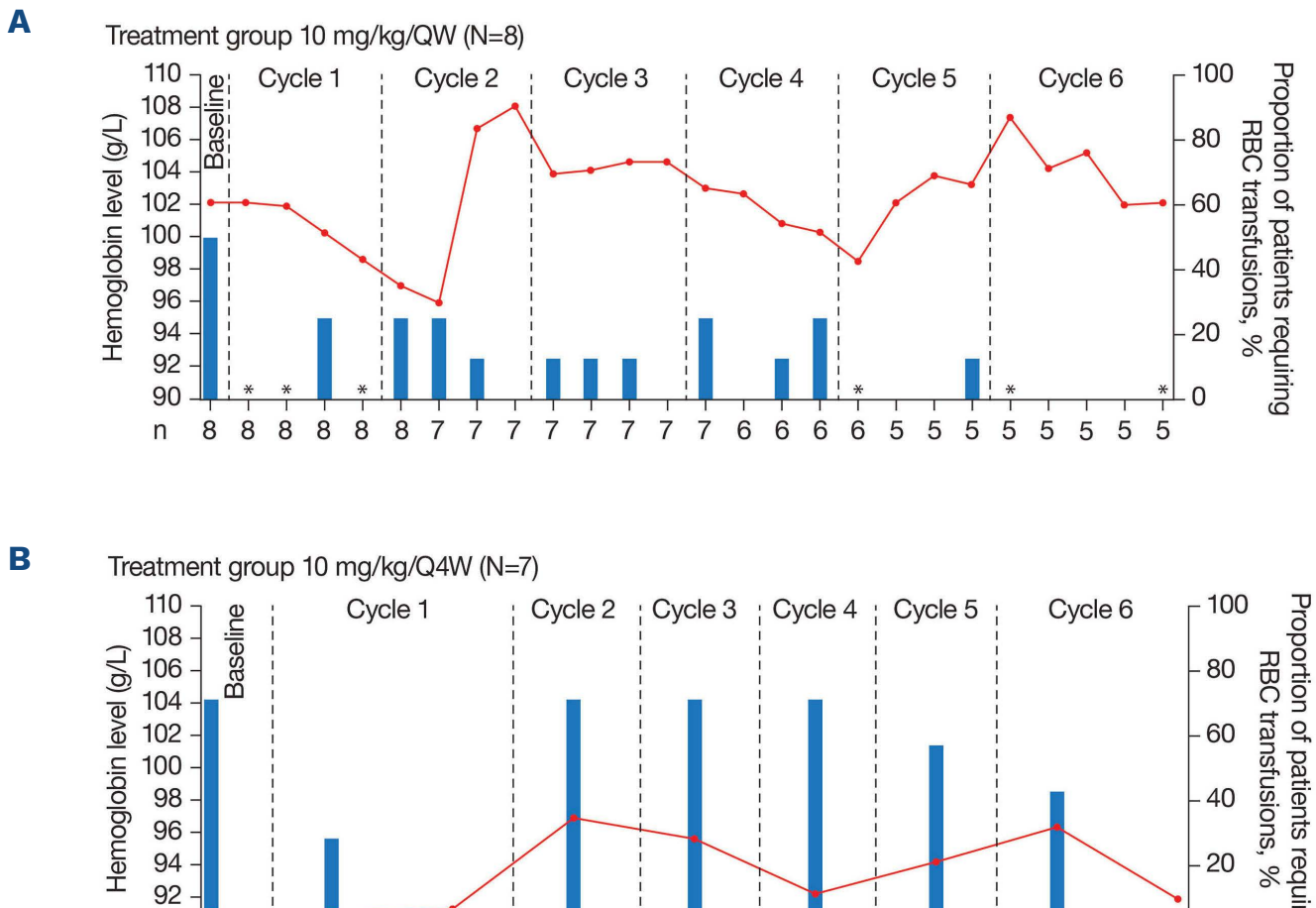

Figure 3. Effect of treatment with zinpentraxin alfa on hemoglobin and red blood cell transfusions. Mean hemoglobin (g/L) levels and proportion of patients with red blood cell (RBC) transfusions over time[a]; all-treated population. *Zero transfusions recorded at scheduled time point. [a](A and C) Time points are day 1, 8, 15, and 22 in each cycle, plus day 29 for cycle 6; (B and D) time points are day 1 and 15 in cycle 1, day 1 in cycles 2 to 5, and days 1 and 29 in cycle 6. Q4W: every 4 weeks; QW: weekly; Rux: ruxolitinib.

Discussion

In this open-label, non-randomized trial, clinical activity with zinpentraxin alfa alone and in combination with ruxolitinib was observed in patients with MF at week 24 and through the OLE for up to 332 weeks (>6 years). During the main phase of the trial, one-third of patients with MF receiving zinpentraxin alfa as monotherapy or in combination with ruxolitinib had a response per protocol-specified criteria, the majority of whom had stable disease with BM improvement, and the ORR further increased during the OLE phase to 59.3% (n=16/27). This trial used an ORR adapted from the IWG-MRT and European LeukemiaNet criteria, which aims to assess the value of new drugs in inducing morphologic remission or improvements in MF-associated signs and symptoms.[42] The IWG-MRT criteria (introduced at the time of protocol development) have not, to our knowledge, been used in other MF clinical trials to date, making it difficult to draw direct comparisons.

Improvements in symptoms were noted following treatment with zinpentraxin alfa, with nearly 20% of evaluable patients experiencing a ≥50% reduction in MPN-SAF TSS at week 24. Six patients (35.3%) had an improvement from baseline in BM fibrosis grade at week 24 and reductions in palpable spleen size were observed in two of 15 patients (13.3%). However, resolution of fibrosis requires complete remodeling of the BM niche, with removal of excessive fibrosis, extracellular matrix remodeling, and cellular migration back to the primary hematopoietic sites, which would be unlikely to occur within 24 weeks; as such, substantial clinical impact on the extra-medullary hematopoiesis and spleen size may not be apparent during this time frame.[43,44] Furthermore, the lack of a placebo arm in this trial prevents comparisons to the natural course of BM fibrosis. Therefore, we were unable to assess the natural variability in BM fibrosis that may be reflected in inter-sample variability (i.e., heterogeneity due to sampling location, or different amounts of material in each sample), though inter-assessor variability was accounted for by the use of central review. In addition, spleen size measured by palpation is less accurate compared with magnetic resonance imaging, even though palpation is util-

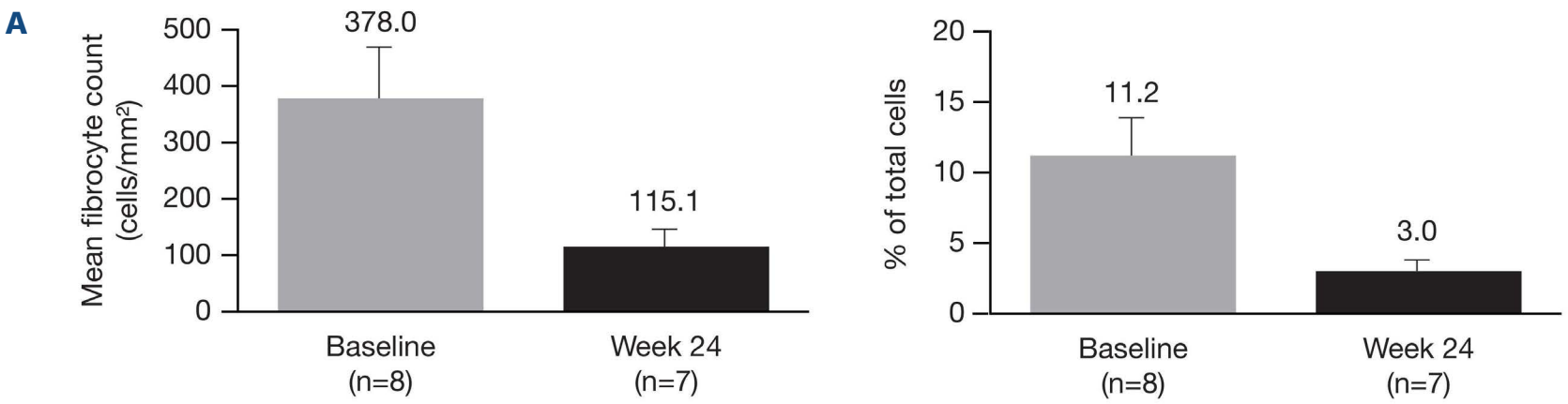

Figure 4. Effect of treatment with zinpentraxin alfa on fibrocytes. (A) Mean fibrocyte count and percentage of fibrocytes as a proportion of total cells in the bone marrow (BM) at baseline and week 24[a] (N=8); (B) representative fluorescence micrographs of BM biopsies of a patient at baseline and at week 24. In (A) data shown for 8 patients from a single center, who had each completed ≥12 cycles of treatment. Error bars represent standard error. In (B), depicted for 1 patient are DNA (Hoechst 33258; blue), CD45 (Opal 690; red), CD68 (Opal 520; green), and procollagen-I (Opal 570; white). Images are shown in pseudo-colors corresponding to individual channel intensities and as a composite of all 4 channels (Merge). Scale bar, 100 μm. Micrographs are provided at a resolution of 600 px/in. [a]Includes 1 patient with data from week 20.

ized frequently in the routine clinical setting. However, these results can be considered promising given this population's likely poor prognosis: the majority of patients had intermediate-2 or high-risk MF, had received prior JAK inhibitor therapy, and had a low platelet count, and some were platelet-transfusion-dependent. Low hemoglobin and platelet counts are both associated with a poor prognosis in patients with MF, and these patients have very limited treatment options.[3,45-47] While the hemoglobin, platelet count, and transfusions fluctuated during therapy, there were no notable downward trends in these hematologic parameters, contrary to the reported anemia and thrombocytopenia progression in patients with such advanced disease.[48] These observations are supported by the number of patients requiring platelet or red blood cell transfusions not increasing during the trial. This is a significant finding as patients with MF often have progressive anemia and thrombocytopenia that are further exacerbated by the use of JAK inhibitors such as ruxolitinib,[49] although the underlying reason for the observed results in the current study cannot be isolated based on the data available.

The safety data from this trial suggest an overall good tolerability of zinpentraxin alfa as monotherapy, as well as in combination with ruxolitinib. The incidence of serious TEAE was numerically higher in the QW cohorts (with or without ruxolitinib) than in the Q4W cohorts (with or without ruxolitinib). However, the small number of patients in each cohort and lack of randomization in this study preclude conclusions regarding any potential differences in the safety of these dosing regimens. Overall, a total of three IRR were reported. All IRR were grade 1–2, non-serious, and resolved, and patients continued study treatment.

Of note, while anemia and thrombocytopenia frequently result from the use of JAK inhibitors as well as MF disease progression itself,[8,27-29,48] zinpentraxin alfa is not anticipated to have myelosuppressive effects based on its mechanism of action. Consistent with this expectation, new onset or exacerbation of anemia was infrequent in all treatment cohorts, occurring in only three patients (one patient each in cohorts 1, 3, and 4) in the main phase of the trial. Treatment-emergent thrombocytopenia was not observed with zinpentraxin alfa monotherapy and occurred in only one patient receiving zinpentraxin alfa in combination with ruxolitinib. Moreover, prolonged administration of zinpentraxin alfa may alleviate the progressive anemia and thrombocytopenia resulting from the BM-associated fibrosis,[8] as well as from the myelosuppressive activity of ruxolitinib and other JAK inhibitors.[27-29] The non-overlapping safety profiles of zinpentraxin alfa and JAK inhibitors with respect to hematologic AE[25,27-29] also provide support for investigating rational combinations of zin-

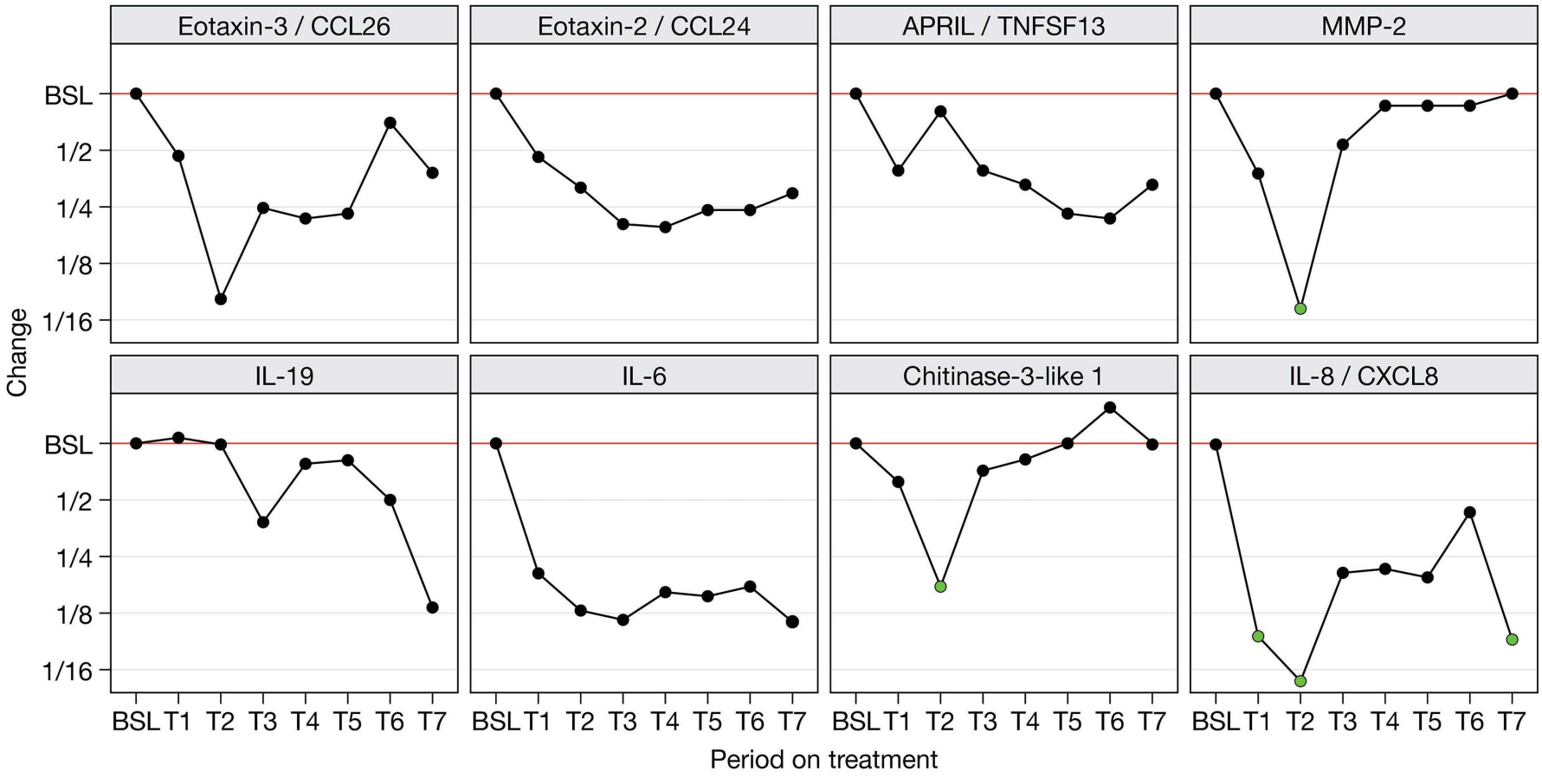

Figure 5. Effect of treatment with zinpentraxin alfa on cytokines. Mean levels of selected cytokines across the treatment period from baseline (BSL) to week 130, showing a significant decrease over the course of treatment (N=5). Plasma was analyzed by combined 40- and 37-plex magnetic bead-based immunoassays. Graphs show mean levels of 8 selected cytokines of interest over time, over the period on treatment (time points T1–7 are defined in the *Online Supplementary Figure S5*). The data points in green denote significant ($P<0.05$) on treatment changes relative to BSL in the 5 patients. *P* values were calculated for each time point using an empirical Bayes moderated *t* test with a Benjamini & Hochberg adjustment for multiple testing.

pentraxin alfa with currently available treatments such as JAK inhibitors. Additionally, the similarity in PK parameters across the four treatment cohorts and the lack of accumulation with more frequent dosing suggests that pentraxin exposure differences across the different dosing schedules were unlikely to impact on zinpentraxin alfa's safety and tolerability profile. Furthermore, PK parameters were comparable between patients with MF in this analysis and patients with IPF in previous research.[50]

In preclinical studies of primary MF, zinpentraxin alfa suppressed the formation of fibrocytes cultured from patients' BM $CD14^+$ monocytes and, in a xenograft murine model, slowed the development of BM fibrosis and prolonged survival.[17] In the current trial, quantification of fibrocytes in BM demonstrated a reduction at week 24 compared with baseline, suggesting that zinpentraxin alfa might induce similar effects in patients with MF. Furthermore, plasma cytokine profiling in a subset of patients showed time-dependent reduction of cytokines associated with differentiation and function of fibrocytes and profibrotic macrophages, such as interleukins (i.e., IL-8), which is in line with previously described anti-inflammatory effects of zinpentraxin alfa.[9,13-15] Both the observed reduction in BM fibrocyte counts and changes in plasma cytokine levels indicate a zinpentraxin alfa drug effect in line with preclinical findings. However, these results are from a *post hoc* analysis in only a small subset of patients from this trial and so further investigation in a larger group of patients is required to draw any firm conclusions, particularly as no obvious differences were observed in the gene expression analysis.

A major limitation of this trial is that there was no randomization of cohorts: patients were assigned zinpentraxin alfa as monotherapy *versus* combination therapy depending on whether they were already receiving ruxolitinib, and the frequency of dosing (QW *vs*. Q4W) was based on the patient's availability for trial visits. As such, there is the possibility of significant investigator bias in assigning patients to cohorts, precluding any meaningful inter-cohort comparisons. Also, for the cohorts receiving concomitant ruxolitinib, ruxolitinib administration was heterogenous, ranging from 5 to 25 mg twice daily, with variability between patients as well as for individual patients throughout the study. Additionally, the absence of a control arm meant that the isolated effect of zinpentraxin alfa could not be delineated. Due to the inclusion criteria permitting the participation of patients with a wide range of prognosis risk scores, there was marked variation in disease severity in the population at baseline leading to heterogeneity within each cohort. Furthermore, few patients in each cohort were treatment-naïve; many patients had taken prior therapies or were transfusion-dependent, indicating they had more advanced disease. Since there were no obvious differences in efficacy or safety between treatment cohorts, and given the small patient numbers and lack of randomization, the results of this phase II trial are difficult to interpret. Hence, any potential differences between zinpentraxin alfa monotherapy and zinpentraxin alfa in combination with ruxolitinib, or differences related to the dosing frequency cannot be determined. Although it is possible that an improvement in hematologic parameters in patients treated with zinpentraxin alfa could have also led to improved tolerably and/or changes in ruxolitinib dosing, no obvious differences were noted between the two combination cohorts. However, further investigations were precluded due to the small number of patients, the significant baseline heterogeneity in the population, lack of randomization, and absence of a ruxolitinib monotherapy control cohort. However, given the consistent ORR across all cohorts, the Q4W schedule was carried into stage 2 of the trial for further monotherapy testing as it offered optimal flexibility and patient convenience. Last but not least, accurate quantification of BM fibrosis is critical for the development of novel compounds.[51] However, accuracy may be compromised due to heterogeneity in fibrosis grade and megakaryocyte clusters within the BM that can lead to inconsistency between different samples and assessments.[41] More global quantitative assessments of changes in BM fibrosis using less invasive tools such as magnetic resonance imaging or positron emission tomography/computed tomography scanning of the skeleton may be useful to study anti-fibrotic effects of novel agents in MF.

In summary, zinpentraxin alfa has the potential to be a valuable treatment as monotherapy or in combination with anti-proliferative drugs such as ruxolitinib, through specifically targeting the BM microenvironment to reduce inflammation and fibrosis with no added toxicity. These factors could enable a long-term treatment plan that remains tolerable, evidenced by one patient continuing up to 83 cycles in the OLE. Notwithstanding the small patient numbers, results from stage 1 of this phase II trial showed evidence of clinical activity and a tolerable safety profile of zinpentraxin alfa as single agent and in combination with ruxolitinib, with QW or Q4W dosing schedules. Downward shifts in the BM fibrosis score in some patients and preliminary *post hoc* subpopulation analyses showing fibrocyte reductions and decreased levels of proinflammatory cytokines support the postulated mechanism of action of zinpentraxin alfa. The results from this trial were used to inform the zinpentraxin alfa monotherapy dosing schedule in stage 2 of this trial, which will be reported separately. These results will advise future investigations of zinpentraxin alfa in select populations and using treatment combinations that have the best chance to ameliorate the disease course for patients.

Disclosures

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Contributions

SV, TM, IV, RM and MU developed the concept and designed the study. JM, SV, TM, IV, LF, ER and RM acquired data. BH, LH, TCE-G, KG, OP and RH (BM morphologic analysis/review), SV, TM, IV, VG, KT, MU, HH, DW, JG and RM analyzed data. BH, LH, JM, TCE-G, KG, SV, TM, IV, VG, BT, LF, KT, MU, HH, RM, ER, DW and JG interpreted results. All authors were involved in the review/revision of the manuscript, approved the final version, and vouch for the accuracy of the content included in the manuscript.

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Data-sharing statement

Qualified researchers may request access to individual patient-level data through the clinical study data request platform (https://vivli.org). Further details on Roche's criteria for eligible studies are available here: https://vivli.org/ourmember/roche/. For further details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see here: https:www.roche.com/innovation/process/clinicaltrials/data-sharing/.

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