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

Srdan Verstovsek,[1°] Lynda Foltz,[2] Vikas Gupta,[3] Robert Hasserjian,[4] Taghi Manshouri,[1] John Mascarenhas,[5] Ruben Mesa,[6] Olga Pozdnyakova,[7] Ellen Ritchie,[8] Ivo Veletic,[1] Katia Gamel,[9] Habib Hamidi,[10] Lyrialle Han,[10] Brian Higgins,[10] Kerstin Trunzer,[9] Marianne Uguen,[9] Dao Wang,[9] Tarec Christoffer El-Galaly,[9°] Boyan Todorov[9] and Jason Gotlib[11]

[1]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [2]St Paul's Hospital, University of British Columbia, Vancouver, British Columbia, Canada; [3]Princess Margaret Cancer Center, University of Toronto, Toronto, Ontario, Canada; [4]Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA; [5]Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [6]Mays Cancer Center at UT Health San Antonio MD Anderson, San Antonio, TX, USA; [7]Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [8]Weill Cornell Medicine, New York, NY, USA; [9]F. Hoffmann-La Roche, Ltd., Basel, Switzerland; [10]Genentech Inc, South San Francisco, CA, USA and [11]Stanford Cancer Institute/Stanford University School of Medicine, Stanford, CA, USA

°SV current address: Kartos Therapeutics Inc, Redwood City, CA, USA.
°TCE-G current address: Department of Hematology, Aalborg University Hospital, Aalborg, Denmark.

Correspondence: S. Verstovsek
srdanverstovsek@gmail.com

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SUPPLEMENTARY MATERIAL

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METHODS

Selection of zinpentraxin alfa dose and administration schedule

The zinpentraxin alfa dose and schedule were chosen based on the results of a phase I multiple ascending dose study in patients with idiopathic pulmonary fibrosis (PRM151A-11EU; NCT01254409).[1] Both a weekly (QW) and every 4-week (Q4W) dosing schedule were investigated in the current trial. Patients received zinpentraxin alfa 10 mg/kg on days 1, 3, 5, 8, 15, and 22 of cycle 1, followed by days 1, 8, 15, and 22 of each subsequent cycle (QW schedule), or on days 1, 3, and 5 of cycle 1, followed by day 1 of each subsequent cycle (Q4W schedule).

Patients who continued into the open-label extension (OLE) continued with their previous zinpentraxin alfa dosing schedule. Patients who did not experience any clinical benefit during the main study were permitted to change schedule to one that had resulted in responses in the main study. Patients were permitted to discontinue ruxolitinib in the OLE, but patients were not allowed to start ruxolitinib if they received monotherapy during the main phase.

Exploratory and post hoc analyses

Key exploratory endpoints included percentage change (≥35% reduction) from baseline in palpable spleen size assessed by palpation, changes in circulating plasma cytokine levels, and changes in whole blood mRNA levels. Additional, post hoc analyses included quantification of fibrocytes in bone marrow (BM) biopsies by multiplexed fluorescence immunohistochemistry, changes in hemoglobin levels and red blood cell transfusions, and changes in platelet counts and platelet transfusions.

Procedures

Patients were assessed for response at cycle 2 through cycle 6 by the investigator, and overall response rate (ORR) modified from International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) criteria (Supplementary Table 1).[2] BM biopsies were performed at baseline, day 1 of cycle 4 (12 weeks) and day 29 of cycle 6 (24 weeks). Analysis included confirmation of diagnosis (at baseline) and determination of fibrosis grade (reticulin and trichrome stain), and standard cytogenetic analysis for marrow biopsy/aspirate. BM fibrosis was graded according to the European Consensus on Grading of Bone Marrow Fibrosis [3] by the site's local pathologist, as well as centrally by a separate committee of two pathologists blinded to the subject, treatment, and time point; the central committee's fibrosis score was used in the response assessments. Spleen size was measured by physical examination palpation in cm below the left costal margin.

The Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF) was administered to evaluate the effects of zinpentraxin alfa treatment on patients' quality of life, expressed as the MPN-SAF total symptom score (MPN-SAF TSS).[4]

Infusion-related reactions (IRR) were considered to be adverse events of special interest and were reportable within 24 hours. All IRR were not pre-defined and were reported based on the investigator's judgment. Study-specific protocol provided possible signs and symptoms of IRR, as well as instructions about interruption, discontinuation, and possible re-starting of drug administration. If a patient experienced an acute IRR, a blood sample was collected for cytokines and antibodies against zinpentraxin alfa.

Blood samples (4 mL) were obtained to determine the pharmacokinetics (PK) of endogenous levels of human pentraxin 2 (hPTX-2) and intravenously administered zinpentraxin alfa at

cycles 1, 2, and 6, day 1 pre-dose, at end of infusion, and 1, 2, 4, and 8 hours post-infusion; cycle 1, day 15 pre-dose, and 0.5, 0.75, 1.5, 3, and 6 hours post infusion; cycle 1, days 3, 5, 8, and 22 pre-dose; cycle 2, days, 8, 15, and 22 pre-dose; cycle 6, day 15 pre-dose; and at any time on cycle 6, day 29. A non-compartmental analysis was used to calculate the PK parameters. Due to lack of a drug-specific quantification assay, all PK parameters were calculated by subtracting the baseline (endogenous) PTX-2 concentration from the measured total PTX-2 value. Absolute concentrations are reported without baseline correction.

BM fibrocytes were evaluated by immunostaining as part of a post hoc analysis in patients at a single center who each had completed ≥12 cycles of treatment with zinpentraxin alfa. Fibrocytes were detected by multiplexed fluorescence immunohistochemistry (CD45, CD68, and procollagen I) performed at a single center. Briefly, decalcified formalin-fixed, paraffin-embedded trephine biopsies were processed through multiple rounds of immunostaining using commercially available monoclonal antibodies and Opal fluorophore-conjugated tyramides, as described previously.[5] Whole tissue sections were imaged using Vectra 3 multispectral imaging system (Akoya Biosciences, MA, USA) and spectrally unmixed based on single-stained controls. Fibrocytes, defined as $CD45^+/CD68^+$/procollagen I^+ cells, were quantitated using an unsupervised tissue and cell segmentation algorithm developed in VIS software (Visiopharm, Denmark). All analyses were performed blinded to the sample identity and clinical outcomes.

To evaluate changes in circulating cytokine levels as part of a post hoc analysis, plasma samples were taken from patients at a single center with ≥12 cycles of treatment with zinpentraxin alfa. Plasma was evaluated by 40- and 37-plex magnetic bead-based immunoassays from Bio-Rad (Bio-Plex Pro Human Chemokine Panel, 40-Plex #171AK99MR2, Bio-Plex Pro Human Inflammation Panel 1, 37-Plex #171AL001M).

Plasma from nine healthy volunteers was used to compare baseline levels between patients in the current trial and healthy controls.

RNA extracted from whole blood collected at trial visits (cycle 1 day 1 [n=25], cycle 2 day 1 [n=25], cycle 3 day 1 [n=25], cycle 4 day 1 [n=25], and cycle 6 day 29 [n=19]) was analyzed by next-generation RNA sequencing. Differences in gene expression were investigated over time (visits) and between treatment cohorts.

Statistical analysis

Efficacy and safety endpoints were assessed in the all-treated and safety populations respectively, which included all patients who received ≥1 dose of zinpentraxin alfa. For the primary ORR endpoint, hypothesis testing was performed separately in each of the four cohorts and overall, at the 5% significance level (one-sided; statistical significance concluded if the lower bound of the two-sided 90% confidence interval is >10%). For other endpoints, no formal hypothesis testing was performed, and results were considered descriptive. Patients with no post-baseline IWG-MRT response assessments were considered non-responders. Non-compartmental PK analysis was performed using WinNonlin (v8; Certara, Princeton, NJ). Changes in whole blood mRNA and circulating plasma cytokine levels were summarized using descriptive statistics and *P*-values (Mood's median test), assessed using SAS software (version 9.4; Cary, NC).

RESULTS

Pharmacokinetics

Baseline-adjusted PK parameters were calculated for zinpentraxin alfa on cycle 1 day 1. Baseline-adjusted geometric mean maximum observed plasma concentration (C_{max}) was 112–164 µg/mL and clearance was 0.233–0.362 L/hour. C_{max} was observed 1.2–1.6 hours post-dose. The geometric mean plasma half-life was approximately 15–18 hours. Concentration–time plots for PTX-2 for the first 8 hours after dosing are shown in Supplementary Figure S6; zinpentraxin alfa exposure on Day 1 Cycle 1 was comparable between cohorts (Supplementary Table S4). The PK profile of zinpentraxin alfa did not vary substantially with different dosing regimens or with/without combined treatment with ruxolitinib. No zinpentraxin alfa accumulation was observed upon QW dosing (data not shown) and there was no evidence of impact of ADA on zinpentraxin alfa exposure following multiple treatment cycles (data not shown).

Supplementary Table S1. Study definition of ORR.

Any one of the following:	**Notes**
Clinical improvement[a]	Any one of the following at a post-baseline assessment of treatment response: • Spleen response (35% volume reduction) • Symptom response (50% reduction in MPN-TSS) • Anemia response (≥20 g/L increase in haemoglobin or becoming transfusion-independent)
Partial remission[a]	At a post-baseline assessment of treatment response
Complete remission[a]	At a post-baseline assessment of treatment response
Stable disease	For three consecutive end-of-cycle response assessments (i.e., day 1 of the subsequent cycle) **AND** An improvement in the BM fibrosis score (determined by central review,[b] blinded to subject, treatment, and time point) relative to baseline by ≥1 grade at any time point during the period of stable disease

[a]Per IWG-MRT criteria (assessed by the investigator).[2]

[b]BM fibrosis was graded according to the European Consensus on Grading of Bone Marrow Fibrosis[3]; the central committee was blinded to patient, treatment, and collection time for centralized grading of fibrosis.

BM, bone marrow; IWG-MRT, International Working Group-Myeloproliferative Neoplasms Research and Treatment; MPN-TSS, Modified Myeloproliferative Neoplasm Total Symptom Score; ORR, overall response rate.

Supplementary Table S2. Demographics and baseline characteristics in the all-treated population.

Characteristic	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)
Median (range) age, years	62.0 (51–85)	71.0 (60–78)	68.0 (52–72)	65.5 (57–78)	67.0 (51–85)
Sex, n (%)					
Male	3 (37.5)	5 (71.4)	3 (50.0)	1 (16.7)	12 (44.4)
Female	5 (62.5)	2 (28.6)	3 (50.0)	5 (83.3)	15 (55.6)
Race, n (%)					
American Indian/Alaskan native	0	0	1 (16.7)	0	1 (3.7)
Asian	0	0	1 (16.7)	0	1 (3.7)
Black/African American	1 (12.5)	0	1 (16.7)	0	2 (7.4)
White	7 (87.5)	7 (100)	3 (50.0)	6 (100)	23 (85.2)
Type of MF, n (%)					
Primary MF	6 (75.0)	3 (42.9)	3 (50.0)	2 (33.3)	14 (51.9)
Post-ET MF	1 (12.5)	2 (28.6)	0	1 (16.7)	4 (14.8)
Post-PV MF	1 (12.5)	2 (28.6)	3 (50.0)	3 (50.0)	9 (33.3)
Median (range) time since diagnosis, years	1.0 (0–3)	6.0 (1–11)	2.0 (1–8)	4.5 (1–9)	3.0 (0–11)
Risk group (IWG-MRT DIPSS), n (%)					
Low	0	0	0	0	0
Intermediate-1	3 (37.5)	2 (28.6)	2 (33.3)	1 (16.7)	8 (29.6)
Intermediate-2	5 (62.5)	4 (57.1)	2 (33.3)	5 (83.3)	16 (59.3)
High	0	1 (14.3)	2 (33.3)	0	3 (11.1)
Baseline BM fibrosis grade,[a] n (%)					
n	7	6	5	6	24
MF – 1	1 (14.3)	0	0	1 (16.7)	2 (8.3)
MF – 2	3 (42.9)	3 (50.0)	0	3 (50.0)	9 (37.5)
MF – 3	3 (42.9)	3 (50.0)	5 (100)	2 (33.3)	13 (54.2)

Spleen size below left costal margin at baseline, cm					
n	8	6	6	6	26
Mean (SD)	17.19 (11.84)	15.92 (5.89)	10.33 (8.64)	13.75 (10.06)	14.52 (9.41)
Hb level at baseline, n (%)					
<100 g/L	4 (50.0)	5 (71.4)	3 (50.0)	3 (50.0)	15 (55.6)
≥100 g/L	4 (50.0)	2 (28.6)	3 (50.0)	3 (50.0)	12 (44.4)
≥1 Hb transfusion in 12 weeks prior to baseline, n (%)	4 (50.0)	5 (71.4)	1 (16.7)	1 (16.7)	11 (40.7)
RBC transfusion dependency at baseline,[b] n (%)					
Dependent	0	3 (42.9)	1 (16.7)	0	4 (14.8)
Independent	8 (100)	4 (57.1)	5 (83.3)	6 (100)	23 (85.2)
Mean (SD) [median] RBC units transfused in the 12 weeks before baseline, n	4.25 (2.63) [4]	7.6 (3.85) [8]	8.00 (NE) [8]	4.00 (NE) [4]	6.09 (3.36) [6]
Platelet count at baseline, n (%)					
≤25 x 10^9/L	2 (25.0)	3 (42.9)	1 (16.7)	0	6 (22.2)
>25 x 10^9/L to ≤50 x 10^9/L	0	2 (28.6)	0	2 (33.3)	4 (14.8)
>50 x 10^9/L to ≤100 x 10^9/L	2 (25.0)	0	1 (16.7)	1 (16.7)	4 (14.8)
>100 x 10^9/L	4 (50.0)	2 (28.6)	4 (66.7)	3 (50.0)	13 (48.1)
Mean (SD) [median] platelet units transfused in the 12 weeks before baseline, n	16.00 (NE) [16]	3.00 (1.41) [3]	25.00 (NE) [25]	NE (NE) [NE]	11.75 (10.78) [10]
Patients with prior MF therapy, n (%)	6 (75.0)	7 (100)	6 (100)	6 (100)	25 (92.6)
Protein kinase inhibitor	4 (50.0)	3 (42.9)	3 (50.0)	3 (50.0)	13 (48.1)

[a]According to central review. [b]Baseline PRBC transfusion dependency is defined as ≥2 units PRBC Q4W for 12 weeks prior to cycle 1 day 1, regardless of baseline Hb level.

BM, bone marrow; Hb, hemoglobin; IWG-MRT DIPSS, International Working Group-Myeloproliferative Neoplasms Research and Treatment Dynamic International Prognostic Scoring System; MF, myelofibrosis; NE, not evaluable; post-ET MF, post-essential thrombocythemia myelofibrosis; post-PV MF, post-polycythemia vera myelofibrosis; PRBC, peripheral red blood cell; Q4W, every 4 weeks; QW, weekly; RBC, red blood cell; SD, standard deviation.

Supplementary Table S3. Summary of TEAE in the safety population during the OLE, by cohort and overall.

TEAE, n (%)	Zinpentraxin alfa Q4W (n=13)	Zinpentraxin alfa Q4W plus ruxolitinib (n=5)	Overall (N=18)
Any TEAE	12 (92.3)	5 (100)	17 (94.4)
Most common TEAE (>10% of patients overall)			
Arthralgia	2 (15.4)	4 (80.0)	6 (33.3)
Nausea	4 (30.8)	2 (40.0)	6 (33.3)
Cough	2 (15.4)	3 (60.0)	5 (27.8)
Headache	2 (15.4)	2 (40.0)	4 (22.2)
Vomiting	2 (15.4)	2 (40.0)	4 (22.2)
Abdominal pain upper	3 (23.1)	0	3 (16.7)
Splenomegaly	3 (23.1)	0	3 (16.7)
Squamous cell carcinoma	0	3 (60.0)	3 (16.7)
URTI	3 (23.1)	0	3 (16.7)
ALT increased	0	2 (40.0)	2 (11.1)
Anemia	1 (7.7)	1 (20.0)	2 (11.1)
Aspartate aminotransferase increased	0	2 (40.0)	2 (11.1)
Back pain	1 (7.7)	1 (20.0)	2 (11.1)
Blood creatine phosphokinase increased	0	2 (40.0)	2 (11.1)
Bone pain	1 (7.7)	1 (20.0)	2 (11.1)
Bronchitis	0	2 (40.0)	2 (11.1)
Contusion	1 (7.7)	1 (20.0)	2 (11.1)

Fatigue	2 (15.4)	0	2 (11.1)
Hematoma	2 (15.4)	0	2 (11.1)
Musculoskeletal chest pain	1 (7.7)	1 (20.0)	2 (11.1)
Edema peripheral	2 (15.4)	0	2 (11.1)
Rash	1 (7.7)	1 (20.0)	2 (11.1)
Urinary tract infection	2 (15.4)	0	2 (11.1)
Wound infection	1 (7.7)	1 (20.0)	2 (11.1)
Grade 3–4 TEAE	6 (46.2)	4 (80.0)	10 (55.6)
Grade 5 TEAE	0	0	0
TEAE leading to discontinuation of zinpentraxin alfa	4 (30.8)	0	4 (22.2)
Serious TEAE	5 (38.5)	2 (40.0)	7 (38.9)
Treatment-related serious TEAE	0	0	0
Infusion-related reactions (grade 1–2)	1 (7.7)	1 (20.0)	2 (11.1)

ALT, alanine aminotransferase; OLE, open-label extension; Q4W, every 4 weeks; QW, weekly; TEAE, treatment-emergent adverse event; URTI, upper respiratory tract infection.

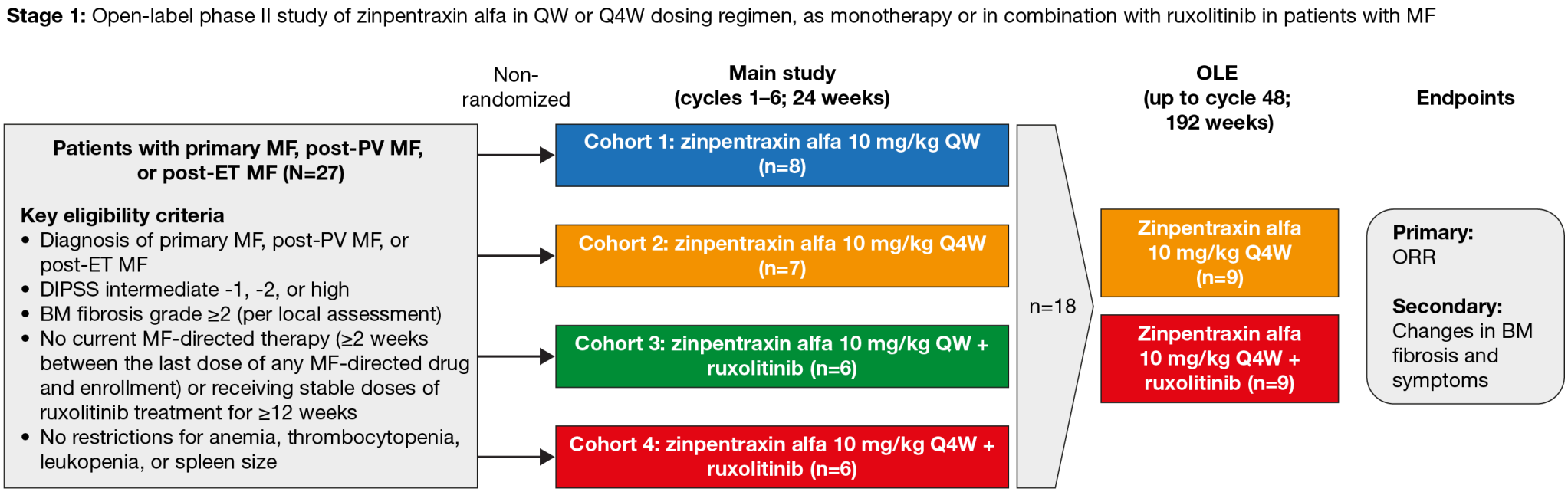
Supplementary Table S4. Baseline-adjusted exposure to zinpentraxin alfa (AUC_{0-9} and C_{max}) by treatment cohort on cycle 1 day 1.

	Cohort 1: zinpentraxin alfa QW (n=7)	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)
AUC_{0-9} (µg*hr/mL)				
Geometric mean	734	632	932	635
CV%[a]	47.7	22.2	24.8	86.3
C_{max} (µg/mL)				
Geometric mean	133	113	164	112
CV%[a]	48.4	28.1	23.9	90.4

[a]CV% of the geometric mean

AUC_{0-9}, area under the plasma concentration-time curve from time 0 to 9 hours from the start of the infusion; C_{max}, maximum observed plasma concentration; CV, coefficient of variation; Q4W, every 4 weeks; QW, weekly.

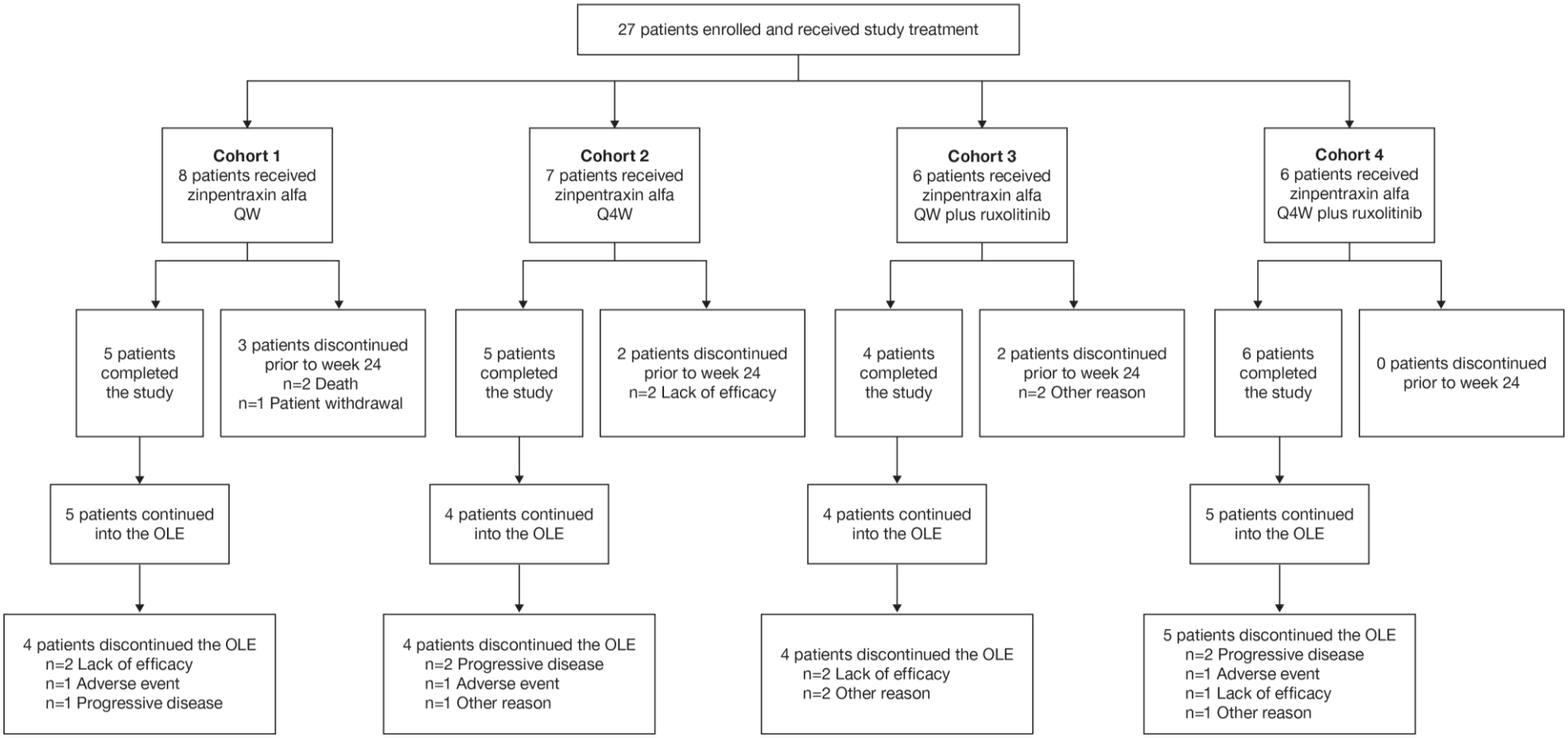
Supplementary Figure S1. Trial design.



Two patients in the OLE initially received zinpentraxin alfa QW before switching to Q4W at cycles 11 and 14, respectively.

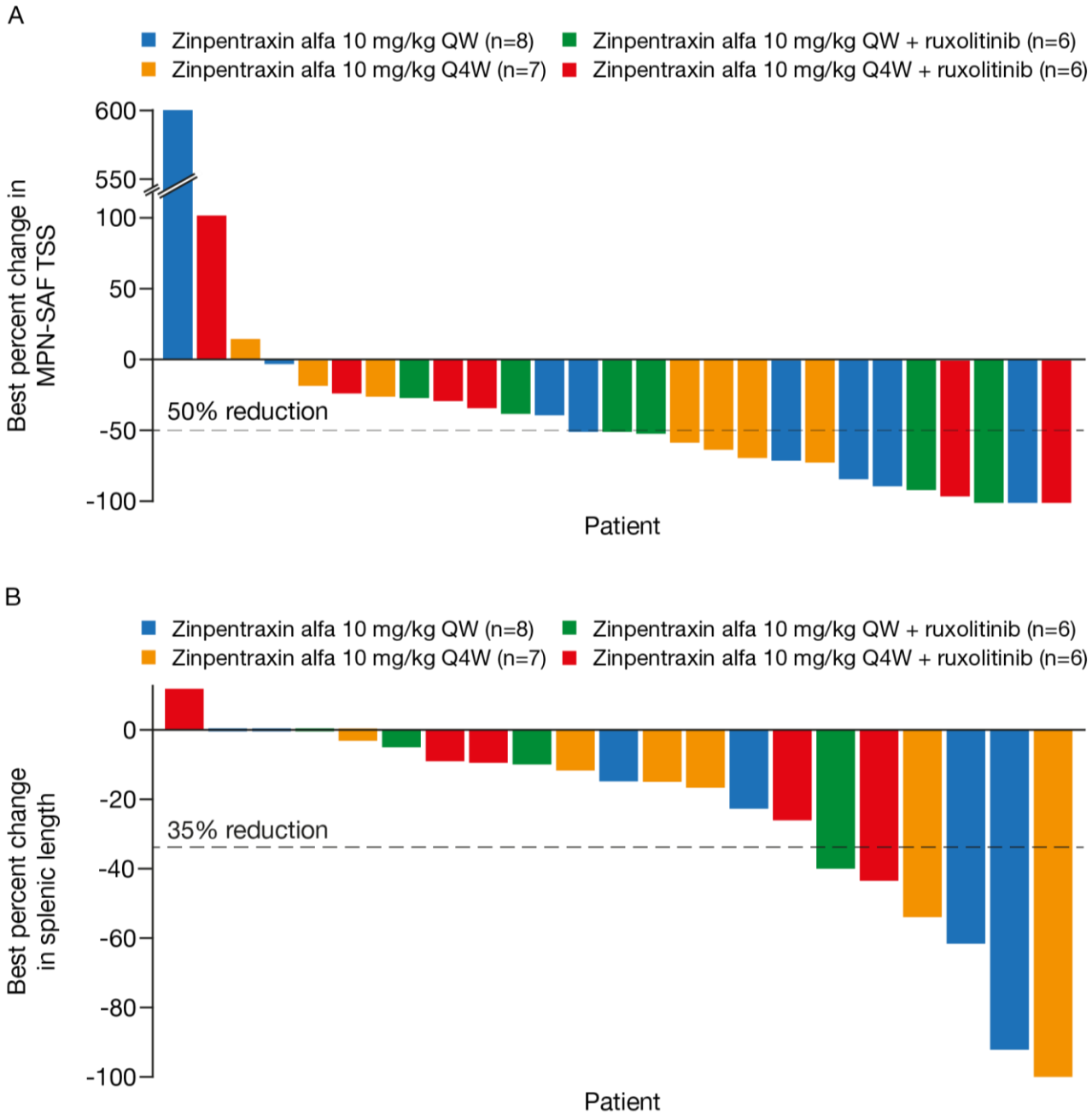
BM, bone marrow; DIPSS, Dynamic International Prognostic Scoring System; MF, myelofibrosis; OLE, open-label extension; ORR, overall response rate; post-ET MF, post-essential thrombocythemia myelofibrosis; post-PV MF, post-polycythemia vera myelofibrosis; Q4W, every 4 weeks; QW, weekly.

Supplementary Figure S2. Patient disposition.



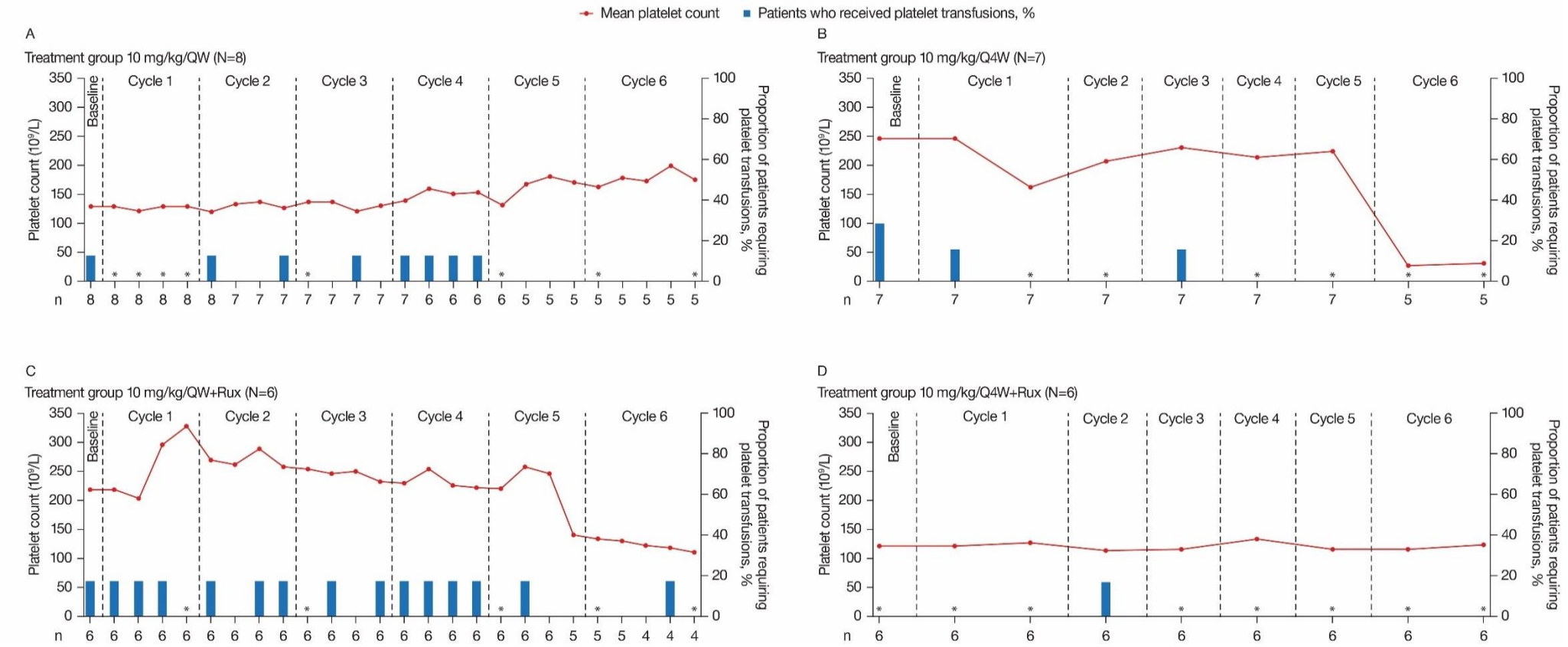
OLE, open-label extension; Q4W, every 4 weeks; QW, weekly.

Supplementary Figure S3. Best percentage change from baseline in MPN-SAF TSS and palpable spleen size during the main phase and OLE. (A) MPN-SAF TSS; (B) palpable spleen size; all treated population.



MPN-SAF TSS, Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score; OLE, open-label extension; Q4W, every 4 weeks; QW, weekly.

Supplementary Figure S4. Effect of treatment with zinpentraxin alfa on platelets. Mean platelet count (10^9/L) and proportion of patients with platelet transfusions over time[a]; all-treated population.

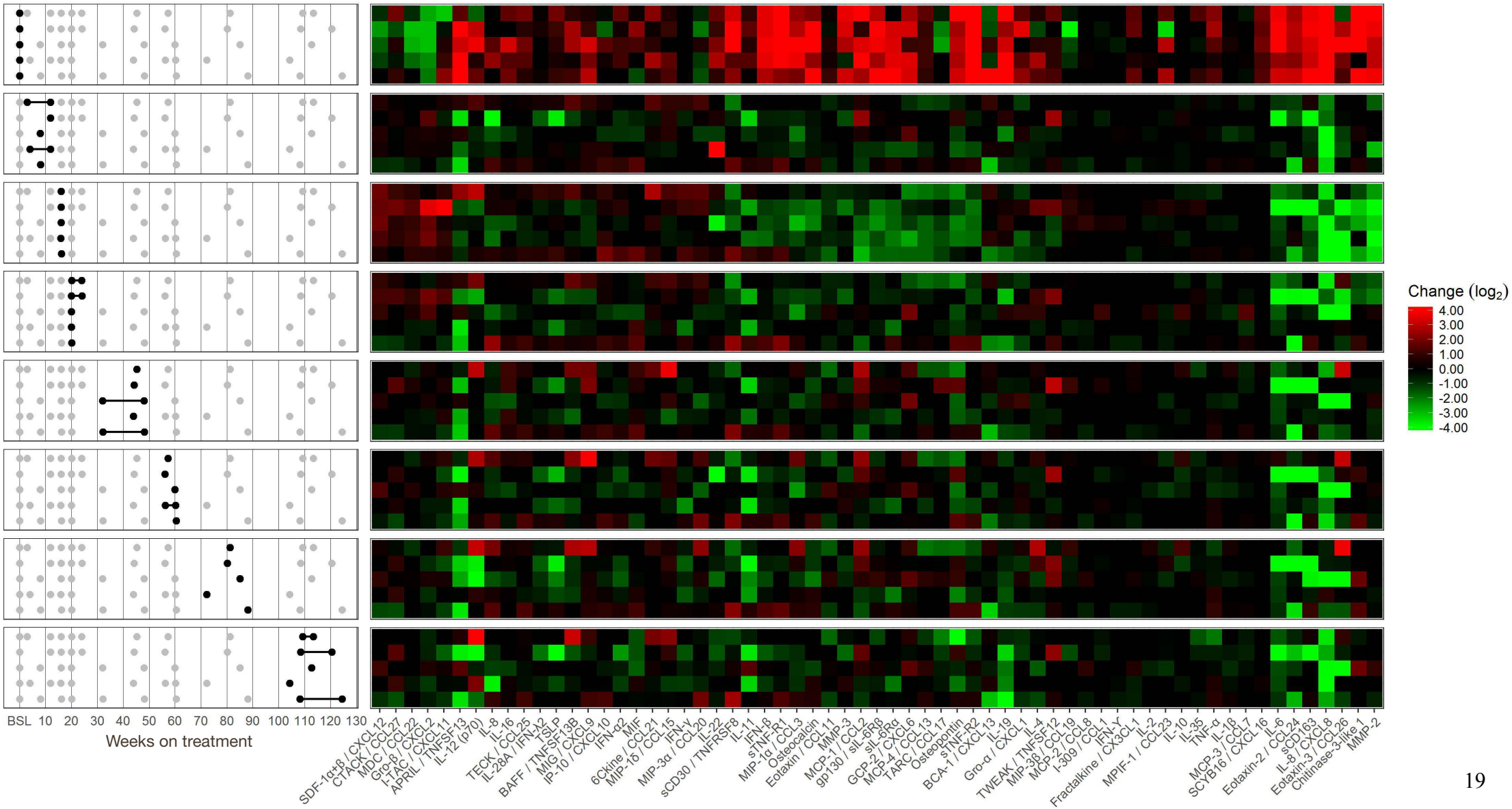


*Zero transfusions recorded at scheduled time point.

[a]Panels A and C: time points are Day 1, 8, 15, and 22 in each cycle, plus Day 29 for Cycle 6; Panels 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.

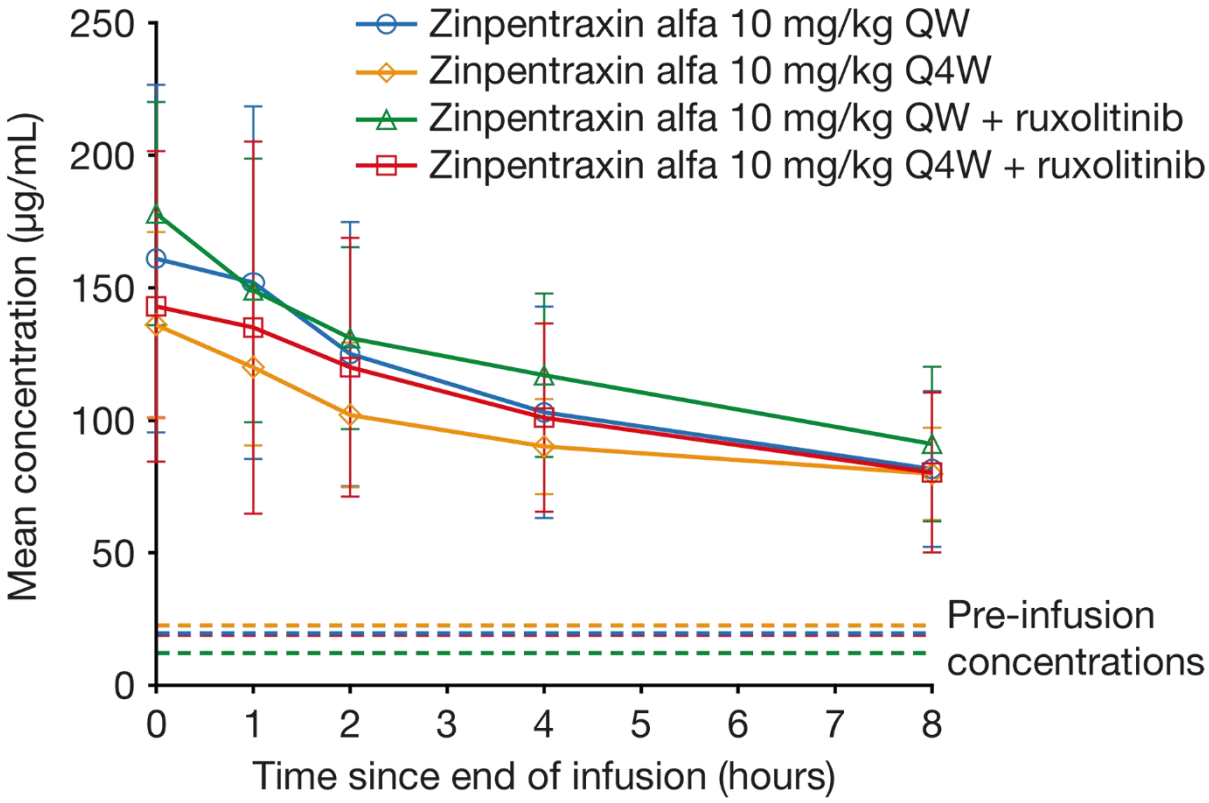
Supplementary Figure S5. Effect of treatment with zinpentraxin alfa on cytokines. Cytokine levels over time in a subset of patients from a single center with ≥12 cycles of treatment (n=5).



Plasma was analyzed by combined 40- and 37-plex magnetic bead-based immunoassays; some biomarkers are duplicated due to being included in both panels. Plasma from nine healthy volunteers was used for comparison of baseline levels in the five patients with MF (listed as one to five on the left-hand side) (top row "BSL"). The other rows show on-treatment changes relative to baseline in the five patients with MF during a specific period on treatment (rows T1–7). Black dots in the left-hand panel indicate the period on treatment when samples were taken during visits; lines across the dots indicate an average of the number of samples taken. Color change ranges from red (up-regulation of cytokines) to green (down-regulation). Three additional patients were excluded from the analysis due to having no appropriate baseline plasma sample.

MF, myelofibrosis.

Supplementary Figure S6. The concentration–time profile of PTX-2[a] by treatment cohort on cycle 1 day 1.



[a]Zinpentraxin alfa could not be distinguished from endogenous PTX-2 during quantification; as such, the measured concentrations include both zinpentraxin alfa and endogenous PTX-2.

PTX-2, pentraxin-2; Q4W, every 4 weeks; QW, weekly.

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