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ACVR1/JAK1/JAK2 inhibitor momelotinib reverses transfusion dependency and suppresses hepcidin in myelofibrosis phase 2 trial

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Key Points

- In a phase 2 study, momelotinib reversed or reduced transfusion dependency in transfusion-dependent myelofibrosis patients.

- Momelotinib inhibited hepcidin, an iron-regulatory hormone associated with restricted erythropoiesis.

Momelotinib (MMB) is a JAK1/2 and ACVR1 inhibitor with demonstrated clinical activity in all 3 hallmarks of myelofibrosis (MF): anemia, constitutional symptoms, and splenomegaly. In this phase 2 open-label translational biology study (NCT02515630) of 41 transfusion-dependent patients with MF, we explored mechanisms underlying the favorable activity of MMB on MF-associated iron-restricted anemia, including its impact on serum hepcidin levels, and markers of iron storage and availability, erythropoiesis, and inflammation. A transfusion-independent response (TI-R), defined as red blood cell transfusion independence (TI) ≥12 weeks at any time on study, occurred in 17 patients (41%; 95% confidence interval [CI], 26%-58%), including 14 patients (34%; 95% CI, 20%-51%) who achieved TI-R by week 24. In addition, 78% of TI nonresponse (TI-NR) patients achieved a ≥50% decrease in transfusion requirement for ≥8 weeks. Adverse events (AEs) were consistent with previous studies of MMB in MF, with cough, diarrhea, and nausea as the most common. Twenty-one patients experienced grade ≥3 AEs, most commonly anemia and neutropenia. Consistent with preclinical data, daily MMB treatment led to an acute and persistent decrease in blood hepcidin associated with increased iron availability and markers of erythropoiesis. Baseline characteristics associated with TI-R were lower inflammation and hepcidin as well as increased markers of erythropoiesis and bone marrow function. Overall, the study demonstrates that MMB treatment decreases hepcidin in conjunction with improving iron metabolism and erythropoiesis, suggesting a mechanistic explanation for the reduced transfusion dependency observed in transfusion-dependent MF patients treated with MMB, thereby addressing the key unmet medical need in the MF population.

Introduction

Myelofibrosis (MF) is a myeloproliferative neoplasm caused by hyperactivation of JAK-STAT signaling and clonal proliferation due primarily to mutations in *JAK2*, *CALR*, or *MPL* with associated inflammatory cytokine production. Cardinal features of the disease include anemia, bone marrow failure and

consequent extramedullary hematopoiesis, splenomegaly, and constitutional symptoms.[1,2] Pervasive JAK-STAT signaling results in the overproduction of multiple cytokines in MF, which are thought to contribute to its clinical features and promote disease evolution.[3-5] Inflammatory cytokines acting locally contribute directly to bone marrow fibrosis and decreased hematopoiesis, whereas systemic inflammation contributes to constitutional symptoms, such as fatigue, cachexia, night sweats, and anemia of inflammation.

Anemia is the most important negative prognostic indicator in MF. One study found that 86% of MF patients are anemic, including 37% who are severely anemic or transfusion dependent, with most progressing to transfusion dependency over time.[6] Patients with severe anemia at diagnosis (defined as hemoglobin <8 g/dL or transfusion dependence) have a median survival of only 2.1 years and a >1.5-fold increase in the risk of death compared with patients with moderate anemia (hemoglobin, 8-10 g/dL), who have a median survival of 3.4 years.[1] Although anemia is typically present at initial diagnosis of MF, development of anemia during the disease course is also strongly associated with poor prognosis.[7] Anemia predicted shortened survival, with a hazard ratio (95% confidence interval [CI]) of 3.4 (2.7-4.3) for severe anemia, 2.1 (1.6-2.8) for moderate anemia, and 1.4 (1.1-1.7) for mild anemia.[6]

The complex and interrelated drivers of anemia in MF include marrow fibrosis fundamental to the disease, anemia of chronic inflammation, and splenic sequestration of red blood cells (RBCs). In addition, anemia can be exacerbated by specific MF treatments, including the JAK inhibitors ruxolitinib and fedratinib.[8,9]

Anemia of chronic inflammation is a complex iron distribution disorder mediated by elevated levels of hepcidin associated with shortened erythrocyte lifespan and restricted erythropoiesis through direct and indirect effects of cytokines.[10] Hepcidin is a hepatic iron-regulatory hormone that reduces duodenal iron absorption and increases iron sequestration in monocytes and macrophages. Elevated serum hepcidin reduces iron availability and restricts erythropoiesis. Inflammation greatly increases synthesis of hepcidin through activation of the JAK2-STAT signaling pathway, a fundamental driver of MF. Aberrant cytokine-driven signaling via activin receptor type 1 (ACVR1), a member of the transforming growth factor-β superfamily of receptors that controls iron storage, also upregulates hepcidin production. Thus, anemia of inflammation is associated with substantially elevated circulating hepcidin levels and perturbed iron homeostasis, which is characterized by a decreased availability of iron for erythropoiesis and iron-restricted anemia.

Like severe anemia itself, elevated hepcidin has been associated with reduced survival in MF patients. These patients have significantly higher circulating hepcidin relative to healthy controls, which correlates with the need for RBC transfusions and shortened life expectancy.[11]

Therapies that can adequately ameliorate anemia in MF are lacking. There are no available interventions to lower elevated hepcidin levels or directly address the iron-restricted anemia of chronic inflammation, 1 of the major drivers of anemia in MF. Consequently, there is a significant unmet medical need in moderate and severely anemic MF patients, especially in those who are transfusion dependent.

In contrast to the myelosuppressive effects of other approved and investigational JAK inhibitors, momelotinib (MMB) therapy elicited a range of anemia benefits in phase 1/2 clinical trials of MF. The benefits included high rates of durable conversion from transfusion dependence to independence in MF patients.[12] These initially unanticipated clinical data were later supported by increased rates of TI, decreased transfusion requirements, and fewer adverse events (AEs) of anemia in the MMB arms of the 2 phase 3 SIMPLIFY studies of MMB vs ruxolitinib and best-available therapy controls.[13,14] These findings suggested that MMB exerted a differentiated but, at that stage, incompletely defined proerythropoietic pharmacology. Further investigation into the mechanism of anemia benefit revealed MMB to be a potent and selective inhibitor of JAK1 and JAK2, as well as of ACVR1. Preclinical studies in a hepatoma cell line and a rodent anemia of chronic disease (ACD) model demonstrated that MMB inhibited the interleukin-6 (IL-6)–JAK/STAT inflammatory pathway and uniquely inhibited the BMP6-ACVR1/SMAD1/5/8 iron-sensing pathway. Both pathways stimulate hepcidin production.[15] Consequently, MMB treatment elicited decreased liver *Hamp* (which encodes hepcidin) messenger RNA expression, reduced serum hepcidin, and increased serum iron, resulting in increased hemoglobin and RBC production in ACD rats but not in normal controls. These preclinical findings suggested that MMB optimally and uniquely inhibits both pathways, thereby suppressing hepcidin production. Therefore, MMB is able to more adequately resolve the iron-restricted anemia typical of MF than is JAK-STAT inhibition alone.

This phase 2 study was designed to further investigate the differentiated ACVR1/hepcidin-mediated anemia benefit in transfusion-dependent MF patients, by measuring circulating hepcidin, as well as biomarkers of iron storage, erythropoiesis, and inflammation.

Methods

Study design and patients

This phase 2 open-label study (NCT02515630) investigated 200 mg of MMB administered orally once daily, for up to 24 weeks, to transfusion-dependent MF patients at 13 sites in the United States and Canada between January 2016 and August 2017. The study was conducted in accordance with Good Clinical Practice guidelines, and the protocol was approved by each site's ethics board or institutional review board. Following completion of the 24-week dosing period, patients who were responding to treatment had the option to continue maintenance MMB treatment in an open-label extension study (NCT02124746).

Eligible patients were ≥18 years of age with a diagnosis of primary MF, postpolycythemia vera, or postessential thrombocythemia MF; required MF therapy; were high risk, intermediate-2 risk, or intermediate-1 risk (per Dynamic International Prognostic Scoring System [DIPSS]) with symptomatic splenomegaly and/or hepatomegaly; and were transfusion dependent at baseline (≥4 U RBC transfusions in the 8 weeks prior to the first dose of MMB). Key exclusion criteria were prior splenectomy, splenic irradiation within 3 months prior to randomization, prior treatment with MMB, and treatment with a JAK inhibitor ≤21 days before the planned first dose of MMB. All patients provided written informed consent.

Clinic visits occurred at screening, baseline, enrollment (first dose), weeks 2, 4, 8, 12, 16, 20, and 24, and at a follow-up visit 30 days

later. Transfusion, physical examinations, and AEs were recorded at all visits. The modified myeloproliferative neoplasm symptom assessment form diary was completed by patients daily for calculation of total symptom score (TSS).[16] Complete blood count with differential, reticulocyte count, and iron studies were done at baseline and at all on-treatment visits. Because of diurnal variation in hepcidin levels, samples were drawn in the morning (normal trough) prior to receiving MMB and in the afternoon, at 6 hours post-MMB dosing, at the following visits: baseline visit (no MMB), enrollment (first MMB dose), and weeks 2, 4, 8, 12, 16, 20, and 24. C-reactive protein (CRP) was measured at baseline and at weeks 2, 12, and 24 using a high-sensitivity CRP test; erythropoietin was measured at baseline and at weeks 8 and 20; and liver iron concentration (LIC) was assessed at baseline and at week 24 via magnetic resonance imaging. Phosphorylated STAT3 (pSTAT3) and total STAT3 (tSTAT3) were measured in IL-6–stimulated whole blood by flow cytometry, and data were reported as the percentage of $CD3^+CD4^+$ T cells positive for pSTAT3 relative to tSTAT3.

For hepcidin testing, blood was collected and allowed to clot at room temperature for ≥2 hours. Hepcidin was measured in serum by weak cation exchange time-of-flight mass spectrometry and quantified relative to a spiked internal standard. The daily change in hepcidin was calculated as the morning value subtracted from the afternoon value. The percentage change in hepcidin daily change is calculated as $100 \times$ ([daily change at postbaseline − daily change at baseline]/[daily change at baseline]).

End points and analyses

The primary efficacy end point was transfusion-independent response (TI-R) by week 24, defined as becoming RBC transfusion independent for ≥12 weeks at any time on study. The key secondary efficacy end point was the transfusion response rate by week 24 (transfusion response defined as becoming transfusion independent for ≥8 weeks at any time on study). Other end points included splenic response rate (SRR) at week 24 (splenic response defined as ≥35% reduction in spleen volume from baseline, as measured by magnetic resonance imaging), and response rate in TSS at week 24 (TSS response defined as achieving a ≥50% reduction from baseline). This exploratory biomarker study was not designed to detect a specific effect size, and a sample size of 40 patients was chosen pragmatically. End points are presented with corresponding 2-sided 95% exact CIs using the binomial distribution.

The safety and tolerability of MMB were characterized by the type, frequency, severity, timing of onset, duration, and relationship of AEs to MMB coded using the Medical Dictionary for Regulatory Activities (version 20.0), serious AEs, or AEs leading to discontinuation of MMB.

Descriptive statistics are presented for baseline and change from baseline for anemia-related biomarkers. Biomarker analyses were conducted to explore differences between the TI-R and transfusion-independent nonresponse (TI-NR) subgroups. For the TI-R population, 2 calculations were performed for hepcidin: the first compared baseline with postbaseline, and the second compared pre–TI-R with post–TI-R, essentially synchronizing individual patient data from the initiation of the TI-R population. All other biomarkers for the TI-R population are

presented as synchronized data. An exploratory analysis was performed to investigate potential prognostic factors influencing the TI-R rate using the logistic regression model approach, which included variables of age, sex, race, DIPSS assessment, type of MF, *JAK2* V617F mutation status, baseline TSS and spleen volume, baseline levels of hemoglobin, morning hepcidin, and LIC, and baseline transferrin saturation. All end point tests were done at a significance level of 0.1, unless otherwise specified. No multiplicity adjustments were made for testing because the end points were considered exploratory in nature.

Results

Patient demographics and disposition

A total of 58 transfusion-dependent patients were screened, 41 of whom received treatment. Common reasons why screened patients were not enrolled in the study were because they did not meet inclusion/exclusion criteria and patient withdrawal of consent. Baseline demographics and disease characteristics are shown in Table 1. Notably, 73% (30/41) of patients had grade 3 bone marrow fibrosis. Five patients (12%) had received prior treatment with a JAK inhibitor (all ruxolitinib) for MF. Adherence to treatment was 99%, and the mean (standard deviation) daily dose was 188.2 (22.4) mg.

Clinical and pharmacodynamics effects of MMB

Anemia benefit. Nearly all patients who remained on study for ≥12 weeks achieved at least a partial reduction in transfusion requirements. Seventeen of 41 transfusion-dependent patients (41%; 95% CI, 26%-58%) achieved TI for ≥12 weeks at any time on study, including 14 (34%; 95% CI, 20%-51%) who met TI criteria by week 24 (TI-R subgroup). In addition, 16 patients (39%; 95% CI, 24%-56%) did not have any RBC transfusions for ≥8 weeks by week 24. The response remained ongoing at the end of the 24-week dosing period in 11 of the 13 TI-R patients who were still active (Figure 1). The median duration of TI was not reached, and the 2 patients who lost TI did so after 12.3 and 22.6 weeks. Six patients who discontinued treatment prior to week 12 were not evaluable. In the TI-evaluable population of patients with ≥12 weeks of follow-up, the TI-R rate at any time on study was effectively 49% (17/35).

Anemia benefit was also observed in patients who retained a transfusion requirement at week 24, with 78% (21/27) of TI-NR patients experiencing a ≥50% decrease in transfusion requirement for any 8-week period.

Biomarker assessments

Markers of iron metabolism and anemia. The hepcidin median intrapatient daily change was 0 nM at the baseline visit (no MMB administered, Figure 2A [left side]). In contrast, MMB administration elicited an acute decrease in hepcidin production at 6 hours after drug administration (Figure 2A [right side]), as well as at each subsequent visit, in the overall population and the TI-R and TI-NR groups (Figure 2B-D), with a downward trend in circulating hepcidin levels over the 24-week dosing period, consistent with MMB-mediated inhibition of hepcidin production. The largest decrease from pre- to postdose was observed following the first MMB dose. Baseline (no MMB administered) hepcidin was higher in TI-NR patients than in TI-R patients at the

Table 1. Patient demographics and disease characteristics

Baseline characteristic	TI-R (n = 14)	TI-NR (n = 27)*	TI-R vs TI-NR†	Overall (N = 41)
Age, y	65 (10.0)	72 (7.5)	nt	70 (9.0)
≥65, n (%)	7 (50.0)	23 (85.2)	$P = .070$	30 (73.2)
Males, n (%)	8 (57.1)	18 (66.7)	ns	26 (63.4)
Race, n (%)			ns	
White	11 (78.6)	25 (92.6)		36 (87.8)
Non-White	3 (21.4)	2 (7.4)		5 (12.2)
Body mass index, kg/m^2	28.9 (5.20)	25.3 (4.05)	nt	26.5 (4.74)
Type of MF, n (%)			ns	
PMF	11 (78.6)	21 (77.8)		32 (78.0)
Post-PV/ET MF	3 (21.4)	6 (22.2)		9 (22.0)
Time since MF diagnosis, y	3.0 (1.96)	3.4 (3.15)	nt	3.3 (2.78)
RBC units transfused ≤8 wk prior to enrollment	5 (1.9)	7 (2.2)	nt	6 (2.3)
Bone marrow fibrosis grade, n (%)			nt	
0 or 1	0	2 (7.4)		2 (4.9)
2	1 (7.1)	5 (18.5)		6 (14.6)
3	12 (85.7)	18 (66.7)		30 (73.2)
Missing	1 (7.1)	2 (7.4)		3 (7.3)
DIPSS risk level, n (%)			$P = .075$	
Intermediate-1	4 (28.6)	1 (3.7)		5 (12.2)
Intermediate-2	8 (57.1)	14 (51.9)		22 (53.7)
High	2 (14.3)	12 (44.4)		14 (34.1)
JAK2V617F mutation positive	9 (64.3)	19 (70.4)	ns	28 (68.3)
Hemoglobin, g/dL	8.8 (1.00)	8.1 (0.87)	$P = .036$	8.3 (0.96)
Hemoglobin <8	2 (14.3)	10 (37.0)		12 (29.3)
Hemoglobin ≥8	12 (87.5)	17 (63.0)		29 (70.7)
White blood cells, ×10^3/μL	7.2 (4.37)	15.2 (26.15)	nt	12.50 (21.58)
ANC, ×10^3/μL	5.28 (3.43)	11.97 (21.88)	nt	9.68 (18.04)
Platelet count, ×10^3/μL	233 (194.8)	155 (69.5)	nt	181 (129.9)
Spleen volume, cm^3	2132.2 (1439.5)	2018.1 (1248.5)	ns	2057.1 (1299.8)
TSS	19.00 (16.14)	21.73 (13.97)	ns	20.73 (14.65)
CRP, mg/dL	1.4 (1.6)	2.5 (2.3)	nt	2.2 (2.1)
EPO, mIU/mL	293 (272)	522 (925)	nt	444 (770)
Ferritin, ng/mL	710 (471)	1648 (1507)	nt	1328 (1324)
Liver iron content, mg/g	3.6 (3.4)	7.6 (8.2)	ns	6.2 (7.2)
Hepcidin, nM				
Morning	22.8 (15.14)	44.3 (24.80)	$P = .013$	36.9 (24.09)
Afternoon	24.5 (11.59)	43.8 (22.50)	nt	37.2 (21.40)
Hepcidin daily change, nM	0.0 (5.52)	−0.9 (7.80)	nt	−0.6 (7.04)

Data are mean (standard deviation) unless otherwise noted.
ANC, absolute neutrophil count; EPO, erythropoietin; ET, essential thrombocythemia; ns, not significant ($P > .1$); nt, not tested; PMF, primary myelofibrosis; PV, polycythemia vera.
*One patient enrolled without a confirmed transfusion history and was categorized as a TI-NR at week 24.
†Significance was tested using a logistic regression model approach.

morning (44.3 vs 22.8 nM, respectively) and afternoon (43.8 vs 24.5nM, respectively) assessments (Table 1), and it remained higher in TI-NR patients for the duration of the study (Figure 2E). However, given the small sample size of this study, the relevance of this finding is equivocal. In addition, the intrapatient daily change in hepcidin at all postbaseline visits was relatively similar in terms of percentage change for the 2 subgroups (data not shown), although it was slightly greater numerically in TI-NR patients than in TI-R patients (Figure 2D). Hepcidin tended to decrease over time in the TI-R population (Figure 2E); this was more apparent when the TI-R rate was synchronized by using the visit prior to the TI-R rate window as the baseline (Figure 2F).
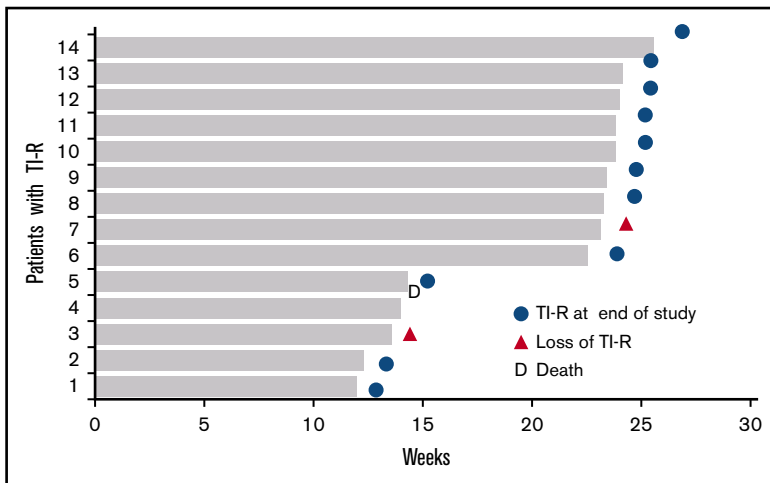
Figure 1. Outcomes in patients with TI-R by week 24. Summary of MMB effects on duration of response and outcome in individual patients who achieved TI-R by week 24.

Consistent with the acute drop in hepcidin levels, an increase in serum iron was noted specifically at week 2 in TI-R patients, with restoration of iron homeostasis consistent with increased RBC production thereafter (Figure 3A-B). Hemoglobin increased sharply at week 2 in TI-R patients (Figure 3C) and continued to climb as serum iron decreased (Figure 3B-C), indicating sustained soluble iron consumption for erythropoiesis. Transferrin (as measured by total iron-binding capacity) increased over time (Figure 3D), demonstrating the need for increased blood iron-carrying capacity to support the increased erythropoiesis. Further demonstrating active erythropoiesis, reticulocyte counts increased, mirroring hemoglobin (supplemental Figure 1A). Erythropoietin levels decreased in TI-R patients, which was indicative of increased blood oxygen capacity (supplemental Figure 1B). In contrast, TI-NR patients had limited evidence of erythropoietic activity (measured by reticulocytes, erythropoietin, transferrin, and iron), despite a small increase in hemoglobin at week 2 (Figure 3C; supplemental Figure 1). Week 2 change for TI-R patients relative to TI-NR patients for the complete panel of anemia biomarkers is summarized in Figure 3E.

As expected for a transfusion-dependent population, baseline transferrin saturation levels were high (supplemental Figure 1C), suggesting significant tissue iron storage. The impact of MMB on the reduction of iron stores was assessed by measuring LIC, which increased by a median of 2.3 mg/g (a median increase of 53%) in the TI-NR population at week 24, consistent with ongoing RBC transfusions, but it was relatively unchanged in the TI-R population (median increase of 0.2 mg/g; a median increase of 5%) (supplemental Table 1). As an additional measure of iron storage, soluble ferritin also decreased steadily in TI-R patients only (supplemental Figure 1D; supplemental Table 1).

TI-R was associated with higher baseline levels of hematopoietic lineage cells, so the impact of MMB on overall hematopoietic function was assessed. Platelet, neutrophil, and lymphocyte counts increased in TI-R patients, whereas platelet and neutrophil cell counts decreased for TI-NR patients (Figure 4).

Markers of inflammation. The impact of MMB on JAK/STAT signaling was measured in patient blood samples before dosing and 2 hours postdose using an assay that measures pSTAT3 in

IL-6–stimulated T cells. MMB treatment reduced pSTAT3 at first dose and at steady-state (4 weeks), consistent with potent inhibition of the JAK-STAT pathway (Figure 5A). The median level of pSTAT3 prior to dosing at first dose and steady-state was similar (supplemental Table 2).

Baseline levels of circulating CRP were >1 mg/dL (the level generally considered to represent significant inflammation). At week 2, CRP for this transfusion-dependent MF population was decreased by a median of 56% and remained consistently suppressed throughout the 24-week dosing period (Figure 5B). Reductions in CRP at week 24 were −60% (95% CI, −79 to −45) and −47% (95% CI, −68 to +65) in the TI-R and TI-NR populations, respectively, again consistent with a systemic anti-inflammatory effect of MMB therapy.

Comparison of baseline characteristics between TI-R and TI-NR subgroups. TI responsiveness was weakly associated with younger age ($P = .070$) and lower DIPSS grade ($P = .075$), but not with sex, type of MF, TSS, spleen volume, or *JAK2* V617F mutation ($P > .10$) (Table 1). Additionally, TI-R patients had lower serum hepcidin (morning/trough), CRP, LIC, serum iron, ferritin, and transferrin saturation and higher baseline hematocrit, erythrocytes, reticulocytes, and platelets, as well as baseline hemoglobin ≥8 g/dL (Figure 6). In a logistic regression model, the difference between TI-R and TI-NR patients was significant for hepcidin and hemoglobin only ($P < .05$) (Table 1). Overall, these biomarkers suggest that TI-R patients tended to be less inflamed and had greater latent erythropoietic potential, although data are limited.

Symptom and spleen response. TSS and SRR results in the intent-to-treat population were 16% (6/38) and 12% (5/41), respectively. Week 24 TSS rates and spleen volume assessments were not available for 17 (45%) and 15 (37%) patients, respectively, because of missing week-24 data or early discontinuation. Assessments of spleen volume reduction and change in symptom burden were not principal objectives of this translational biology study, and heavy biomarker sampling may have inadvertently led to reduced compliance. In those patients with available week-24 data, the TSS and SRR results were 29% (6/21) and 19% (5/26), respectively. Notably, 2 of the patients excluded
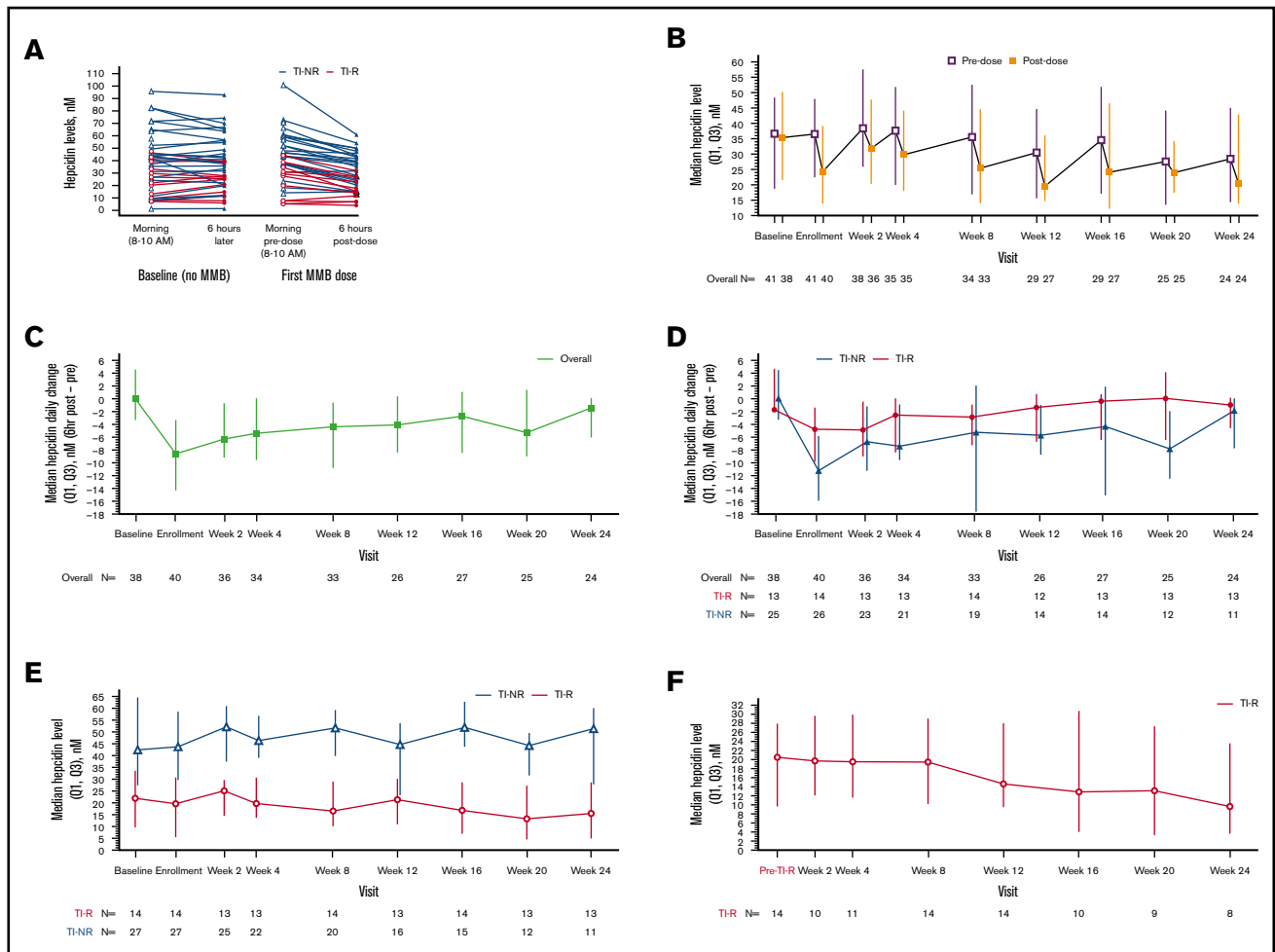
Figure 2. MMB dosing and hepcidin levels over time. (A) Individual intrapatient change in hepcidin levels at baseline (no MMB treatment) and at enrollment (first MMB dose). (B) Overall population hepcidin levels over time. (C) Hepcidin daily change (median of the individual changes in hepcidin from morning to 6 hours later per time point) in the overall population. (D) Hepcidin daily change (median of the individual changes in hepcidin from morning to 6 hours later per time point) in the TI-R and TI-NR subgroups. (E) Morning/predose hepcidin levels in TI-R and TI-NR subgroups relative to baseline. (F) Morning/predose hepcidin levels in the TI-R subgroup relative to pre−TI-R levels (pre−TI-R is the last visit with data before the start of TI response, week 2 is the visit 2 weeks after the pre-TI response visit; the same applies to the other post-TI response visits). In all panels, open symbols are predose, and filled symbols are 6 hours postdose.

from the analysis because of missing week-24 TSS assessments had a ≥50% reduction in TSS from baseline at study discontinuation; however, they were considered nonresponders because their last recorded TSS assessment was just 1 day prior to the required cutoff for evaluability at week 24.

Safety analyses

AEs in this transfusion-dependent MF population were consistent with previous studies of MMB in MF.[13,17] The most common AEs were cough (29%), diarrhea (24%), nausea (22%), and fatigue (20%), as well as dizziness, pruritus, thrombocytopenia, and vomiting (17% each). Grade ≥3 AEs occurred in 21 patients (51%), most commonly anemia and neutropenia (12% each). Serious AEs occurred in 14 patients (34%) and led to premature discontinuation of MMB in 6 patients (15%). Two patients (5%) had AEs that resulted in death (1 because of progressive disease on day 112 and 1 had an AE [acute myeloid leukemia] on day 76, discontinued MMB on day 77, and died on day 102 from septic

shock). Peripheral neuropathy occurred in 5 patients (12%); all events were grade 1, and none resulted in discontinuation of MMB.

Sixteen patients discontinued study treatment prematurely. The most common reasons were patient decision, AE, and investigator discretion.

Pharmacokinetics. The plasma concentration of MMB was determined at 2, 4, and 6 hours post-MMB dose at enrollment, week 4, and week 24 (data not shown). MMB plasma exposure was similar to that observed in other studies of patients with MF.

Discussion

Almost all patients with MF ultimately develop anemia, and most become transfusion dependent. Severe anemia, transfusion dependency, and elevated hepcidin levels are among the most important negative prognostic indicators in MF; consequently, a substantive unmet medical need for cytopenic MF patients
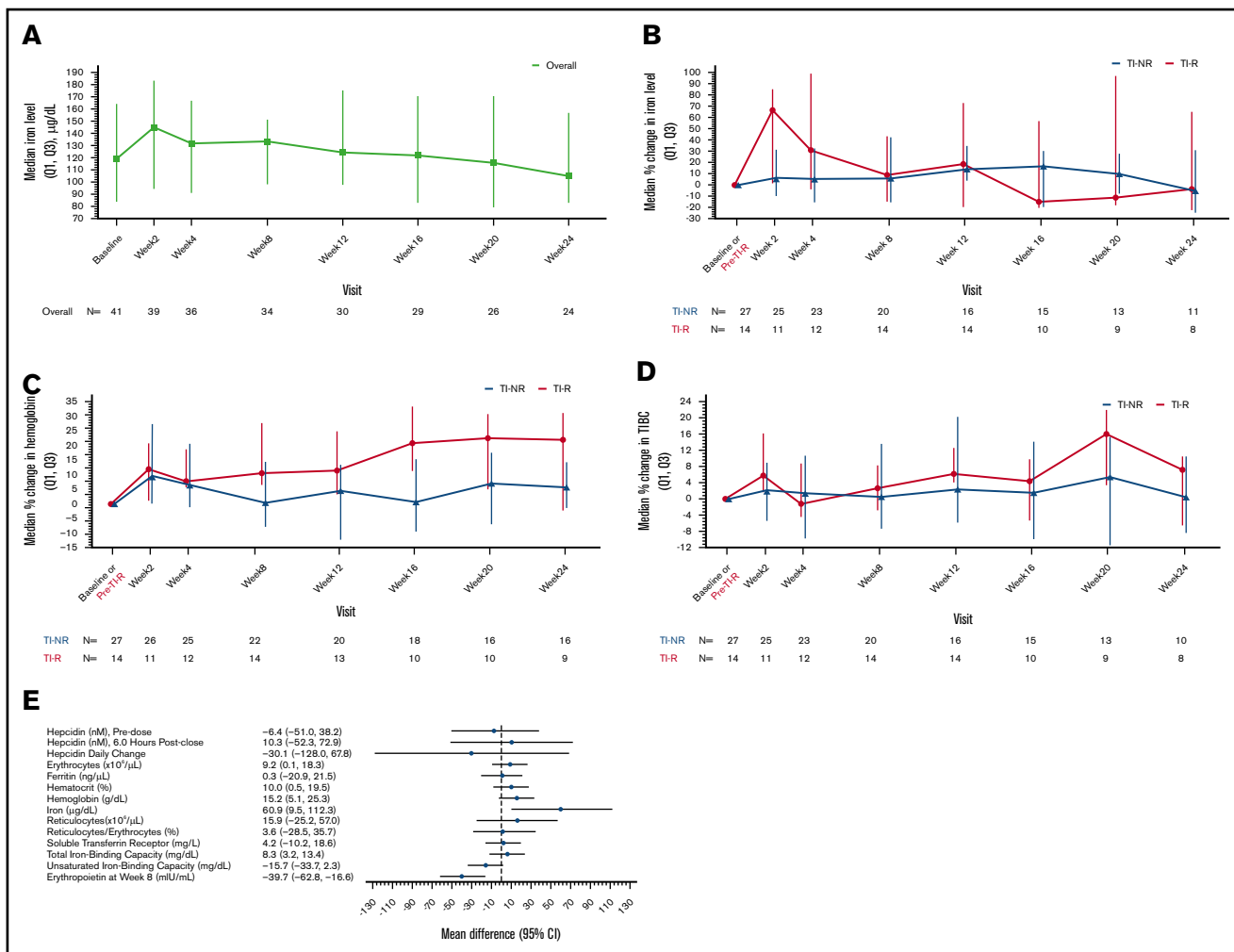
Figure 3. Iron, hemoglobin, and transferrin changes over time. Iron levels in the overall population (A) and percentage change in iron levels in TI-R and TI-NR subgroups (B). Other markers related to iron metabolism in the TI-R and TI-NR subgroups: hemoglobin (C), and total iron-binding capacity (D). For the TI-R group only, pre–TI-R is the last visit with data before the start of TI response, and week 2 is the visit 2 weeks after the pre-TI response visit; the same applies to the other post-TI response visits. (E) Forest plot of percentage change in anemia biomarkers from pre-TI response for TI-R patients at 2 weeks; 95% CI is based on the Student *t* test for paired samples.

continues to exist. Unlike other JAK inhibitors, which induce and worsen anemia and thrombocytopenia, MMB has demonstrated a range of anemia benefits, including new and maintained TI, decreased transfusion dependency, and decreased transfusion burden, while retaining constitutional symptom and splenomegaly activity,[12,13] comparable to ruxolitinib.

Because there is no available therapy aimed at inhibiting ACVR1, this represents a critical unexploited target in MF. Preclinical studies indicate that MMB ameliorates anemia by decreasing hepatic hepcidin secretion and restoring iron homeostasis for efficient erythropoiesis, consistent with the compound's optimal inhibition of JAK1, JAK2, and, uniquely among the JAK inhibitors, ACVR1. This phase 2 translational biology study was designed to further investigate and characterize the differentiated ACVR1/hepcidin-mediated anemia benefit in advanced prospectively defined transfusion-dependent MF patients.

MMB therapy elicited a significant anemia benefit in this advanced transfusion-dependent population, with TI for any period ≥12 weeks

at any time on study observed in 41% of patients, including 34% who achieved TI-R by week 24. Additionally, by week 24, 39% of patients did not experience any RBC transfusions for a period ≥8 weeks. Anemia benefit was also observed in the remaining patients who retained a transfusion requirement, with 78% of all TI-NR patients and 86% of the patients who received ≥12 weeks of MMB therapy experiencing a ≥50% reduction in 8-week transfusion burden by week 24. In this exploratory study, TI-R was associated with lower baseline hepcidin, LIC, ferritin, serum iron, inflammatory markers (CRP), and higher hematocrit, erythrocytes, reticulocytes, platelets, and hemoglobin compared with TI-NR, although a statistically significant difference could not always be demonstrated.

MMB therapy resulted in an acute reduction in hepcidin after every dose and a downward trend in hepcidin over the 24-week dosing period. Two patients experienced treatment failure, defined as loss of TI, which was associated with increased hepcidin at the time of loss of TI compared with baseline (data not shown). The rapid
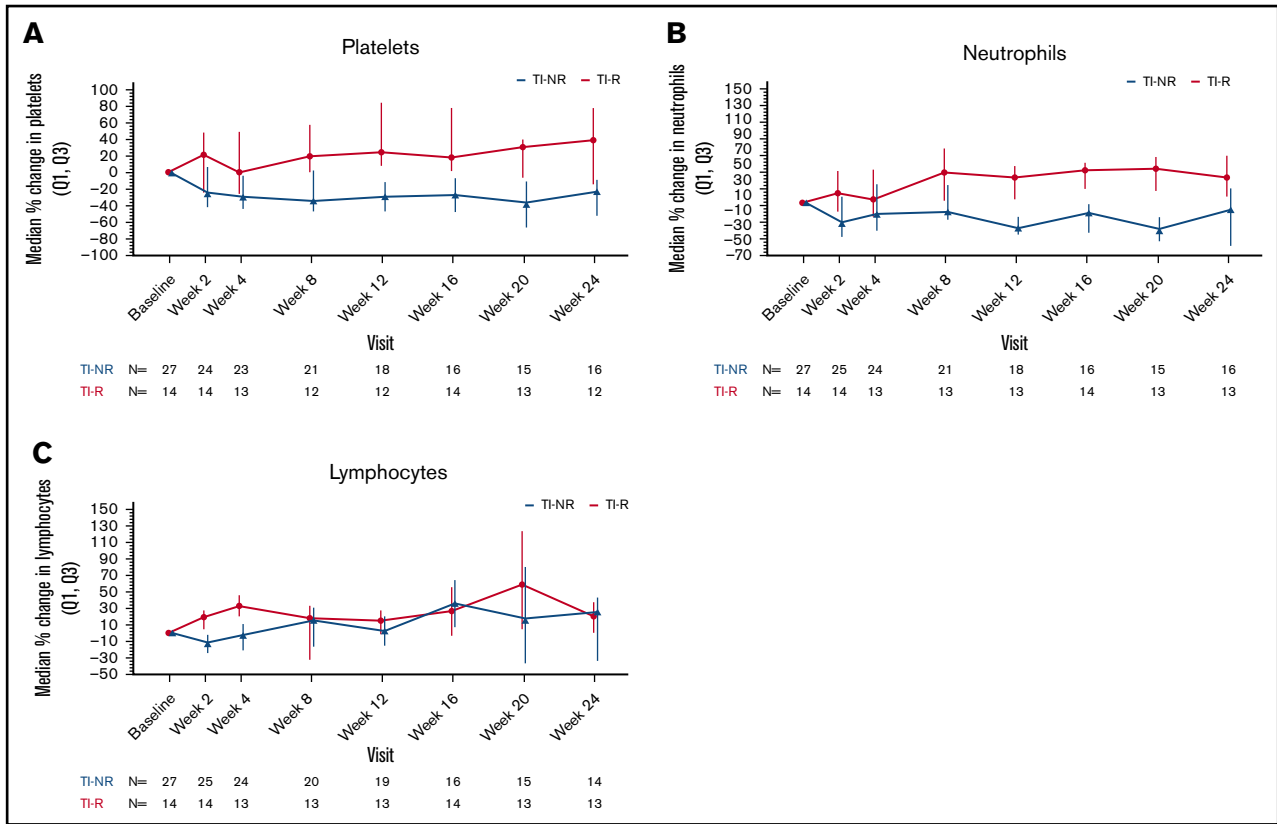
Figure 4. Effects of MMB on hematopoietic function. Changes in platelets (A), neutrophils (B), and lymphocytes (C) in TI-R and TI-NR subgroups.

decrease in peripheral hepcidin is consistent with the impact of MMB on *Hamp* demonstrated in the rat ACD model[15]; however, the relative contributions of the BMP6-ACVR1/SMAD1/5/8 and IL-6–JAK/STAT pathways cannot be elucidated without liver biopsies. Decreased hepcidin correlated with an acute increase in serum iron in TI-R at week 2. This was accompanied by increased hemoglobin, erythrocytes, and reticulocytes, suggesting that increased iron availability contributed to improved erythropoiesis. Although serum iron declined after week 2, the increase in iron-carrying capacity and increased hemoglobin levels from week 2 to week 24 suggest that serum iron was sustaining erythropoiesis. The large reduction in erythropoietin demonstrated the impact of improved oxygen-carrying capacity.

The impact of MMB on hepcidin was also observed in the TI-NR population. There was less evidence of increased erythropoiesis in this population, perhaps related to their higher baseline level of inflammation, lower erythropoietic potential, or early treatment discontinuation. However, reduced transfusion requirements were observed, to some degree, in all evaluable patients.

In contrast to the known thrombocytopenic effect of ruxolitinib and other JAK inhibitors,[18,19] the mean platelet count also increased in the TI-R population. An improvement in neutrophil levels was also observed. The mechanism underlying these favorable hematological changes is not known, but it may include a general disinhibition of hematopoiesis as a result of decreased inflammation.
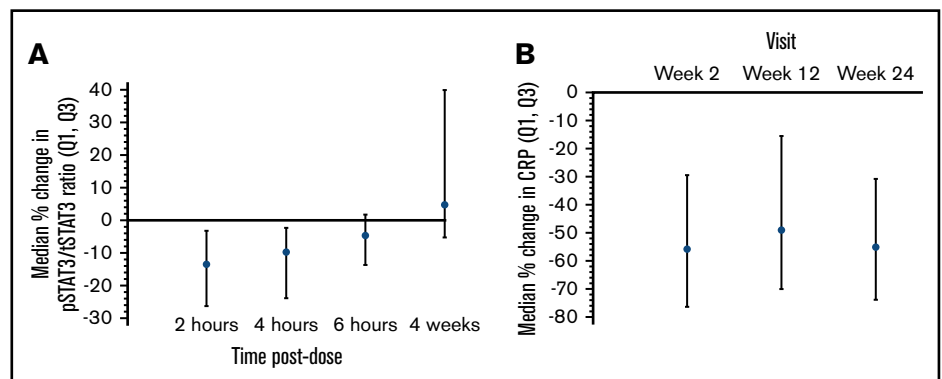

Figure 5. Inflammation markers post-MMB. (A) Median percentage change from baseline for pSTAT3/tSTAT3 ratio. (B) Median percentage change in CRP from baseline over time.
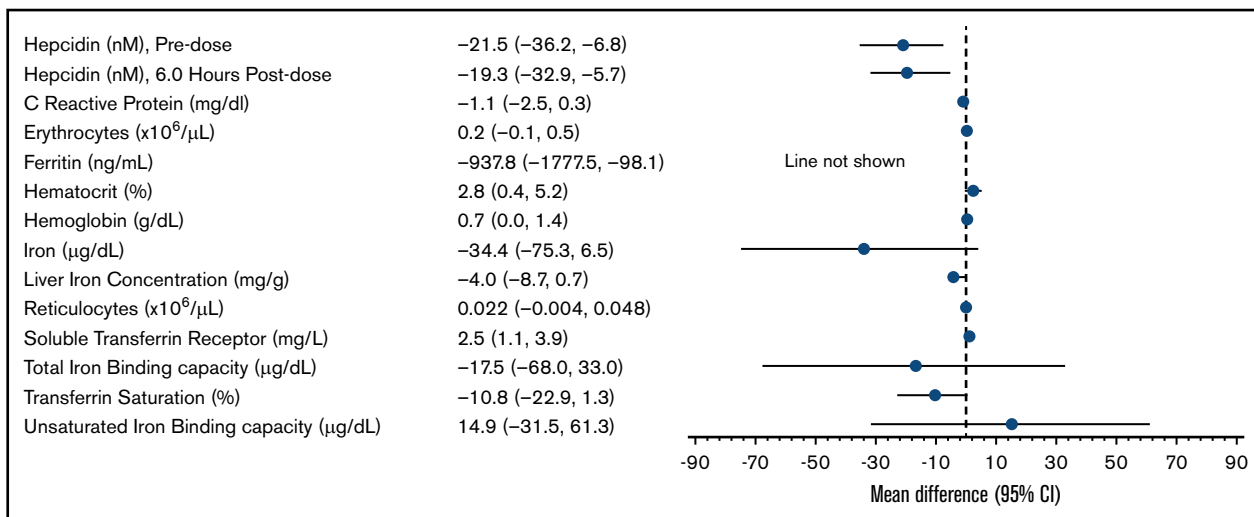
Hepcidin (nM), Pre-dose	−21.5 (−36.2, −6.8)		
Hepcidin (nM), 6.0 Hours Post-dose	−19.3 (−32.9, −5.7)		
C Reactive Protein (mg/dl)	−1.1 (−2.5, 0.3)		
Erythrocytes (x10^6/μL)	0.2 (−0.1, 0.5)		
Ferritin (ng/mL)	−937.8 (−1777.5, −98.1)	Line not shown	
Hematocrit (%)	2.8 (0.4, 5.2)		
Hemoglobin (g/dL)	0.7 (0.0, 1.4)		
Iron (μg/dL)	−34.4 (−75.3, 6.5)		
Liver Iron Concentration (mg/g)	−4.0 (−8.7, 0.7)		
Reticulocytes (x10^6/μL)	0.022 (−0.004, 0.048)		
Soluble Transferrin Receptor (mg/L)	2.5 (1.1, 3.9)		
Total Iron Binding capacity (μg/dL)	−17.5 (−68.0, 33.0)		
Transferrin Saturation (%)	−10.8 (−22.9, 1.3)		
Unsaturated Iron Binding capacity (μg/dL)	14.9 (−31.5, 61.3)		

Mean difference (95% CI)
-90 -70 -50 -30 -10 10 30 50 70 90

Figure 6. Baseline biomarkers in TI-R and TI-NR subgroups. Forest plot of baseline differences between subgroups; 95% CI is based on 2-sample Student *t* test. Positive values represent higher baseline values in the TI-R subgroup than in the TI-NR subgroup, and negative values are lower in the TI-R subgroup than in the TI-NR subgroup.

Together, these findings suggest that anemia of chronic inflammation plays a significant role in MF and that MMB can provide improvements in transfusion-dependent patients who have a greater residual erythropoietic potential by targeting this previously unaddressed driver of anemia. Despite the extremely poor prognosis for the targeted patient population based on the transfusion-dependency requirement at baseline, MMB treatment resulted in an appreciable TSS response and SRR of 29% and 19%, respectively, in patients with week-24 data. MMB safety, tolerability, and pharmacokinetics were consistent with earlier MMB studies. Thirty-nine percent of patients did not complete 24 weeks of therapy in this translational biology study, which may have reflected the high protocol complexity with a requirement for full-day clinic visits every 2 to 4 weeks throughout the duration in a study of advanced poor-prognosis patients in whom the mean age was 70 years. This lack of compliance highlights the challenge of conducting intensive pharmacodynamics biomarker studies in advanced MF.

In conclusion, treatment with MMB led to TI in a significant proportion of patients (TI-R subgroup) with advanced transfusion-dependent MF and a reduction in the need for transfusions in the TI-NR subgroup, similar to prior studies. These anemia benefits were observed in conjunction with decreased plasma hepcidin and improved iron homeostasis likely contributing to increased erythropoiesis, consistent with the differentiated activity of MMB against JAK1, JAK2, and ACVR1. Overall, the study suggests that modulation of hepcidin by MMB stimulates erythropoiesis to ameliorate transfusion-dependent anemia. MMB's anemia benefit and ACVR1 inhibition differentiate it from other JAK inhibitors, supporting its ability to address this important unmet need for patients with MF. Its anemia benefits will be evaluated further in MOMENTUM, an ongoing phase 3 clinical trial intended to support potential registration of MMB for the treatment of patients with MF who previously received JAK inhibitor. In addition to assessments of constitutional symptoms, landmark anemia rates (ie, TI), and splenomegaly, MOMENTUM will provide an opportunity to further evaluate the associations among anemia benefit, transfusion burden, and patient-reported measures of clinical benefit.

Authorship

Contribution: S.T.O., C.B.B., T.G., and Y.Z. designed the study; all authors participated in the acquisition, analysis, or interpretation of data; and S.T.O., Y.Z., J.K., T.G., and C.B.B. wrote the initial draft of the manuscript, which was subsequently reviewed, revised, and approved by all authors.

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