

# Pelabresib plus ruxolitinib for JAK inhibitor-naïve myelofibrosis: a randomized phase 3 trial

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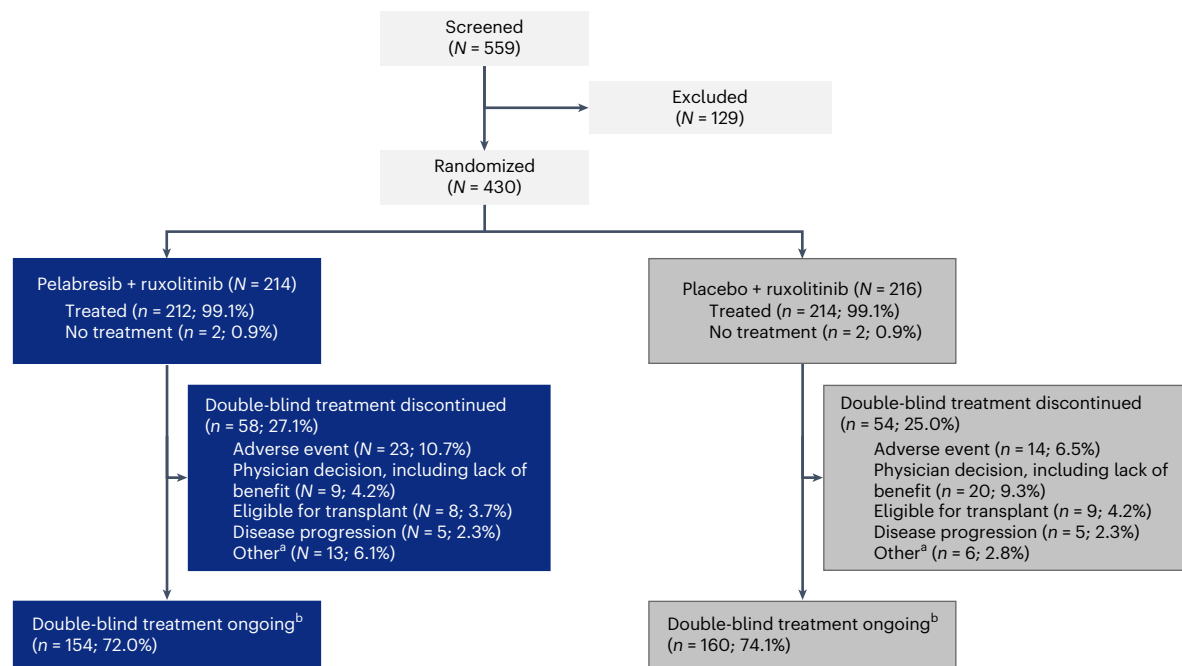
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Janus kinase (JAK) inhibitors provide limited depth and durability of response in myelofibrosis. We evaluated pelabresib—a bromodomain and extraterminal domain (BET) inhibitor—plus ruxolitinib (a JAK inhibitor) compared with placebo plus ruxolitinib as first-line therapy. In this phase 3 study (MANIFEST-2), JAK inhibitor-naïve patients with myelofibrosis were randomized 1:1 to pelabresib 125 mg once daily (QD; 50–175 mg QD permitted) for 14 days followed by a 7-day break (21-day cycle), or to placebo in combination with ruxolitinib 10 or 15 mg twice daily (BID; 5 mg QD–25 mg BID permitted). Primary endpoint was reduction in spleen volume of  $\geq 35\%$  from baseline at week 24. Key secondary endpoints were absolute change in total symptom score (TSS) and TSS50 response ( $\geq 50\%$  reduction in TSS from baseline at week 24). The primary endpoint was met in 65.9% of patients randomized to pelabresib–ruxolitinib ( $n = 214$ ) versus 35.2% to placebo–ruxolitinib ( $n = 216$ ) (difference, 30.4%; 95% confidence interval (CI), 21.6, 39.3;  $P < 0.001$ ). Absolute change in TSS was  $-15.99$  versus  $-14.05$  (difference,  $-1.94$ ; 95% CI,  $-3.92, 0.04$ ;  $P = 0.0545$ ) and TSS50 was achieved in 52.3% versus 46.3% (difference, 6.0%; 95% CI,  $-3.5, 15.5$ ) with pelabresib–ruxolitinib versus placebo–ruxolitinib. Exploratory analyses of proinflammatory cytokine amounts and bone marrow morphology showed greater improvement with the combination. Thrombocytopenia and anemia were the most common treatment-emergent adverse events, occurring in 52.8% (13.2% grade  $\geq 3$ ) versus 37.4% (6.1% grade  $\geq 3$ ) and 44.8% (23.1% grade  $\geq 3$ ) versus 55.1% (36.5% grade  $\geq 3$ ), respectively. Pelabresib in combination with ruxolitinib is well tolerated, improves signs of underlying myelofibrosis pathobiology and provides substantial clinical benefit over standard-of-care JAK inhibitor monotherapy. ClinicalTrials.gov identifier: [NCT04603495](https://clinicaltrials.gov/ct2/show/study/NCT04603495).

Myelofibrosis is a myeloproliferative neoplasm that manifests as aberrant activation of the JAK–STAT signaling pathway, often driven by mutations in *JAK2*, *CALR* or *MPL*<sup>1</sup>. Cardinal clinical features of myelofibrosis include hepatosplenomegaly, anemia, weight loss, bone pain and myelofibrosis-associated symptoms such as night sweats and fatigue<sup>2</sup>. Key histopathological features include bone marrow reticulin fibrosis and osteosclerosis, and megakaryocyte proliferation, atypia and tight clustering<sup>3,4</sup>. JAK inhibitor monotherapy is the

standard of care for myelofibrosis in patients with splenomegaly and/or symptoms<sup>1,5</sup>. However, JAK inhibitors do not consistently resolve pathobiological features, such as molecular markers of clonal burden and disrupted bone marrow morphology, particularly with short-term treatment<sup>6–8</sup>. Therefore, an unmet medical need persists due to limited depth and durability of clinical response, and frequency of treatment-emergent adverse events (TEAEs) with JAK inhibitor monotherapy<sup>9</sup>.

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**Fig. 1 | Patient disposition.** The study opened for enrollment in November 2020; the first patient received their initial treatment on 22 April 2021, and the last patient was enrolled on 2 March 2023. Percentages reported are based on the number of patients randomized (intent-to-treat set). <sup>a</sup>Other

(noncompliance/protocol violation ( $n = 2$  in each arm) or withdrawal of consent ( $n = 11$  in the pelabresib–ruxolitinib arm, and  $n = 4$  in the placebo–ruxolitinib arm)). <sup>b</sup>Treatment ongoing as of 31 August 2023.

Aberrant increases in levels of proinflammatory cytokines are a hallmark of myelofibrosis pathogenesis<sup>2,10</sup>. Such cytokines have a fundamental role in disease establishment and progression, contribute to the constitutional symptom profile of patients and are prognostic of outcomes<sup>2,10–12</sup>. The tumor necrosis factor (TNF)–nuclear factor- $\kappa$ B (NF- $\kappa$ B) signaling network promotes proinflammatory cytokine production in myelofibrosis, with the BET protein BRD4 having a key role in regulating NF- $\kappa$ B-mediated inflammation<sup>13,14</sup>. In an adoptive transfer mouse model of myelofibrosis, BET inhibition (using the prototypical BET inhibitor JQ1) demonstrated reductions in spleen size, bone marrow fibrosis, proinflammatory cytokine amounts and NF- $\kappa$ B activation, and significantly prolonged survival compared with a vehicle control<sup>13</sup>. In the same model, BET inhibition with JQ1 in combination with JAK inhibition (using ruxolitinib) demonstrated greater reductions in these parameters than achieved by either drug alone<sup>13</sup>. These preclinical observations support simultaneous BET and JAK inhibition as a potent therapeutic strategy to exceed the effects of JAK inhibitor monotherapy, and provide a strong rationale to evaluate this approach in patients<sup>15</sup>.

Pelabresib (CPI-0610) is an investigational, oral, small molecule BET inhibitor under evaluation in clinical trials for patients with myelofibrosis<sup>15,16</sup>. Data from the phase 2 MANIFEST study (NCT02158858) demonstrated substantial and durable improvements in splenomegaly and symptoms with pelabresib plus ruxolitinib in JAK inhibitor-naïve patients with myelofibrosis ( $n = 84$ ): a reduction in spleen volume of  $\geq 35\%$  was observed in 68% of patients; a  $\geq 50\%$  reduction in symptoms at week 24 was observed in 56% of patients and the combination was generally well tolerated<sup>17,18</sup>.

Here, we report the primary analysis of the randomized phase 3 MANIFEST-2 study (NCT04603495), evaluating the efficacy and safety of pelabresib–ruxolitinib versus placebo–ruxolitinib in JAK inhibitor-naïve patients with myelofibrosis<sup>19</sup>.

## Results

### Study design

MANIFEST-2 is a global, phase 3, randomized, double-blind active-control study of pelabresib–ruxolitinib versus placebo–ruxolitinib in

JAK inhibitor-naïve patients with myelofibrosis (Extended Data Fig. 1). Key eligibility criteria included confirmed diagnosis of myelofibrosis (primary myelofibrosis, postpolycythemia vera myelofibrosis or postessential thrombocythemia myelofibrosis); JAK inhibitor treatment-naïve; dynamic international prognostic scoring system (DIPSS) score  $\geq$ Int-1; platelet count  $\geq 100 \times 10^9 \text{ l}^{-1}$ ; spleen volume  $\geq 450 \text{ cm}^3$  by magnetic resonance imaging (MRI) or computed tomography (CT); at least two symptoms measurable (score  $\geq 3$ ) or a total symptom score (TSS) of  $\geq 10$  using the Myelofibrosis Symptom Assessment Form (MFSAF) v.4.0; peripheral blast count  $< 5\%$ ; Eastern Cooperative Oncology Group (ECOG) performance status  $\leq 2$ . The primary endpoint was splenic response at week 24. Key secondary endpoints were absolute change in TSS at week 24 compared with baseline and  $\geq 50\%$  decrease from baseline in TSS response at week 24 (TSS50). Other pre-specified secondary endpoints were: percent change in TSS at week 24 compared with baseline; improvement in bone marrow fibrosis by at least one grade at week 24 compared with baseline; splenic response at week 48; TSS50 response at week 48; absolute change in TSS at week 48 compared with baseline; rate of red blood cell (RBC) transfusion over the first 24 weeks of treatment; conversion from RBC transfusion dependence at baseline to independence; categorical change of Patient Global Impression of Change (PGIC) at week 24 compared with baseline; progression-free survival; overall survival; safety (adverse events of all grades and serious adverse events); proportion of patients with transformation to blast phase; pharmacokinetics; descriptive assessment of ruxolitinib plasma concentrations in the presence or absence of pelabresib; duration of splenic response; modified TSS response at week 24 and duration of TSS50 response.

### Patients

Between November 2020 and March 2023, 430 patients were enrolled in the MANIFEST-2 study (first patient enrolled, 23 April 2021; last patient enrolled, 2 March 2023) and randomized; 214 to pelabresib plus ruxolitinib and 216 to placebo plus ruxolitinib. At the data cutoff (31 August 2023), two patients in each arm had not received study treatment; 58 patients in the pelabresib–ruxolitinib arm and 54 patients in the

**Table 1 | Baseline characteristics of the study patients**

Characteristic	Pelabresib + ruxolitinib (N=214)	Placebo + ruxolitinib (N=216)
Median age (range), years	66 (19–84)	66 (26–88)
Sex, no. (%)		
Female	85 (39.7)	94 (43.5)
Male	129 (60.3)	122 (56.5)
Race, no. (%)		
White	160 (74.8)	163 (75.5)
Asian	35 (16.4)	42 (19.4)
Black or African American	2 (0.9)	0
American Indian or Alaska Native	1 (0.5)	0
Not reported	15 (7.0)	11 (5.1)
Unknown	1 (0.5)	0
Myelofibrosis subtype, no. (%)		
Primary myelofibrosis	107 (50.0)	110 (50.9)
Postpolycythemia vera myelofibrosis	45 (21.0)	53 (24.5)
Postessential thrombocytopenia myelofibrosis	62 (29.0)	53 (24.5)
DIPSS, no. (%)		
Intermediate-1	128 (59.8)	127 (58.8)
Intermediate-2	75 (35.0)	74 (34.3)
High-risk	11 (5.1)	15 (6.9)
Mutations, no. (%)		
JAK2 V617F	125 (58.4)	122 (56.5)
CALR	45 (21.0)	50 (23.2)
MPL	11 (5.1)	13 (6.0)
Triple negative <sup>a</sup>	8 (3.7)	6 (2.8)
High-molecular-risk mutations	72 (33.6)	88 (40.7)
ASXL1	55 (25.7)	75 (34.7)
EZH2	12 (5.6)	19 (8.8)
IDH1	8 (3.7)	5 (2.3)
IDH2	7 (3.3)	5 (2.3)
SRSF2	15 (7.0)	18 (8.3)
U2AF1	12 (5.6)	10 (4.6)
Hemoglobin, g dL <sup>-1</sup>		
Median (range)	10.9 (5.8–18.0)	11.0 (6.7–17.9)
≤10, no. (%)	70 (32.7)	76 (35.2)
Platelets, ×10 <sup>9</sup> L <sup>-1</sup>		
Median (range)	285 (99–1303)	287 (66–1084)
>200, no. (%)	154 (72)	157 (72.7)
Peripheral blasts, mean (s.d.)	0.8 (1.18) <sup>b</sup>	0.8 (1.25) <sup>c</sup>
RBC transfusions, no. (%)		
Requiring RBC transfusion at baseline	22 (10.3)	21 (9.7)
ECOG performance status, no. (%)		
0	107 (50.0)	109 (50.5)
1	97 (45.3)	95 (44.0)
2	10 (4.7)	10 (4.6)
Missing	0	2 (0.9)
Spleen volume (central read) <sup>d</sup>		
Median spleen volume, cc (range)	1,308.89 (200.24–7,117.03)	1,382.97 (277.87–5,540.45)

**Table 1 (continued) | Baseline characteristics of the study patients**

Characteristic	Pelabresib + ruxolitinib (N=214)	Placebo + ruxolitinib (N=216)
Total symptom score <sup>e</sup>		
Median total symptom score (range)	26.6 (7.3–66.4)	24.7 (9.0–68.4)
Bone marrow fibrosis, no. (%)		
Grade 0	5 (2.3)	4 (1.9)
Grade 1	26 (12.1)	27 (12.5)
Grade 2	56 (26.2)	51 (23.6)
Grade 3	58 (27.1)	67 (31.0)
Missing	69 (32.2)	67 (31.0)

<sup>a</sup>Triple negative status was missing for 55 patients (28 in the pelabresib–ruxolitinib arm and 27 in the placebo–ruxolitinib arm). <sup>b</sup>n=208. <sup>c</sup>n=207. <sup>d</sup>Randomization to treatment arms was based on local read for spleen volume, whereas stratification for the primary analysis was based on central read. <sup>e</sup>Patients with baseline TSS values of <10 have at least two individual symptom scores ≥3 at baseline.

placebo–ruxolitinib arm had discontinued study treatment (patient disposition shown in Fig. 1). The median duration on study was 45.5 weeks; 154 patients (72.0%) receiving pelabresib–ruxolitinib and 160 patients (74.1%) receiving placebo–ruxolitinib were continuing treatment. Baseline characteristics in each treatment arm are shown in Table 1.

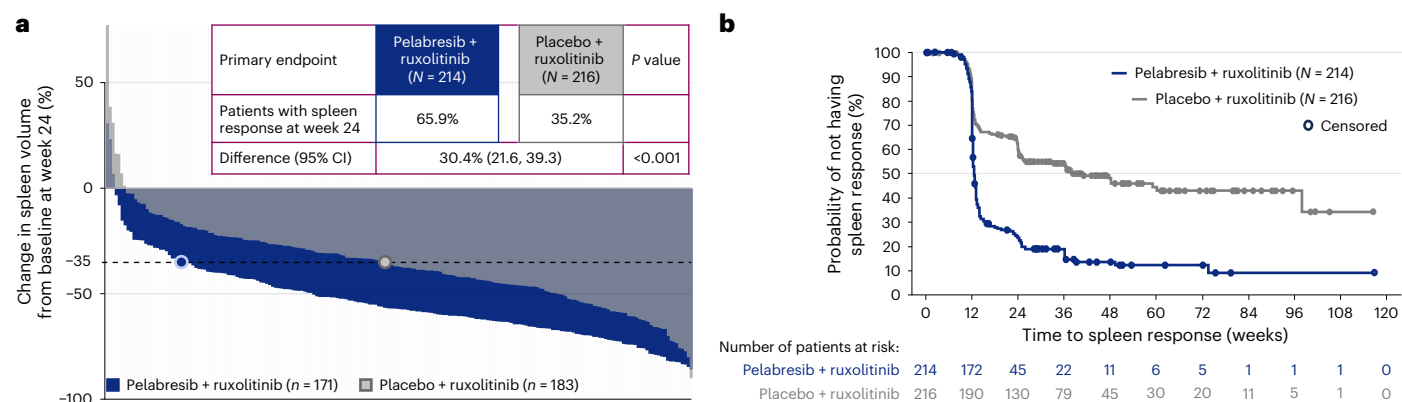
In the pelabresib–ruxolitinib arm, mean (s.d.) daily pelabresib dose in treated patients (N = 212) was 108.4 mg (27.6) and daily ruxolitinib dose was 30.5 mg (16.9). In the placebo–ruxolitinib arm (N = 214), mean (s.d.) ruxolitinib dose was 29.8 mg (12.9).

### Efficacy: spleen volume reduction

The primary endpoint of reduction in spleen volume of ≥35% at week 24 was observed in a statistically significantly larger proportion of patients in the pelabresib–ruxolitinib arm than in the placebo–ruxolitinib arm; 65.9% versus 35.2%, respectively (Cochran–Mantel–Haenszel difference, 30.4%; 95% CI, 21.6, 39.3; *P* < 0.001) (Fig. 2a). Mean percentage change in spleen volume at week 24 was –50.6% (95% CI, –53.2, –48.0) in the pelabresib–ruxolitinib arm versus –30.6% (95% CI, –33.7, –27.5) in the placebo–ruxolitinib arm. Spleen volume response rate at week 24 was consistently higher with pelabresib–ruxolitinib across predefined subgroups (Extended Data Fig. 2a). Spleen volume reduction of ≥35% at any time was observed in 172 patients (80%) treated with pelabresib–ruxolitinib and in 108 (50%) treated with placebo–ruxolitinib. Time to splenic response is shown in Fig. 2b. Duration of splenic response is shown in Extended Data Fig. 3a.

### Efficacy: patient-reported outcomes

The key secondary endpoint of absolute change in TSS at week 24 demonstrated substantial improvements for patients in both treatment arms, with a trend toward greater benefit with pelabresib–ruxolitinib versus placebo–ruxolitinib. Least squares mean change from baseline at week 24 was –15.99 versus –14.05, respectively (difference, –1.94; 95% CI, –3.92, 0.04; *P* = 0.0545) (Fig. 3a). The proportion of patients with ≥50% reduction in TSS at week 24 (TSS50; key secondary endpoint) was greater in the pelabresib–ruxolitinib arm, with 52.3% versus 46.3%, respectively (Cochran–Mantel–Haenszel difference, 6.0%; 95% CI, –3.5, 15.5) (Fig. 3b and see Extended Data Fig. 3b for duration of TSS50 response). Reductions were consistent across symptom score domains (Extended Data Table 1). Mean percentage change in TSS from baseline at week 24 was –50.3% (95% CI, –56.6, –44.0) in the pelabresib–ruxolitinib arm versus –45.9% (95% CI, –51.8, –40.0) in the placebo–ruxolitinib arm. Similar trends in symptom scores were observed across predefined subgroups, except for the small subgroup of patients with



**Fig. 2 | Splenic response.** **a**, Percentage change in spleen volume from baseline at week 24, overlaid with the proportion of patients with spleen response (defined as a  $\geq 35\%$  reduction in spleen volume from baseline, by central read). Patients without week 24 change from baseline assessment are not shown and were

considered nonresponders for spleen response. Difference between treatment arms was compared by stratified Cochran–Mantel–Haenszel test (performed two-sided Cochran–Mantel–Haenszel test at the alpha level 5%). The exact P value is  $1.64 \times 10^{-10}$ . **b**, Kaplan–Meier time-to-event estimate for spleen response.

high-risk (DIPSS) myelofibrosis at baseline (Extended Data Fig. 2b,c). Regarding the self-reported PGIC, 46.9% of patients in the pelabresib–ruxolitinib arm reported their condition as ‘much improved’ or ‘very much improved’ compared with 50.0% in the placebo–ruxolitinib arm (no statistical comparison performed) (Extended Data Table 2). There was more than a twofold difference in patients achieving both spleen and TSS50 responses at week 24 with pelabresib–ruxolitinib compared with placebo–ruxolitinib (40.2% versus 18.5%) (Extended Data Fig. 4).

### Efficacy: hemoglobin and transfusions

A numerically greater proportion of patients achieved hemoglobin (Hb) response with pelabresib–ruxolitinib compared with placebo–ruxolitinib, with a persistent difference in hemoglobin over time between treatment arms (Fig. 4). In patients with anemia ( $\text{Hb} < 10 \text{ g dl}^{-1}$ ) at baseline, hemoglobin concentrations were modestly and consistently higher across weeks 2–24 in the pelabresib–ruxolitinib arm versus the placebo–ruxolitinib arm. Transfusions were received during the first 24 weeks on treatment by 27.6% of patients treated with pelabresib–ruxolitinib and 37.5% with placebo–ruxolitinib.

### Disease-relevant proinflammatory cytokines

Changes in proinflammatory cytokines, including NF- $\kappa$ B-regulated cytokines, TNF, interleukin-6 (IL-6), and IL-8 are shown in Extended Data Fig. 5a. Amounts of the NF- $\kappa$ B-regulated cytokines TNF and IL-6 decreased in both treatment arms, with a greater magnitude of reduction observed with pelabresib–ruxolitinib compared with placebo–ruxolitinib. Concentrations of IL-8 decreased in the pelabresib–ruxolitinib arm but increased in the placebo–ruxolitinib arm. Independent of treatment, lower amounts of proinflammatory cytokines were correlated with spleen responses (Extended Data Fig. 6).

### Bone marrow morphology

Bone marrow samples were assessed for fibrosis, megakaryocytic density and proliferation, and erythropoiesis. At week 24, 192 patients treated with pelabresib–ruxolitinib and 188 patients treated with placebo–ruxolitinib were evaluable for change from baseline in bone marrow fibrosis. Among them, 98 out of 192 (51.0%) and 105 out of 188 (55.9%) patients, respectively, had missing data. Improvement of at least one grade was observed in 18.8% (36 out of 192) of patients treated with pelabresib–ruxolitinib and in 11.2% (21 out of 188) of patients treated with placebo–ruxolitinib (Extended Data Fig. 5b). Considering only patients with results available at baseline and week 24, improvement of at least one grade was observed in 38.3% (36 out of 94) and 25.3% (21 out of 83) of patients, respectively. There was a greater reduction in

density of reticulin fiber ( $-5.2$  (95% CI,  $-6.9$ ,  $-3.5$ ) versus  $-1.0$  (95% CI,  $-2.7$ ,  $0.6$ )) and  $\text{CD}61^+$  megakaryocytes ( $-54.5 \text{ cells mm}^{-2}$  (95% CI,  $-70.8$ ,  $-38.3$ ) versus  $-27.4 \text{ cells mm}^{-2}$  (95% CI,  $-43.0$ ,  $-11.9$ )) in the pelabresib–ruxolitinib arm versus placebo–ruxolitinib, respectively (Extended Data Fig. 5c,d and Supplementary Fig. 1). Erythrocyte progenitor cell proportions increased by 11.4% (95% CI,  $-2.4$ ,  $27.2$ ) from baseline at week 24 with pelabresib–ruxolitinib, and decreased by  $-9.8\%$  (95% CI,  $-20.6$ ,  $2.6$ ) with placebo–ruxolitinib. A greater increase in the proportion of erythrocyte progenitor cells was observed in patients who did not require RBC transfusions compared with patients who did require transfusions (Extended Data Fig. 5e).

### Mutational analyses

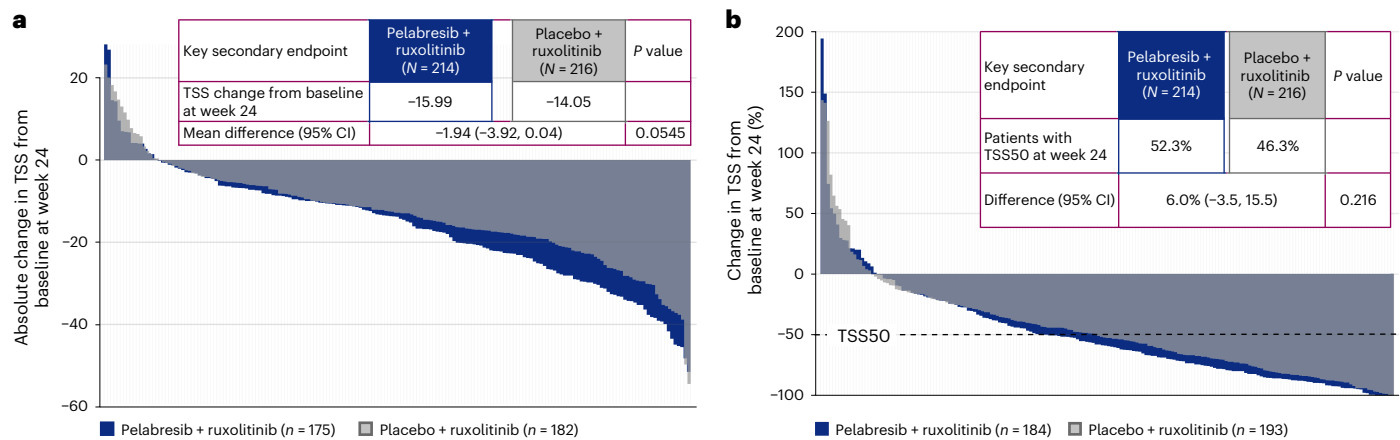
At baseline, the pattern of driver and high-molecular-risk mutations was broadly comparable between arms, with the exception of higher frequency of *ASXL1* and *EZH2* in the placebo–ruxolitinib arm (Table 1). Nevertheless, treatment responses with pelabresib–ruxolitinib were independent of driver and high-molecular-risk mutation status (Extended Data Fig. 7). At week 48, a total of 83 of 247 (33.6%) patients with a *JAK2*V617F mutation were included in the variant allele fraction (VAF) analysis. There was an early trend towards greater reduction in the *JAK2*V617F VAF with pelabresib–ruxolitinib ( $n = 46$ ;  $-23.9\%$ ), compared with placebo–ruxolitinib ( $n = 37$ ;  $-16.8\%$ ) (difference,  $-9.5\%$ ; 95% CI,  $-23.6$ ,  $2.9$ ; Supplementary Fig. 2a). Independent of treatment, patients with a spleen response at week 24 ( $n = 55$ ) had a greater reduction from baseline in the *JAK2*V617F VAF compared with nonresponders ( $n = 28$ ) (Supplementary Fig. 2b).

### Safety

In total, 212 patients in the pelabresib–ruxolitinib arm and 214 in the placebo–ruxolitinib arm received treatment and were included in safety analyses. At least one TEAE was experienced by 96.7% (205 out of 212) of patients in the pelabresib–ruxolitinib arm versus 96.7% (207 out of 214) of patients in the placebo–ruxolitinib arm. Most TEAEs were considered related to pelabresib or placebo treatment (77.4% ( $n = 164$  out of 212) versus 74.8% ( $n = 160$  out of 214), respectively) (Extended Data Table 3). The overall frequency of any grade  $\geq 3$  TEAEs was lower with pelabresib–ruxolitinib (49.1%) than with placebo–ruxolitinib (57.0%). Mean (s.d.) time to onset for any grade TEAEs was 23 days (45.6) in the pelabresib–ruxolitinib arm and 27 days (45.6) in the placebo–ruxolitinib arm. Time to onset for grade  $\geq 3$  was 98 days (123.7) versus 113 days (130.8), respectively.

The most frequent ( $\geq 10\%$ ) hematological TEAEs (Table 2) in the pelabresib–ruxolitinib arm were thrombocytopenia (composite





**Fig. 3 | Symptom response. a**, Absolute change in TSS score from baseline at week 24; patients without week 24 data are not shown. Change from baseline was determined by ANCOVA model using multiple imputation. Least square mean difference was determined from the ANCOVA model using baseline DIPSS, baseline platelet count and baseline spleen volume as factors and baseline TSS as covariate, performed two-sided Cochran–Mantel–Haenszel test at the alpha

level 5%. **b**, Percentage change in TSS score from baseline at week 24, overlaid with the proportion of patients experiencing a TSS50 response. Patients without baseline and week 24 data are not shown. Difference between treatment arms was compared by stratified Cochran–Mantel–Haenszel test (performed two-sided Cochran–Mantel–Haenszel test at the alpha level 5%; weighted 95% CI adjusted across strata).

including preferred terms of thrombocytopenia and platelet count decrease, 52.8% any grade; 13.2% grade  $\geq 3$ ) and anemia (composite including preferred terms of anemia and hemoglobin decrease; 44.8% any grade; 23.1% grade  $\geq 3$ ). In the placebo–ruxolitinib arm, the most frequent ( $\geq 10\%$ ) hematological TEAEs were anemia (composite term, 55.1%; 36.5% grade  $\geq 3$ ) and thrombocytopenia (composite term, 37.4%; 6.1% grade  $\geq 3$ ).

Cytopenias were managed mostly with dose reduction or interruption, according to guidance provided in the protocol. There were no clinically significant bleeding events associated with grade  $\geq 3$  thrombocytopenia. Four patients in the pelabresib–ruxolitinib arm and two patients in the placebo–ruxolitinib arm discontinued treatment due to thrombocytopenia or platelet count decrease, and one patient in each arm discontinued due to anemia or hemoglobin decrease.

Among the nonhematological TEAEs occurring in  $\geq 10\%$  of patients (listed in Table 2), the most frequent were diarrhea in the pelabresib–ruxolitinib arm (23.1%; 0.5% grade  $\geq 3$ ) and constipation in the placebo–ruxolitinib arm (24.3%; 0 grade  $\geq 3$ ). Dysgeusia occurred in 18.4% (0.5% grade  $\geq 3$ ) and 3.7% (0% grade  $\geq 3$ ) of patients in the pelabresib–ruxolitinib and placebo–ruxolitinib arms, respectively (Table 2). The single event of grade  $\geq 3$  dysgeusia in the pelabresib–ruxolitinib arm was considered a nonserious event, related to pelabresib; the event was managed by treatment interruption.

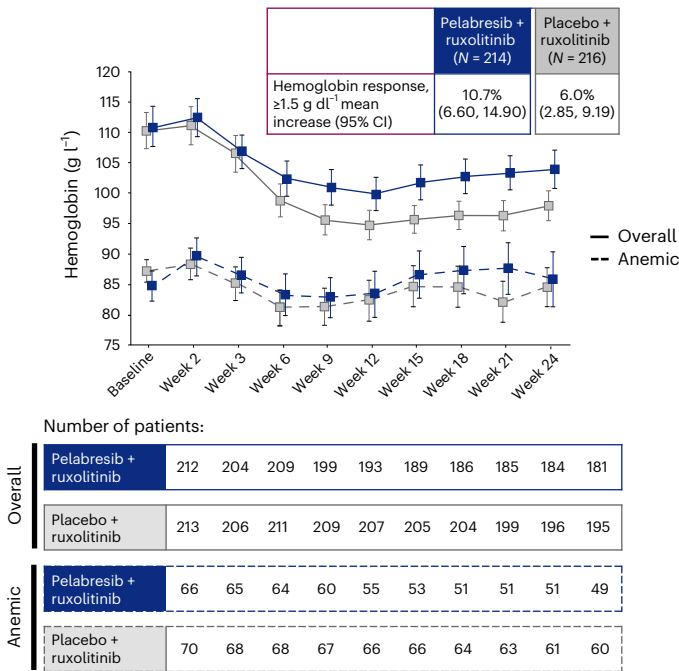
TEAEs leading to dose reduction of ruxolitinib, pelabresib or placebo occurred in 51.9% and 44.9% of patients in the pelabresib–ruxolitinib and placebo–ruxolitinib arms, respectively. The most frequent TEAEs resulting in dose reduction were thrombocytopenia (23.1% and 15.0%, respectively), platelet count decrease (17.0% and 13.1%, respectively) and anemia (10.4% and 16.4%, respectively). TEAEs leading to ruxolitinib, pelabresib or placebo dose interruptions occurred in 32.5% and 24.8% in the pelabresib–ruxolitinib and placebo–ruxolitinib arms, respectively. The most frequent TEAEs resulting in dose interruption were thrombocytopenia (6.6% and 4.7%, respectively), COVID-19 (0.9% and 4.2%, respectively), platelet count decrease (3.3% and 1.4%, respectively) and anemia (2.4% and 2.3%, respectively). TEAEs leading to withdrawal of pelabresib or placebo occurred in 12.3% and 7.5% of patients in the pelabresib–ruxolitinib and placebo–ruxolitinib arms, respectively (Supplementary Table 1). TEAEs leading to withdrawal of ruxolitinib occurred in 9.9% of patients in the pelabresib–ruxolitinib arm and in 6.1% of patients in the placebo–ruxolitinib arm. Serious TEAEs occurred in 29.7% of patients in the pelabresib–ruxolitinib arm

and 29.4% in the placebo–ruxolitinib arm. The most frequent ( $\geq 2\%$ ) serious adverse events were pneumonia (3.3% of patients in the pelabresib–ruxolitinib arm and 2.8% of patients in the placebo–ruxolitinib arm) and anemia (2.4% and 2.3%, respectively). There were 11 deaths during treatment (5 in the pelabresib–ruxolitinib arm and 6 in the placebo–ruxolitinib arm). In the pelabresib–ruxolitinib arm, fatal events of pneumonia, sepsis, cardiac arrest, intracranial hemorrhage (attributed to underlying hypertension, considered unrelated to study treatment) and septic shock (co-recorded as a fatal event with pneumonia) were each reported in one patient, except cardiac arrest, which was reported in two patients. In the placebo–ruxolitinib arm, fatal events of pneumonia, sepsis, cardiac failure, road traffic accident, small intestinal obstruction and sudden cardiac death were each reported in one patient. All deaths were assessed as not related to study treatment. One patient died of a road traffic accident and all other deaths had medical history that may have been considered a contributory factor, or were attributable to concurrent disease.

Disease progression to accelerated phase or transformation to blast phase occurred in 3.3% of patients (7 out of 212) receiving pelabresib–ruxolitinib versus 2.3% (5 out of 214) receiving placebo–ruxolitinib. This assessment was based on local laboratory results, adverse events and documented disease progression. A numerical imbalance was observed in the number of patients with blast phase based on reported disease progression, with 2.4% (5 out of 212) versus 0.5% (1 out of 214) of patients in the pelabresib–ruxolitinib versus placebo–ruxolitinib arms, respectively. One of the five patients treated with pelabresib–ruxolitinib with blast phase according to this assessment was ultimately diagnosed with lymphoproliferative neoplasm and not blast phase transformation of myelofibrosis.

## Discussion

The MANIFEST-2 study of pelabresib–ruxolitinib in JAK inhibitor-naïve patients with myelofibrosis met its primary endpoint, with 65.9% of patients experiencing spleen response ( $\geq 35\%$  volume reduction) at week 24 with the combination. Indeed, the spleen response rate in patients treated with pelabresib–ruxolitinib was approximately double that seen with placebo–ruxolitinib in the MANIFEST-2 study (35.2%;  $P < 0.001$ ), and in previous studies of JAK inhibitor monotherapy (ranging from 18% to 42%)<sup>20–24</sup>. Furthermore, symptom improvements were also observed with placebo–ruxolitinib and pelabresib–ruxolitinib, with trends towards further benefit with the combination.



**Fig. 4 | Hemoglobin change over time.** Graph shows the mean change in hemoglobin over time in the overall study population and in the subgroup of patients with anemia (defined as patients with a baseline hemoglobin of  $<10$  g dl<sup>-1</sup>). Hemoglobin response is defined as a mean increase in hemoglobin from baseline of  $\geq 1.5$  g dl<sup>-1</sup> in the absence of transfusions during the previous 12 weeks (baseline hemoglobin defined as the last assessment before or on cycle 1 day 1, regardless of blood transfusions). Patients who received transfusions are included in these data. Data are presented as mean  $\pm$  95% CI. In accordance with preplanned hierarchical statistical testing, for these data, we report descriptive statistical analyses only.

These clinical observations are consistent with the proposed mechanism of action of combined BET and JAK inhibition, and with our understanding of proinflammatory cytokine involvement in myelofibrosis pathogenesis. In line with preclinical findings<sup>13</sup>, combined BET and JAK inhibition with pelabresib–ruxolitinib treatment in the MANIFEST-2 study led to stronger and more frequent improvements in proinflammatory cytokine amounts than JAK inhibition alone with ruxolitinib. These include NF- $\kappa$ B-regulated cytokines, TNF, IL-6 and IL-8, which have a key role in myelofibrosis pathogenesis and are associated with a detrimental prognosis<sup>11,12</sup>. IL-8, in particular, has been associated with reduced overall survival<sup>11,12</sup>; in this study, amounts of IL-8 increased in the placebo–ruxolitinib arm. Consistent with proinflammatory cytokines analysis, improvements in fibrosis and overall bone marrow morphology were also observed to a greater degree in the pelabresib–ruxolitinib arm than in the ruxolitinib–placebo arm. Collectively, these exploratory data indicate a biological benefit of the pelabresib–ruxolitinib combination versus ruxolitinib monotherapy, which supports the clinical benefits.

We observed increased erythrocyte progenitor proportions in the pelabresib–ruxolitinib arm, beyond those observed in the placebo–ruxolitinib arm, which may explain the amelioration of anemia with pelabresib–ruxolitinib<sup>25</sup>. Reduced transfusions associated with anemia improvements are likely to have a direct benefit on the clinical and economic burden of myelofibrosis<sup>26</sup>. The clinically and biologically meaningful benefits of the pelabresib–ruxolitinib combination represent valuable short-term outcomes for patients, which may translate into more profound longer-term treatment effects than ruxolitinib monotherapy. A survival benefit would further demonstrate pelabresib–ruxolitinib as a clinically meaningful therapeutic option.

**Table 2 | TEAEs in  $\geq 10\%$  of patients in either treatment arm**

Adverse events	Pelabresib + ruxolitinib (N=212) <sup>a</sup>		Placebo + ruxolitinib (N=214) <sup>a</sup>	
	All grades	Grade $\geq 3$	All grades	Grade $\geq 3$
Hematological events, no. patients (%)				
Thrombocytopenia <sup>b</sup>	112 (52.8)	28 (13.2)	80 (37.4)	13 (6.1)
Anemia <sup>c</sup>	95 (44.8)	49 (23.1)	118 (55.1)	78 (36.5)
Nonhematological events, no. patients (%)				
Diarrhea	49 (23.1)	1 (0.5)	39 (18.2)	3 (1.4)
Dysgeusia	39 (18.4)	1 (0.5)	8 (3.7)	0
Constipation	39 (18.4)	0	52 (24.3)	0
Nausea	30 (14.2)	1 (0.5)	32 (15.0)	0
Cough	27 (12.7)	0	24 (11.2)	0
Asthenia	25 (11.8)	1 (0.5)	30 (14.0)	0
Fatigue	25 (11.8)	1 (0.5)	35 (16.4)	2 (0.9)
Dizziness	24 (11.3)	0	19 (8.9)	0
Headache	24 (11.3)	1 (0.5)	23 (10.7)	0
COVID-19	24 (11.3)	0	34 (15.9)	4 (1.9)
Muscle spasms	24 (11.3)	0	9 (4.2)	0
Dyspnea	19 (9.0)	1 (0.5)	28 (13.1)	3 (1.4)
Arthralgia	17 (8.0)	0	24 (11.2)	0

TEAEs reported in  $\geq 10\%$  of patients in either treatment arm who received at least one dose of treatment. <sup>a</sup>Safety population includes all patients who received at least one dose of study drug. <sup>b</sup>Thrombocytopenia includes preferred terms of thrombocytopenia and platelet count decrease. <sup>c</sup>Anemia includes preferred terms of anemia and hemoglobin decrease. A TEAE for the double-blinded treatment period is defined as an adverse event that has a start date on or after the first dose of pelabresib/placebo and before 30 days after the last dose of pelabresib/placebo or before the start of alternative (off-study) treatment for myelofibrosis, whichever occurs first. For any adverse event for which Common Terminology Criteria for Adverse Events grading does not exist, the investigator assessed severity using the WHO grading system, in which grades 1–4 correspond to the severity of mild, moderate, severe and life-threatening.

Spleen volume reduction and spleen length reduction observed with ruxolitinib treatment have both correlated with an overall survival benefit in patients with myelofibrosis<sup>20,27</sup>. Durability of response, survival and correlation with clinical outcomes will be further evaluated with ongoing, long-term follow-up of this study.

Similar to the phase 2 MANIFEST study<sup>17</sup>, safety analyses in the phase 3 MANIFEST-2 study suggest that combining pelabresib with ruxolitinib improves clinical response<sup>28</sup>, with overall fewer grade 3 or higher adverse events reported with the combination. A higher incidence of thrombocytopenia events was reported for the combination arm versus placebo–ruxolitinib; however, these were well managed with dose modifications, with only one patient in each arm discontinuing study treatment due to thrombocytopenia. Dosing of ruxolitinib was equivalent between the two arms, indicating that dose intensity was unaffected by combining with pelabresib. Constipation adverse events in both treatment arms were reported at higher incidence than observed with ruxolitinib monotherapy in the COMFORT-I (12.9%) and COMFORT-II (12 out of 146 patients) trials, or with other JAK inhibitors (momelotinib pooled analysis, 11.2%)<sup>6,20,29</sup>. Here, most constipation adverse events were grade 1 or 2, similar to the pelabresib–ruxolitinib cohort of the MANIFEST trial (25%) in which the combination treatment was considered well tolerated<sup>17</sup>, and less than half in both arms were considered related to pelabresib or placebo. Rates of leukemic transformation were within a similar range to those reported in COMFORT-I (2 out of 155 patients receiving ruxolitinib monotherapy), COMFORT-II (8 out of 146 patients receiving ruxolitinib monotherapy) and SIMPLIFY-I (2 out of 216 patients receiving ruxolitinib monotherapy and 1 out of 214 patients receiving momelotinib monotherapy)<sup>6,20,23</sup>.

Disease transformation observations from the MANIFEST-2 study warrant further biologic evaluation, continued clinical monitoring and additional follow-up.

Although the primary endpoint of spleen volume reduction represents a valuable short-term clinical benefit, one limitation of this study is that we have not yet reported long-term durability of response data. At data cutoff for this primary analysis, long-term durability of response data were not mature. Durability of response and survival data will be investigated in a long-term follow-up of this study. A second limitation is the potential subjectivity and sensitivity restrictions of the MFSAF for assessment of symptoms. However, this instrument captures the broad spectrum of symptoms of myelofibrosis and has been comprehensively validated, which has led to its use in clinical trials for myeloproliferative neoplasm patients<sup>30</sup>.

In conclusion, pelabresib plus ruxolitinib provided robust clinical benefit, resulting in a statistically significant improvement in the primary endpoint of spleen response, with trends of improvement noted across other principal hallmarks of myelofibrosis, including symptom control, proinflammatory cytokine amounts and bone marrow morphology. The phase 3 MANIFEST-2 study provides important insights into disease biology and modification, supporting the combination of pelabresib plus ruxolitinib for JAK inhibitor-naïve patients with myelofibrosis.

## Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41591-025-03572-3>.

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## Methods

### Patients

Eligible adult patients had myelofibrosis (primary, postpolycythemia vera or postessential thrombocythemia) and a spleen volume of  $\geq 450 \text{ cm}^3$  by MRI or CT scan,  $\geq 2$  symptoms with an average score of  $\geq 3$  or an average TSS  $\geq 10$  over the 7-day period before randomization using MFSAF v.4.0, and a prognostic risk-factor score of intermediate-1 or higher per DIPSS<sup>25</sup>. Additional inclusion criteria were a platelet count of  $\geq 100 \times 10^9 \text{ l}^{-1}$  in the absence of growth factors or transfusions for the previous 4 weeks, an ECOG performance status of  $\leq 2$  and peripheral blast count  $< 5\%$ . Patients were excluded if they had a previous splenectomy, splenic irradiation within 6 months of treatment initiation, received previous treatment with any JAK or BET inhibitors, or were currently candidates for allogeneic hematopoietic stem cell transplant. In this study, a patient's sex refers to biological attribute; gender was not reported, as there is no analysis related to gender identity.

### Study design

In this double-blind active-control study, patients were randomized 1:1 to pelabresib plus ruxolitinib or placebo plus ruxolitinib (Extended Data Fig. 1), by a centralized interactive voice response/interactive web response system. Stratification was based on DIPSS category (intermediate-1 versus intermediate-2 versus high-risk), platelet count ( $> 200 \times 10^9 \text{ l}^{-1}$  versus  $100 \times 10^9$ – $200 \times 10^9 \text{ l}^{-1}$ ) and spleen volume ( $\geq 1,800 \text{ cm}^3$  versus  $< 1,800 \text{ cm}^3$ ). Patients randomized to placebo–ruxolitinib could cross over to pelabresib–ruxolitinib if progressive splenomegaly occurred at or after week 24 (defined as enlargement of spleen volume by  $\geq 25\%$  from baseline). All patients, investigators and the sponsor were blinded to treatment allocation with study drugs packaged identically. The study was conducted at sites across North America, Asia, Europe and Australia (Supplementary Table 2).

### Treatment

Pelabresib or matching placebo was administered orally at a starting dose of 125 mg orally once daily for 14 consecutive days, followed by a 7-day break (21-day cycle). Pelabresib or placebo dose could be increased for lack of spleen response after four cycles in 25 mg increments, up to 175 mg once daily. Ruxolitinib was administered orally at an initial dose of either 10 mg or 15 mg twice daily depending on baseline platelet counts (5 mg twice daily lower than the approved dose), with a mandatory 5 mg per dose increase after one cycle if prespecified criteria were met. Ruxolitinib dose could be further increased for lack of spleen response by 5 mg per dose increments, up to 25 mg twice daily, provided adequate blood counts. To manage adverse events, both treatments could be dose reduced to a minimum of 50 mg once daily for pelabresib–placebo and 5 mg once daily for ruxolitinib.

### Endpoints

The primary endpoint was spleen response, defined as a reduction of  $\geq 35\%$  in spleen volume from baseline at week 24 by independent central review. Spleen volume was measured using MRI or CT, performed during screening and every 12 weeks from the first day of treatment, with MRI being the preferred method.

Key secondary endpoints were (1) absolute change in TSS from baseline, and (2) TSS50, defined as reduction of  $\geq 50\%$  from baseline in TSS; both at week 24 using MFSAF v.4.0. MFSAF was completed electronically daily until 12 weeks after end of treatment.

Other secondary endpoints included percentage change in TSS at week 24, duration of spleen response, improvement in bone marrow fibrosis by at least one grade from baseline at week 24, rate of transfusions over the first 24 weeks of treatment, category change of PGIC at week 24 from baseline using a single question completed weekly until 12 weeks after end of treatment, rate of transformation to blast phase (acute myeloid leukemia) and safety (adverse events, regardless

of attribution, were assessed according to National Cancer Institute Common Terminology Criteria for Adverse Events v.5.0).

Exploratory endpoints included percentage change in spleen volume at week 24, hemoglobin response ( $\geq 1.5 \text{ g dl}^{-1}$  mean increase in hemoglobin from baseline in the absence of transfusions during the previous 12 weeks), time to spleen response, assessment of myelofibrosis-related features associated with response (including bone marrow morphology and hematopoietic cell populations), changes in proinflammatory cytokines (measured by a bead-based multiplex assay from plasma obtained pretreatment at baseline and at week 24) and molecular response.

### Bone marrow biopsy assessment

Bone marrow biopsy was performed at baseline and every 24 weeks on treatment (reduced to every 48 weeks after week 72), and assessed by a local hematopathologist for fibrosis grading according to the European classification<sup>26</sup>. Disease progression to accelerated phase was defined as at least two consecutive peripheral blast counts of 10–19% or a bone marrow myeloblast of 10–19%. Transformation to blast phase was defined as a bone marrow blast count of  $\geq 20\%$  or a peripheral blood blast count of  $\geq 20\%$  associated with an absolute blast count of  $\geq 1 \times 10^9 \text{ l}^{-1}$  that persists for at least 2 weeks. Three stained slides used for local review and six to ten unstained slides were provided for central pathology review and exploratory analyses. Central bone marrow immunohistochemistry staining was conducted for reticulin fiber density, CD61<sup>+</sup> megakaryocytes, and CD71<sup>+</sup> erythrocyte progenitor cells. Digital images were analyzed by an automated quantitative cell-specific detection analysis.

### Mutant allele burden evaluation

Mutational analyses were conducted centrally from peripheral whole blood samples at baseline and at week 48 by targeted next-generation sequencing with the Rapid Heme Panel v.3 assay.

### Statistical analysis

For the primary and key secondary endpoint of TSS50, response rates were compared using the Cochran–Mantel–Haenszel test controlling for baseline prognostic score (DIPSS Int-1, Int-2, High), platelet count ( $100$ – $200 \times 10^9 \text{ l}^{-1}$  versus  $> 200 \times 10^9 \text{ l}^{-1}$ ) and spleen volume ( $< 1,800 \text{ cm}^3$  versus  $\geq 1,800 \text{ cm}^3$ ). An analysis of covariance (ANCOVA) model was used to analyze the continuous key secondary endpoint of absolute change in TSS, controlling for baseline prognostic score (DIPSS Int-1, Int-2, High), platelet count ( $100$ – $200 \times 10^9 \text{ l}^{-1}$  versus  $> 200 \times 10^9 \text{ l}^{-1}$ ) and spleen volume ( $< 1,800 \text{ cm}^3$  versus  $\geq 1,800 \text{ cm}^3$ ) and baseline TSS. SAS v.9.4 was used to assess the impact of missing data. Hypothesis tests were performed sequentially: primary endpoint of spleen response; key secondary endpoint of absolute change in TSS; key secondary endpoint of TSS50. Formal testing for statistical significance was halted if the *P* value was greater than 0.05 for a given endpoint. Descriptive statistics are used to report other secondary endpoints and exploratory analyses.

Assuming respective spleen/TSS50 response rates of 62%/57% with pelabresib–ruxolitinib and 29%/42.2% with placebo–ruxolitinib<sup>20,27</sup>, a sample size of approximately 400 patients (200 in each treatment arm) was estimated to provide over 99% power for testing the primary endpoint and 81% power for the key secondary endpoint of TSS50 using the two-group continuity corrected  $\chi^2$  test, with a 5% two-sided significance level and accounting for 2% nonevaluable patients. The sample size provided 90% power to test the key secondary endpoint of absolute change in TSS, assuming a treatment difference of 4 points based on reported median percentage changes. Mixed models for repeated measures were used to estimate the percentage change from baseline in proinflammatory cytokines, reticulin fiber density, erythrocyte progenitor cell proportions and *AK2V617F* VAF. A mixed model for repeated measure controlling for treatment was used to estimate

the relative concentrations in proinflammatory cytokines according to spleen response ( $\geq 35\%$  reduction in spleen volume).

Continuous variables were summarized with descriptive statistics and categorical variables were summarized by numbers and percentages of patients, with two-sided 95% CIs as appropriate. The primary analysis took place after all randomized patients completed their week 24 visit or prematurely discontinued.

### Study oversight

The study protocol can be found at [https://cdn.clinicaltrials.gov/large-docs/95/NCT04603495/Prot\\_000.pdf](https://cdn.clinicaltrials.gov/large-docs/95/NCT04603495/Prot_000.pdf). The date of preregistration was 10 October 2020. See Supplementary Table 3 for a list of protocol amendments. The MANIFEST-2 study was sponsored by Constellation Pharmaceuticals, a Novartis Company (formally part of MorphoSys). The study was approved by the institutional review board or independent ethics committee at each participating center and conducted in accordance with the International Council for Harmonisation E6 Guideline for Good Clinical Practice, which originates from the Declaration of Helsinki. All patients provided written informed consent. Patients did not receive compensation for their participation in the study. Data were analyzed and interpreted by the sponsors in collaboration with the authors. The first and senior authors prepared the first draft of the manuscript with assistance from a medical writer employed by Syneos Health, and funded by MorphoSys, a Novartis Company. All authors reviewed the manuscript and confirmed the accuracy and completeness of the data.

### Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

### Data availability

Novartis is committed to sharing with qualified external researchers, access to patient-level data and supporting clinical documents from eligible studies. These requests are reviewed and approved by an independent review panel on the basis of scientific merit. All data provided is anonymized to respect the privacy of patients who have participated in the trial in line with applicable laws and regulations. This trial data availability is according to the criteria and process described on <https://www.clinicalstudydatarequest.com/>.

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### Author contributions

M.H., S.-K.K., A.-M.J., Q.L., R.B., B.B. and J.M. contributed to the conception and/or design of the study and the development of the study protocol. R.K.R., S.G., D.C., E.A., P.B., A.T.G., A.M.V., F.P., S.-E.L., V.G., A.L., S.T.O., A.T.K., A.P., A.Á.-L., R.M., J.-J.K., M.T., J.M.S., D.L., C.N.H. and J.M. enrolled and treated patients. M.H., S.-K.K., A.-M.J., Q.L., R.B. and B.B. performed data/statistical analysis. All authors participated in data interpretation, manuscript writing and reviewing, and approved the final manuscript for submission.

### Competing interests

The authors report the following competing interests. R.K.R. has received grants from MorphoSys/Constellation, Ryvu, Stemline and Zentalis, consultation fees from GlaxoSmithKline, Incyte, AbbVie,

BMS-Celgene, Novartis, Zentalis, Promedior, CTI, Blueprint, Stemline, Galectco, PharmEssentia, Disc Medicines, Sunimoto Dainippon, Servier, Karyopharm and Cogent Bio, honoraria fees from Karyopharm, Sunimoto Dainippon and Protagonist, participated on a Data Safety Monitoring Board or Advisory Board for Disc Medicines and Merck, and received advisory fees from the MPN Research Foundation. D.C. has received consultation fees from Janssen, honoraria fees from Astellas, Amgen, BMS, Janssen, AstraZeneca and AbbVie, and support for attending meetings and/or travel from Gilead Sciences, Ryvu Therapeutics and AbbVie. E.A. has received consulting/advisory board fees from Ascentage, BMS, GlaxoSmithKline, Incyte, MorphoSys/Novartis, Pfizer and Takeda. P.B. has received research support from MorphoSys to his institution for the conduct of this clinical trial, institutional grants from Incyte, BMS, CTI BioPharma (a Sobi company), MorphoSys, Kartos, Telios, Karyopharm, Sumitomo, Janssen, Geron, Ionis, Disc, Blueprint and Cogent; consultation fees from Incyte, BMS, CTI BioPharma (a Sobi company), GlaxoSmithKline, AbbVie, MorphoSys, Karyopharm, Sumitomo, PharmaEssentia, Morphic, Jubilant, Ionis, Disc, Blueprint, Cogent, Ono and Novartis; honoraria fees from Incyte, Sumitomo, GlaxoSmithKline, PharmaEssentia, AbbVie, CTI BioPharma (a Sobi company) and Novartis; holds a study steering committee membership with Blueprint, Geron and Karyopharm, a steering committee membership with Sumitomo, Keros, GlaxoSmithKline and Karyopharm and a leadership role in a scientific advisory board with PharmaEssentia; is an advisory board member for Raythera; and had received MD Anderson Cancer Center Support grant P30 CA016672 from the National Institutes of Health (National Cancer Institute). A.T.G. has received consulting fees from Novartis (MorphoSys), PharmaEssentia, GlaxoSmithKline (Sierra Oncology), Sobi (CTI Biopharma), AbbVie, Merck (Imago Biosciences), Kartos, Telios, BMS, Rain Oncology, Disc Medicine and Agios. A.M.V. has received honoraria fees from Incyte, Novartis, AbbVie, GlaxoSmithKline, BMS, MorphoSys and AOP, participated on a Data Safety Monitoring Board or Advisory Board from Incyte, Novartis, AOP, MorphoSys and Roche. F.P. has received consultation and honoraria fees from Novartis, Celgene, AOP, Sierra Oncology and CTI. S.-E.L. declares no personal or financial conflicts of interests related to this manuscript, has received consulting/advisory board fees from BMS, GSK, Novartis and PharmaEssentia, and received an institutional grant from PharmaEssentia. V.G. has received grants from AbbVie and Novartis, consultation fees from GlaxoSmithKline, Incyte, AbbVie, BMS-Celgene and Novartis, honoraria fees from GlaxoSmithKline, Novartis and AbbVie, support for attending meetings and/or travel from GlaxoSmithKline and participated on a Data Safety Monitoring Board or Advisory Board from BMS-Celgene, Incyte, Daichi-Sankyo, AbbVie, Novartis and GlaxoSmithKline. A.L. has received consulting fees from AOP and Sanofi, honoraria fees from Grifols, Incyte, Novartis, Amgen, Pfizer, BMS, Sanofi and SOBI, support for attending meetings and/or travel from Sanofi and BeiGene, and participated on a Data Safety Monitoring Board or Advisory Board for MorphoSys, Amgen, Protagonist, Grifols, SOBI, Novartis and Sanofi. S.T.O. has received consulting fees from Novartis, Kartos Therapeutics, Disc Medicine, Blueprint Medicines, AbbVie, Constellation/MorphoSys, CTI BioPharma, Bristol Myers Squibb, Geron, GlaxoSmithKline/Sierra Oncology, Cogent and Incyte. A.T.K. has received institutional grants from MorphoSys, Novartis, BMS, GlaxoSmithKline, Protagonist, Geron and Janssen, consultation fees from AbbVie, MorphoSys and Karyopharm, honoraria fees from PharmaEssentia, Incyte, BMS and CTI Biopharma, support for attending meetings and/or travel support from PharmaEssentia, and participated on a Data Safety Monitoring Board or Advisory Board with Incyte. A.P. has received consulting fees from Sanofi and Sobi, honoraria fees from Sobi, Sanofi, Pfizer, Incyte, Alexion, Takeda, Novartis and BMS, and support for attending meetings and/or travel from Alexion, Sobi and Sanofi. A.Á.-L. received honoraria fees from AOP Health for participating in an advisory board

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Galecto and CTI, honoraria fees from Novartis, MSD, Karyopharm, Sobi, GlaxoSmithKline and BMS, support for attending meetings and/or travel from Novartis, participated on a Data Safety Monitoring Board or Advisory Board for BMS and Galecto, leadership role with Blood Cancer UK (Trustee; unpaid), EHA (Deputy Editor-in-Chief remunerated) and MPN Voice (Medical Director; unpaid), and holds stock or stock options with Chakana Medical Limited. J.M. has received grants from Incyte, Novartis, Geron, BMS, AbbVie, CTI/SOBI, Karyopharm, Disc Medicines, Ajax, PharmaEssentia and Kartos, consultation fees from Incyte, CTI/SOBI, BMS, MorphoSys, GlaxoSmithKline, AbbVie, Novartis, Roche, Merck, Pfizer, Geron, Karyopharm, PharmaEssentia, Disc Medicines, Blueprint Medicines, Keros, Galecto and Sumitomo, support for attending meetings and/or travel from Kartos, and participated on a Data Safety Monitoring or Advisory Board with Galecto and Incyte. The remaining author(s) declare no competing interests.

## Additional information

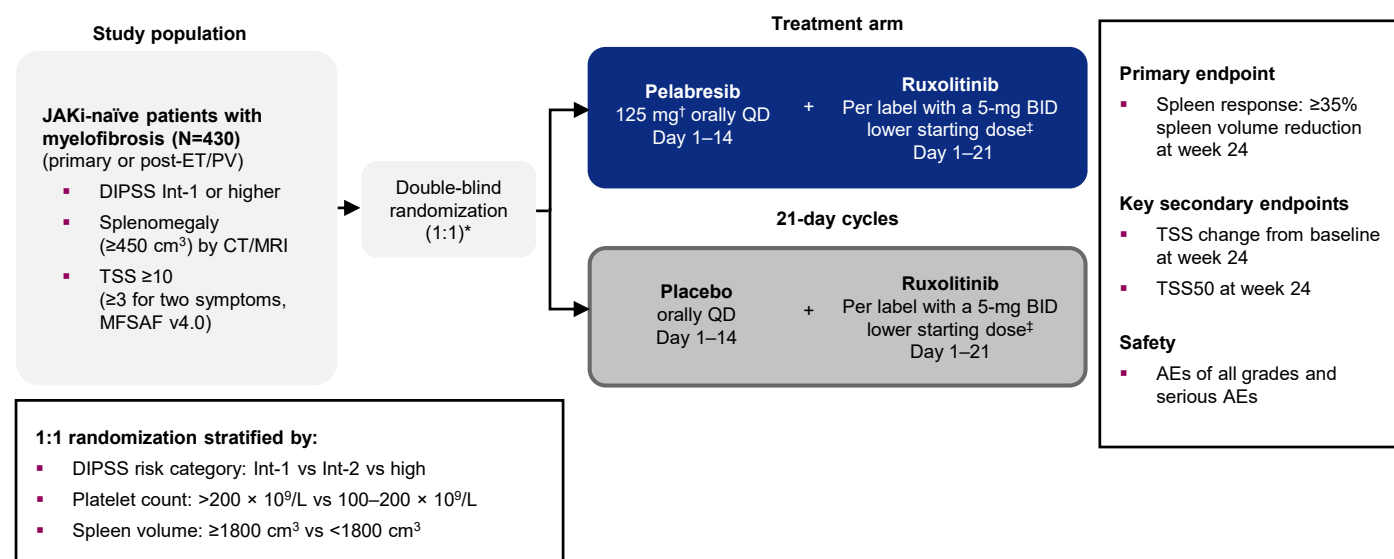
**Extended data** is available for this paper at <https://doi.org/10.1038/s41591-025-03572-3>.

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**Correspondence and requests for materials** should be addressed to John Mascarenhas.

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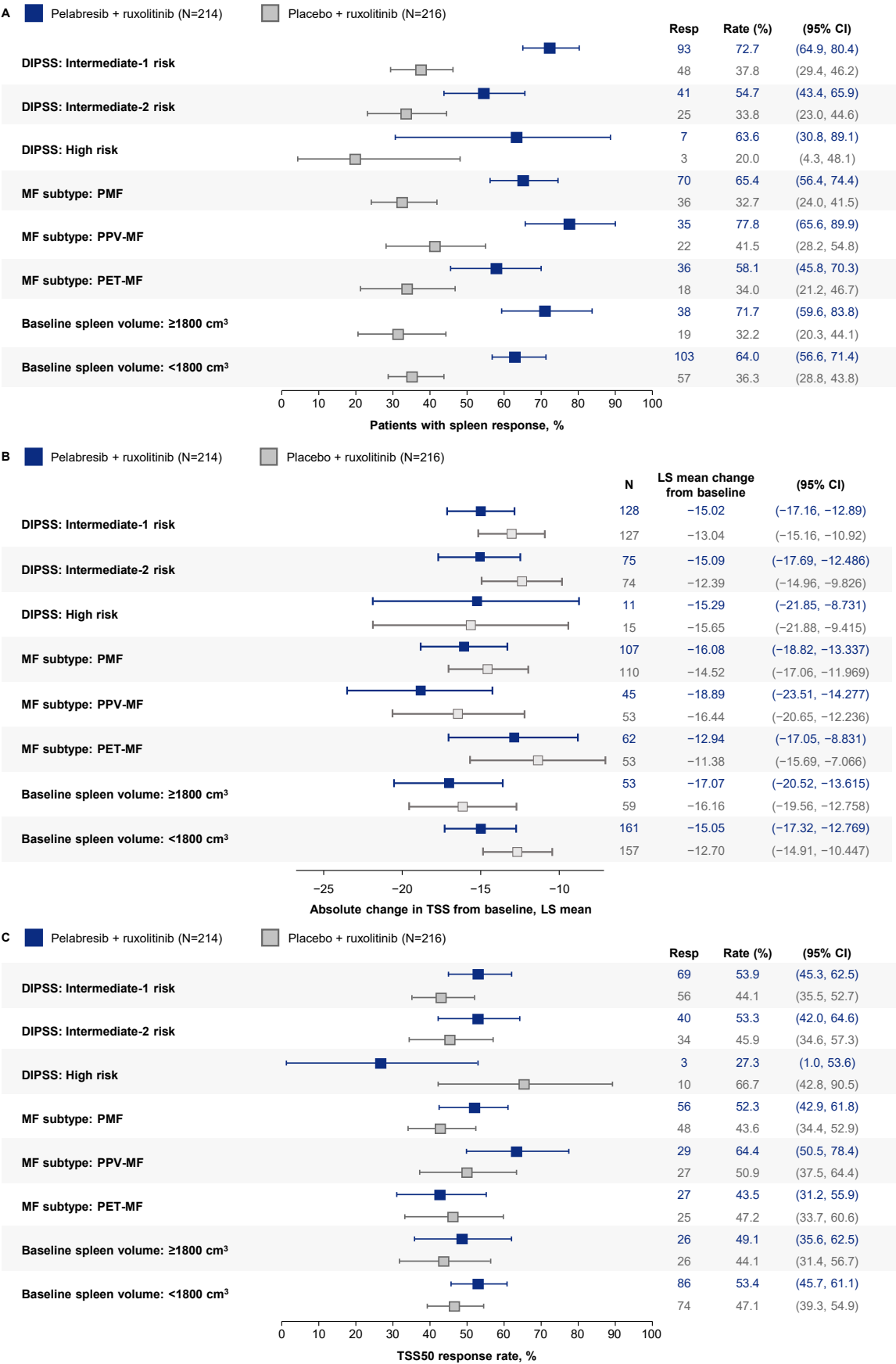
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**Extended Data Fig. 1 | MANIFEST-2 study design.** \*Patients enrolled in the placebo plus ruxolitinib arm could cross over to the pelabresib plus ruxolitinib arm if progressive splenomegaly occurred at or after 24 weeks of treatment (defined as enlargement of spleen volume by at least 25% compared with baseline); there were no crossovers as of 31 August 2023. <sup>†</sup>The starting dose for pelabresib was 125 mg QD and protocol-defined dose modifications based on AEs and treatment response allowed a dose range between 50 mg and 175 mg QD. <sup>‡</sup>Ruxolitinib was started at 10 mg BID (baseline platelet count  $100\text{--}200 \times 10^9/\text{L}$ ) or

15 mg BID (baseline platelet count  $>200 \times 10^9/\text{L}$ ) with a mandatory dose increase by 5 mg BID after one cycle and a maximum dose of 25 mg BID as per the label. AE, adverse event; BID, twice daily; CT, computed tomography; DIPSS, Dynamic International Prognostic Scoring System; ET, essential thrombocythemia; Int-1, Intermediate-1; Int-2, Intermediate-2; MRI, magnetic resonance imaging; MFSAF, Myelofibrosis Symptom Assessment Form; PV, polycythemia vera; QD, once daily; TSS, total symptom score; TSS50,  $\geq 50\%$  TSS reduction from baseline.

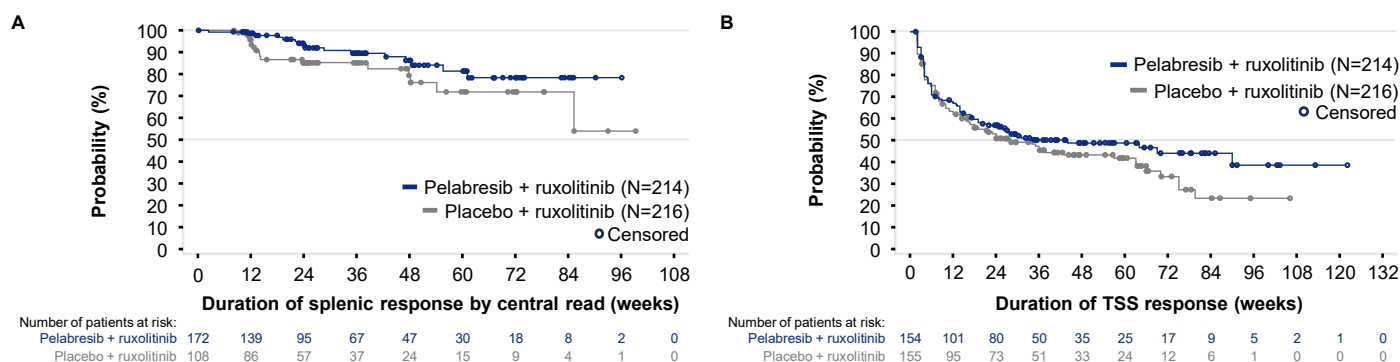




Extended Data Fig. 2 | See next page for caption.

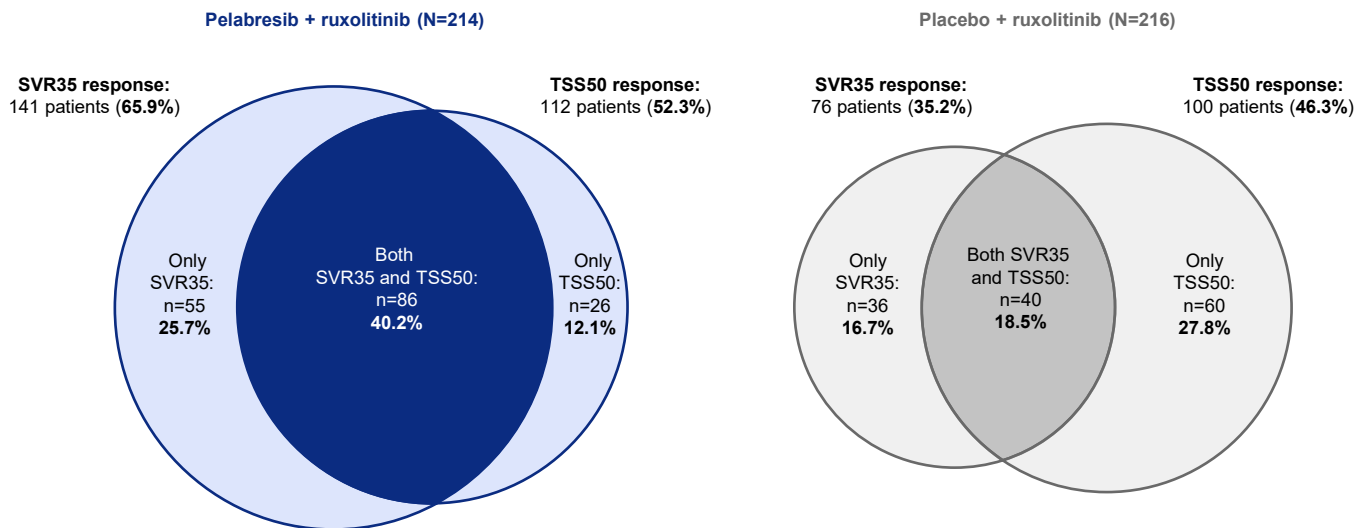
**Extended Data Fig. 2 | Spleen (central read) response and symptom score at week 24 according to pre-defined subgroups.** **(A)** Shows spleen response (defined as  $\geq 35\%$  reduction in spleen volume), according to subgroups. **(B)** Shows the mean absolute change in TSS from baseline to week 24, according to subgroups. **(C)** Shows the TSS50 response rate at week 24, according to subgroups. Data

are presented as mean value  $\pm$  95% CI. CI, confidence interval; DIPSS, Dynamic International Prognostic Scoring System; LS, least squares; MF, myelofibrosis; PET-MF, post-essential thrombocythemia myelofibrosis; PMF, primary myelofibrosis; PPV-MF, post-polycythemia vera myelofibrosis; Resp, number of responders; TSS, total symptom score; TSS50,  $\geq 50\%$  TSS reduction from baseline.



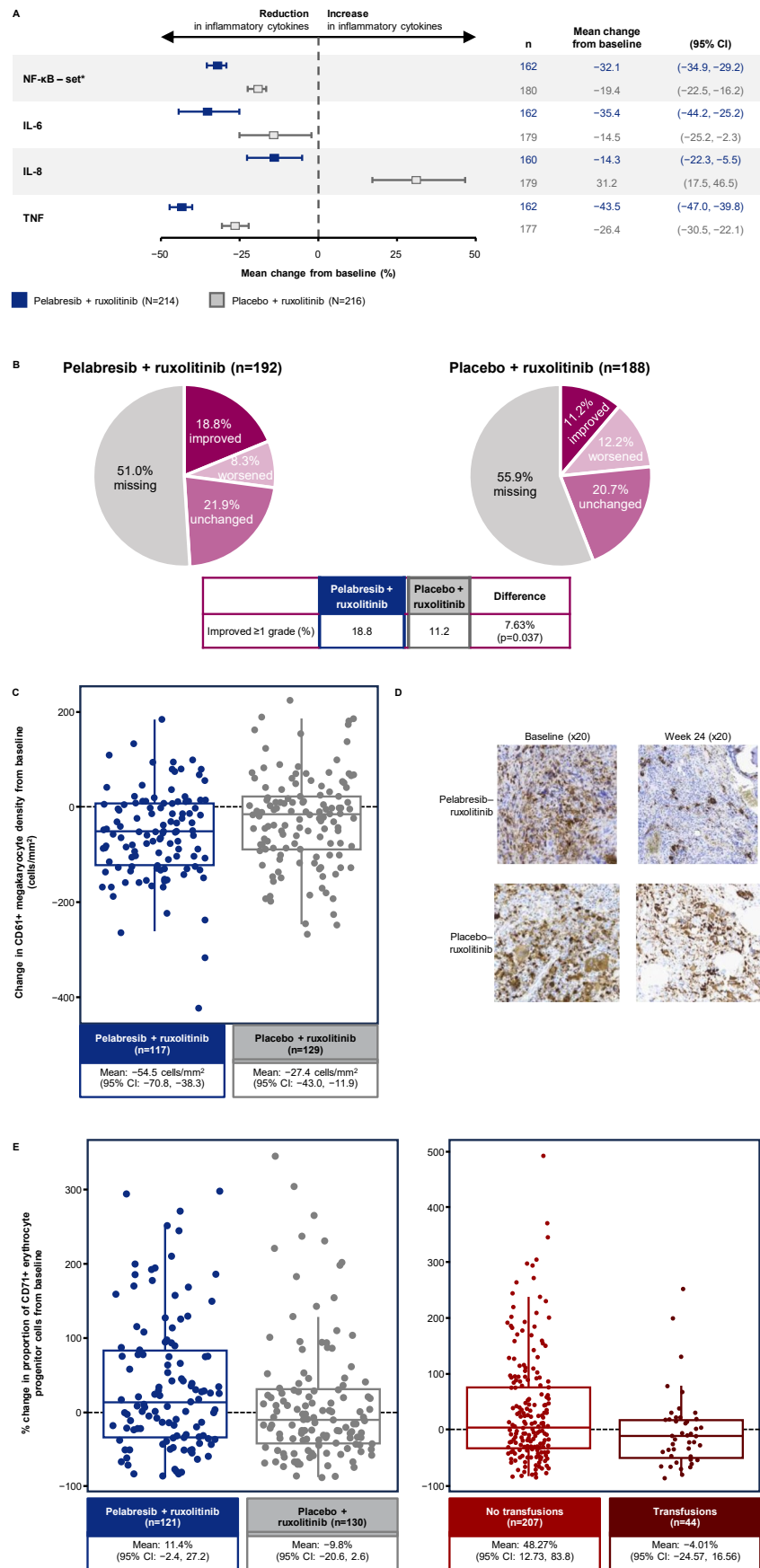
**Extended Data Fig. 3 | Duration of spleen and TSS50 responses.** (A) Shows Kaplan–Meier estimate of duration of spleen response by central read, defined as the time from first spleen response ( $\geq 35\%$  reduction in spleen volume) until a spleen volume reduction of  $<35\%$  from baseline and an increase of  $>25\%$  from nadir was first documented. Median duration of response using Kaplan–Meier was not estimable in either treatment arm at the data cut-off. The median duration of follow-up of splenic response was 27.143 weeks (95% CI: 24.143,

36.143) in the pelabresib–ruxolitinib arm and 25.429 weeks (95% CI: 24.143, 35.857) in the placebo–ruxolitinib arm. (B) Shows Kaplan–Meier estimate of the duration of symptom response, defined as the time from onset of TSS50 response until the time at which a  $< 50\%$  reduction in TSS from baseline and an increase of  $\geq 25\%$  from nadir is first observed. These data are not mature. TSS, total symptom score; TSS50,  $\geq 50\%$  TSS reduction from baseline.



**Extended Data Fig. 4 | Dual spleen and symptom responses.** SVR35,  $\geq 35\%$  reduction in spleen volume; TSS50,  $\geq 50\%$  reduction in total symptom score.

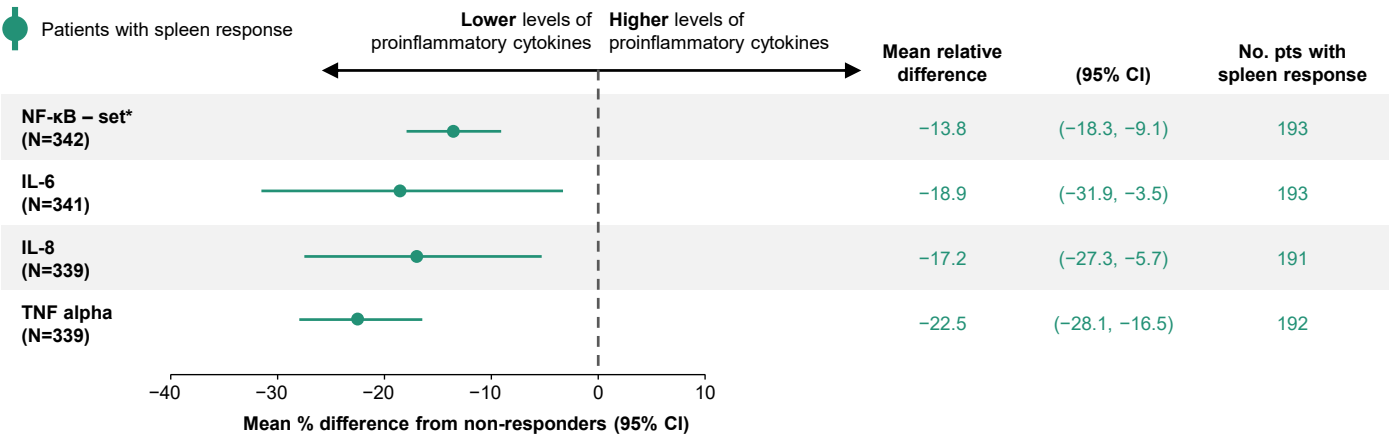




Extended Data Fig. 5 | See next page for caption.

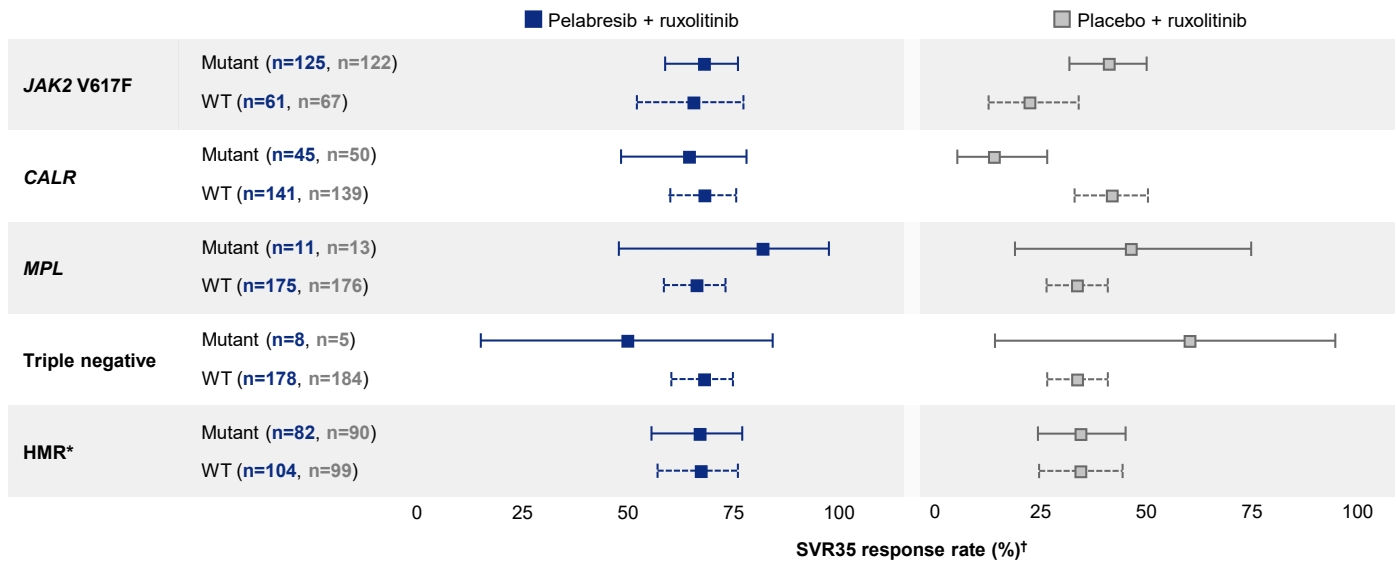
**Extended Data Fig. 5 | Pro-inflammatory cytokines, bone marrow morphology, and erythrocyte progenitors.** For exploratory endpoints, we report descriptive statistical analyses only. **(A)** shows the mean change in inflammatory cytokines from baseline at week 24. Data are presented as mean change from baseline  $\pm$  95% CI. \*NF- $\kappa$ B – set included B2M, CRP, CD40-L, hepcidin, IL-6, IL-12p40, MIP-1 beta, MPIF-1, RANTES, TNFR2, TNF, and VCAM-1. **(B)** shows the secondary endpoint change in reticulin fibrosis grade (graded according to the WHO classification) by central read at week 24. Percentages are based on the number of patients with bone marrow assessments in the intent-to-treat population at each timepoint; non-evaluable patients are those who are ongoing study treatment and have not yet reached that timepoint or opted out of bone marrow assessment. **(C)** shows the change in megakaryocytes (CD61 + ) density from baseline in the bone marrow at week 24, overlayed with individual observations. Density assessed by digital pathology. The horizontal line within the boxplot indicates the mean. The lower and upper ends of the box represent the 25th and 75th percentiles, respectively. The boxplot whiskers indicate the Tukey interval. **(D)** shows examples of immunohistochemistry stainings for megakaryocytes (CD61 + ) representative for observed mean changes of megakaryocyte densities in samples from a single patient randomized

to pelabresib–ruxolitinib and a single patient randomized to placebo–ruxolitinib, respectively. There were 117 patients in the pelabresib–ruxolitinib arm and 129 patients in the placebo–ruxolitinib arm with megakaryocyte density data. Immunohistochemistry staining was performed, and digital images analyzed by an automated quantitative cell-specific detection analysis. **(E)** shows the percentage change in erythrocyte progenitors (CD71+ cells) in the bone marrow according to treatment arm and red blood cell transfusions, overlayed with individual observations. Red blood cell transfusions refer to the number of patients who received any red blood cell transfusion between week 20 and week 24 on treatment. The horizontal line within the boxplots indicates the mean. The lower and upper ends of the box represent the 25th and 75th percentiles, respectively. The boxplot whiskers indicate the Tukey interval. The y-axis encompasses the 99 percentiles of all values. B2M, beta-2 microglobulin; CI, confidence interval; CRP, C-reactive protein; CD40-L, CD40 ligand; IL, interleukin; MIP, macrophage inflammatory protein; MPIF, myeloid progenitor inhibitory factor; NF- $\kappa$ B, Nuclear factor kappa B; RANTES, regulated upon activation, normal T-cell expressed and secreted; TNF, tumor necrosis factor; TNFR, TNF receptor; VCAM, vascular cell adhesion molecule; WHO, World Health Organization.



**Extended Data Fig. 6 | Change in pro-inflammatory cytokine levels according to spleen response (  $\geq 35\%$  reduction in spleen volume ).** Figure shows the mean relative difference in inflammatory cytokine changes from baseline at week 24 in patients with spleen response compared to patients without spleen response. Data are presented as mean relative difference  $\pm$  95% CI. For exploratory endpoints, we report descriptive statistical analyses only. \*NF- $\kappa$ B set included

B2M, CRP, CD40-L, hepcidin, IL-6, IL-12p40, MIP-1 beta, MPIF-1, RANTES, TNFR2, TNF, VCAM-1. B2M, beta-2 microglobulin; CI, confidence interval; CRP, C-reactive protein; IL, interleukin; MIP, macrophage inflammatory protein; MPIF, myeloid progenitor inhibitory factor; NF- $\kappa$ B, nuclear factor kappa B; RANTES, regulated upon activation, normal T cell expressed and secreted; TNF, tumor necrosis factor; TNFR, TNF receptor; VCAM, vascular cell adhesion protein.



**Extended Data Fig. 7 | Impact of driver and high-molecular risk mutations at baseline on clinical response rates at week 24.** Data are presented as mean value  $\pm$  95% CI. \*HMR mutations include *ASXL1*, *EZH2*, *IDH1*, *IDH2*, *SRSF2*, and *U2AF1* mutations. †Clopper–Pearson 95% CI. ASXL1, ASXL transcriptional regulator 1; CALR, calreticulin; CI, confidence interval; EZH2, enhancer of zeste

2 polycomb repressive complex 2 subunit; HMR, high molecular risk; IDH1/2, isocitrate dehydrogenase 1/2; JAK2, Janus kinase 2; MPL, MPL proto-oncogene, thrombopoietin receptor; SRSF2, serine and arginine rich splicing factor 2; SVR35,  $\geq 35\%$  reduction in spleen volume from baseline; U2AF1, U2 small nuclear RNA auxiliary factor 1; WT, wild type.



Extended Data Table 1 | Mean change in symptom score across TSS domains

TSS sub-domain	Pelabresib + ruxolitinib (N=214)	Placebo + ruxolitinib (N=216)
<b>Bone pain, n</b>	163	162
Mean percent change from baseline, %	−39.51	−35.20
SD percent change from baseline	86.48	75.63
<b>Fullness after beginning to eat, n</b>	178	191
Mean percent change from baseline, %	−47.46	−31.05
SD percent change from baseline	83.05	173.41
<b>Pain under ribs left side, n</b>	171	176
Mean percent change from baseline, %	−53.27	−45.18
SD percent change from baseline	124.93	92.26
<b>Abdominal discomfort, n</b>	182	191
Mean percent change from baseline, %	−43.19	−36.31
SD percent change from baseline	69.10	64.94
<b>Itching, n</b>	162	168
Mean percent change from baseline, %	−56.48	−41.37
SD percent change from baseline	100.05	171.13
<b>Night sweats, n</b>	165	178
Mean percent change from baseline, %	−52.27	−50.45
SD percent change from baseline	104.61	85.98
<b>Fatigue, n</b>	184	192
Mean percent change from baseline, %	−38.55	−34.90
SD percent change from baseline	47.57	52.14

In accordance with preplanned hierarchical statistical testing, for symptom scores across TSS domains, we report descriptive statistical analyses only. SD, standard deviation; TSS, total symptom score.

**Extended Data Table 2 | Patient Global Impression of Change (PGIC) at week 24**

<b>Category, n (%)</b>	<b>Pelabresib + ruxolitinib (N=214)</b>	<b>Placebo + ruxolitinib (N=216)</b>
Very much improved	23 (13.0)	24 (12.4)
Much improved	60 (33.9)	73 (37.6)
Minimally improved	61 (34.5)	70 (36.1)
No change	19 (10.7)	16 (8.2)
Minimally worse	10 (5.6)	11 (5.7)
Much worse	3 (1.7)	0
Very much worse	1 (0.6)	0

At week 24, 177 patients in the pelabresib plus ruxolitinib arm and 194 patients in the placebo plus ruxolitinib arm had non-missing results; percentages are calculated based on patients with non-missing results.

**Extended Data Table 3 | Most frequent treatment-emergent adverse events related to pelabresib or placebo reported in at least 5% of patients in either arm**

Preferred term, n (%)	Pelabresib + ruxolitinib (N=212)*	Placebo + ruxolitinib (N=214)*
Any event	164 (77.4)	160 (74.8)
Anemia <sup>†</sup>	60 (28.3)	67 (31.3)
Thrombocytopenia <sup>‡</sup>	56 (26.4)	41 (19.2)
Platelet count decreased	29 (13.7)	28 (13.1)
Diarrhea	27 (12.7)	24 (11.2)
Dysgeusia	33 (15.6)	8 (3.7)
Nausea	21 (9.9)	18 (8.4)
Constipation	10 (4.7)	22 (10.3)
Alanine aminotransferase increased	13 (6.1)	16 (7.5)
Fatigue	12 (5.7)	16 (7.5)

\*Safety population includes all patients who received at least one dose of study drug. <sup>†</sup>'Anemia' includes preferred terms of anemia and hemoglobin decrease. <sup>‡</sup>'Thrombocytopenia' includes preferred terms of thrombocytopenia and platelet count decrease. A treatment-emergent adverse event for the double-blinded treatment period is defined as an adverse event that has a start date on or after the first dose of pelabresib/placebo and before 30 days after the last dose of pelabresib/placebo or before the start of alternative (off-study) treatment for myelofibrosis, whichever occurs first. For any adverse event for which Common Terminology Criteria for Adverse Events grading does not exist, the Investigator assessed severity using the WHO grading system, where Grades 1–4 correspond to the severity of mild, moderate, severe, and life-threatening. WHO, World Health Organization.

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Software and code

Policy information about [availability of computer code](#)

Data collection	Medidata's Rave EDC (Electronic Data Capture), Electronic Clinical Outcome Assessment (eCOA)
Data analysis	SAS version 9.4

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

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Reporting on sex and gender	In the manuscript, the word 'sex' is used appropriately, to refer to biological attribute. The word 'gender' does not appear, as we did not research anything related to identity. This has also been added to the Methods section of the manuscript.
Reporting on race, ethnicity, or other socially relevant groupings	We report race/ethnicity subgroups as follows: White, Asian, Black or African American, American Indian or Alaska Native, Not reported, Unknown
Population characteristics	Covariate-relevant population characteristics are reported in Table 1 of the manuscript.
Recruitment	Patients were recruited by participating clinical trial sites based on the eligibility criteria specified in the clinical trial protocol. Recruitment and enrollment strategies for this study may include recruitment from the Investigators' local practices or referrals from other physicians.
Ethics oversight	The study was approved by the institutional review board or independent ethics committee at each participating center. A full list of participating study centers can be found in Supplementary Table 3.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Assuming respective spleen/TSS50 response rates of 62%/57% with pelabresib–ruxolitinib and 29%/42.2% with placebo–ruxolitinib, a sample size of approximately 400 patients (200 in each treatment arm) was estimated to provide over 99% power for testing the primary endpoint and 81% power for the key secondary endpoint of TSS50 using the two-group continuity corrected $\chi^2$ test, with a 5% two-sided significance level and accounting for 2% non-evaluable patients. The sample size provided 90% power to test the key secondary endpoint of absolute change in TSS, assuming a treatment difference of 4 points based on reported median percentage changes.
Data exclusions	None
Replication	Double programming were performed and passed quality control checks successfully
Randomization	In this double-blind active-control study, patients were randomized 1:1 to pelabresib plus ruxolitinib or placebo plus ruxolitinib, by a centralized interactive voice response/interactive web response system.
Blinding	All patients, investigators, and the sponsor were blinded to treatment allocation with study drugs packaged identically.

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input type="checkbox"/>	<input checked="" type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

### Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

## Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	NCT04603495
Study protocol	The study protocol can be found here: <a href="https://cdn.clinicaltrials.gov/large-docs/95/NCT04603495/Prot_000.pdf">https://cdn.clinicaltrials.gov/large-docs/95/NCT04603495/Prot_000.pdf</a> .
Data collection	The study was conducted at sites across North America, Asia, Europe, and Australia. Patients were enrolled between November 2020 and March 2023. A full list of study sites can be found in Supplementary Table 3.
Outcomes	The primary endpoint was spleen response, defined as a reduction of $\geq 35\%$ in spleen volume from baseline at week 24 by independent central review. Spleen volume was measured using magnetic resonance imaging or computed tomography, performed during screening and every 12 weeks from the first day of treatment, with magnetic resonance imaging being the preferred method. Key secondary endpoints were (i) absolute change in TSS from baseline, and (ii) TSS50, defined as reduction of $\geq 50\%$ from baseline in TSS; both at week 24 using MFSAF Version 4.0. MFSAF was completed electronically daily until 12 weeks after end of treatment.

## Plants

Seed stocks	<i>Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.</i>
Novel plant genotypes	<i>Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.</i>
Authentication	<i>Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.</i>