MDM2 antagonist idasanutlin in patients with polycythemia vera: results from a single-arm phase 2 study

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Abstract:
Idasanutlin, an MDM2 antagonist, showed clinical activity and rapid reduction in JAK2 V617F allele burden in patients with polycythemia vera (PV) in a phase 1 study. This open-label, phase 2 study evaluated idasanutlin in patients with hydroxyurea (HU)-resistant/intolerant PV, per the European LeukemiaNet criteria, and phlebotomy dependence; prior ruxolitinib exposure was permitted. Idasanutlin was administered once daily, days 1–5 of each 28-day cycle. The primary endpoint was composite response (hematocrit control and spleen volume reduction >35%) in patients with splenomegaly, and hematocrit control in patients without splenomegaly at week 32. Key secondary endpoints included safety, complete hematologic response (CHR), patient-reported outcomes, and molecular responses. All patients (n=27) received idasanutlin; 16 had response assessment (week 32). Among responders with baseline splenomegaly (n=13), 9 (69%) attained any spleen volume reduction and 1 achieved composite response. Nine patients (56%) achieved hematocrit control, and 8 patients (50%) achieved CHR. Overall, 43% of evaluable patients (n=6/14) showed a >50% reduction in the Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (week 32). Nausea (93%), diarrhea (78%), and vomiting (41%) were the most common adverse events, with grade 3 nausea and vomiting experienced in 3 patients (11%) and 1 patient (4%), respectively. Reduced JAK2 V617F allele burden occurred early (after 3 cycles), with a median reduction of 76%, and associated with achieving CHR and hematocrit control. Overall, the idasanutlin dosing regimen showed clinical activity and rapidly reduced JAK2 allele burden in patients with HU-resistant/intolerant PV but was associated with low-grade gastrointestinal toxicity, leading to poor long-term tolerability. Registration: NCT03287245.

Conflict of interest: COI declared - see note

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**Short title:** Idasanutlin in patients with polycythemia vera

**Scientific category:** Clinical trials and observations

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**Footnotes:**
Presented in abstract form at the 62nd Annual meeting of the American Society of Hematology1,2
Key points:

- Idasanutlin showed clinical activity in patients with HU-resistant/intolerant PV, but chronic toxicity led to a high discontinuation rate.
- Significant reductions in JAK2 allele burden occurred after 3 treatment cycles and were greatest in patients with clinical response.

Visual abstract
Abstract

Idasanutlin, an MDM2 antagonist, showed clinical activity and rapid reduction in JAK2 V617F allele burden in patients with polycythemia vera (PV) in a phase 1 study. This open-label, phase 2 study evaluated idasanutlin in patients with hydroxyurea (HU)-resistant/intolerant PV, per the European LeukemiaNet criteria, and phlebotomy dependence; prior ruxolitinib exposure was permitted. Idasanutlin was administered once daily, days 1-5 of each 28-day cycle. The primary endpoint was composite response (hematocrit control and spleen volume reduction >35%) in patients with splenomegaly, and hematocrit control in patients without splenomegaly at week 32. Key secondary endpoints included safety, complete hematologic response (CHR), patient-reported outcomes, and molecular responses. All patients (n=27) received idasanutlin; 16 had response assessment (week 32). Among responders with baseline splenomegaly (n=13), 9 (69%) attained any spleen volume reduction and 1 achieved composite response. Nine patients (56%) achieved hematocrit control, and 8 patients (50%) achieved CHR. Overall, 43% of evaluable patients (n=6/14) showed a ≥50% reduction in the Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (week 32). Nausea (93%), diarrhea (78%), and vomiting (41%) were the most common adverse events, with grade ≥3 nausea and vomiting experienced in 3 patients (11%) and 1 patient (4%), respectively. Reduced JAK2 V617F allele burden occurred early (after 3 cycles), with a median reduction of 76%, and associated with achieving CHR and hematocrit control. Overall, the idasanutlin dosing regimen showed clinical activity and rapidly reduced JAK2 allele burden in patients with HU-resistant/intolerant PV but was associated with low-grade gastrointestinal toxicity, leading to poor long-term tolerability. Registration: NCT03287245.
Introduction

Polycythemia vera (PV) is a BCR-ABL1− chronic myeloproliferative neoplasm (MPN) characterized by the near-universal presence of an acquired mutation in JAK2 (JAK2 V617F), with a resultant increase in blood cell production, a heightened risk of thrombosis and, in some patients, progression to post-PV myelofibrosis or acute myeloid leukemia (AML).3-6 About 30% to 40% of patients with PV present with splenomegaly,7,8 and most experience significant constitutional symptoms, adversely affecting quality of life (QoL).9 Median overall survival (OS) in patients with PV (≈16 years) is longer than in patients with other cancers. Early deaths, primarily driven by cardiovascular events and progression to myelofibrosis or AML, occur in ≈5% of patients.5,10 New therapeutic strategies for PV need not only to reduce thrombotic risk and improve constitutional symptoms, but also to modify the natural history of PV and prevent disease progression.

The E3 ubiquitin ligase MDM2 targets tumor suppressor p53 for degradation.11 Abnormal MDM2 upregulation through gene amplification, increased transcription, and translation has been observed in some cancers, resulting in increased p53 degradation.12 Thus, inhibition of the p53-MDM2 interaction to increase functional p53 protein levels is an appealing treatment strategy in cancers without inactivating mutations in TP53.11 Idasanutlin is a potent, small-molecule MDM2 antagonist that disrupts the p53-MDM2 interaction and has shown clinical activity in patients with AML in a phase 1 study.13

MDM2 expression is higher in patients with PV than in healthy individuals.14 Preclinical studies have demonstrated a potential role for idasanutlin in the treatment of PV through enhancement of p53 activity and downstream mediators of this pathway, resulting in depletion of JAK2 V617F MPN cells.14 In a phase 1 study evaluating idasanutlin in patients with high-risk JAK2 V617F+ PV or essential thrombocythemia, promising on-target clinical activity and rapid reduction of the JAK2 V617F variant allele frequency (VAF) was observed in 9 of 12 patients who received treatment.15 Encouraging results from this study prompted this larger, international phase 2 clinical trial exploring the effect of idasanutlin monotherapy in patients with hydroxyurea (HU)-resistant/intolerant PV, reported herein.
Methods

Study design and participants

This open-label, single-arm, non-randomized, phase 2 study (NCT03287245; NP39761) investigating the efficacy, safety, pharmacokinetics (PK) and pharmacodynamics of single-agent idasanutlin in patients with HU-resistant/intolerant PV (Figure S1) was conducted across 9 sites in Canada, Europe, Australia, and the United States.

Eligible patients were ≥18 years of age, met the 2016 World Health Organization criteria for the diagnosis of PV (Supplemental methods), and had an Eastern Cooperative Oncology Group performance status of 0-1.16 Phlebotomy dependence, defined as ≥1 phlebotomy within 16 weeks prior to screening, and hematocrit >40% at screening were required. Patients could have splenomegaly (spleen volume ≥450 cm³), no splenomegaly (spleen volume <450 cm³), or prior splenectomy. HU resistance, intolerance, or both was required according to the 2010 European LeukemiaNet (ELN) consensus criteria;17 patients could have received initial cytoreductive therapy due to an increased risk of thrombosis or to treat disease-related symptoms.18 Consistent with the ELN criteria, resistance to HU was defined as failure to maintain hematocrit <45%, control myeloproliferation, or reduce massive splenomegaly by >50% with an HU dose of ≥2 g/day or a maximum tolerated dose of <2 g/day.17,19 Intolerance of HU was defined as the presence of unacceptable toxicities, such as hematotoxicity, or non-hematologic toxicities, like leg ulcers or other mucocutaneous toxicities.17,19 Patients were enrolled regardless of prior ruxolitinib or interferon-α exposure. Patients previously exposed to ruxolitinib were required to have treatment-resistant disease after ≥6 months of ruxolitinib therapy or ruxolitinib intolerance. Resistance to ruxolitinib was defined by the occurrence of ≥1 of the following:20 the need for ≥2 phlebotomies over a period of 6 months, to achieve hematocrit <45%; (2) uncontrolled leukocytosis (white blood cell count >10 x 10⁹/L; (3) uncontrolled thrombocytosis (platelet count >400 x 10⁹/L; (4) failure to achieve a >50% reduction in palpable splenomegaly measuring >5 cm from the left costal margin, or failure to become non-palpable in palpable splenomegaly measuring 0-5 cm; and (5) inadequately controlled disease-related symptoms (e.g., pruritus, headache, night sweats, and excluding fatigue) after excluding other causes. Ruxolitinib intolerance was defined as the occurrence of ≥1 of the following at the lowest ruxolitinib dose required for adequate response:20 cytopenia, defined as neutopenia (absolute neutrophil count <1.0 x 10⁹/L, and/or thrombocytopenia (platelet count <100 x 10⁹/L) and/or anemia (hemoglobin...
<10 g/dL; (2) life-threatening or other infections (shingles, tuberculosis, or hepatitis reactivation) considered to be associated with ruxolitinib at any time during study treatment; and (3) recurrent or multiple non-melanoma skin cancer at any time during study treatment.

Patients who met the International Working Group-Myeloproliferative Neoplasms Research and Treatment criteria for post-PV myelofibrosis were excluded from the study. Other exclusion criteria included blast phase disease (>20% blasts in the marrow or peripheral blood), or clinically significant thrombosis ≤3 months before screening. Patients who received HU ≤1 day, or prior treatment with MDM2 antagonists, interferon-α, anagrelide, ruxolitinib or other cytoreductive or investigational agents ≤28 days (or 5 half-lives whichever was shorter) of initial dose were also excluded.

The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice guidelines. Ethics approval was obtained from the independent ethics committees and institutional review boards of each participating site before trial initiation. All patients provided informed consent prior to trial participation, in accordance with the principles of the Declaration of Helsinki.

Treatment

Idasanutlin was orally administered once daily on days (D) 1 through 5, followed by a treatment-free period of 23 days, in a 28-day treatment cycle, for up to 2 years (24 treatment cycles) (Figure S1). Based on association with hematologic response in patients with PV in a previous phase 1 study, the starting dose of idasanutlin was 150 mg/day, with dose reduction to 100 mg/day permitted in cases of toxicity or in patients showing response at cycle (C) 13, to allow long-term tolerability assessment. Intra-patient dose escalation to the maximum-allowed dose of 200 mg/day for 5 days was permitted after C3 but before C6 in patients demonstrating no hematocrit control and/or patients with inadequately controlled leukocytosis and/or thrombocytosis.

To mitigate gastrointestinal toxicities such as nausea and vomiting, antiemetic prophylaxis consisting of a minimum of oral dexamethasone and a 5HT3 antagonist was mandatory on treatment days during C1. Subsequent protocol amendment made this treatment mandatory in all treatment cycles, unless otherwise decided by the investigator and sponsor. Antidiarrheal
therapy was recommended as secondary prophylaxis for all patients who manifested grade ≥2 diarrhea during a previous treatment cycle.

Endpoints

Primary efficacy endpoints in patients with ruxolitinib-naive PV were composite response in patients with baseline splenomegaly (hematocrit control and ≥35% reduction in spleen volume), hematocrit control in patients without baseline splenomegaly, and hematocrit control in all patients (with and without baseline splenomegaly) at week 32, defined as protocol-specified ineligibility for therapeutic phlebotomy between weeks 8 and 32 and ≤1 instance of phlebotomy eligibility between the first dose and week 8. The primary efficacy endpoint in patients with ruxolitinib-resistant/intolerant PV was hematocrit control, as assessed by the investigator.

Secondary endpoints included safety, where incidence, nature, and severity of adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events v4.0. Other secondary endpoints were complete hematologic response (CHR, defined as hematocrit control, a white blood cell count of ≤10×10⁹/L, and a platelet count of ≤400×10⁹/L), response by a modified version of the ELN hematologic response criteria for PV (Table S1), and mean change from baseline in patient-reported clinical outcome assessments (COA). Exploratory endpoints included correlation between PK exposure and clinical responses, percentage change from baseline in serum macrophage inhibitory cytokine 1 (MIC-1) profile (indicator of p53 pathway activation), and molecular response evaluation by reduction of JAK2 V617F VAF.

Response assessments were performed at C3D28, C5D28, and C8D28 (week 32). After week 32, response assessments were performed every 3 cycles.

Assessments and procedures

A bone marrow biopsy was performed to exclude the presence of post-PV myelofibrosis during screening and prior to the first treatment cycle. Baseline spleen volume was assessed by magnetic resonance imaging or computed tomography imaging. In patients with splenomegaly at baseline, spleen volume was reassessed by imaging at C3D28, C5D28, and week 32 and every 3 months thereafter. BM biopsy was repeated at week 32, but subsequent evaluations
were at the discretion of the investigator and only upon complete remission when assessed at week 32.

Patient-reported COAs were measured using the Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS),\textsuperscript{24-26} the European Organization for Research and Treatment of Cancer QoL Questionnaire–Core 30 (EORTC QLQ-C30),\textsuperscript{21,27} and the Patient Global Impression of Change (PGIC)\textsuperscript{28} scales. Further details are outlined in the Supplemental methods.

Plasma samples for PK analyses and serum samples to measure the MIC-1 levels were collected at prespecified time points (Supplemental methods). PK samples collected for this study were included in a population PK analysis and were analyzed in combination with PK data obtained from patients with AML treated with idasanutlin in the spray-dried powder formulation.\textsuperscript{13} Individual PK parameter estimates were subsequently used to explore exposure-response relationships to MIC-1 induction in patients with PV.

\textit{JAK2 V617F VAF} was analyzed using qPCR at screening, at the end of C3 and C5, and at week 32. Blood for centralized genetic testing was obtained during screening; however, results were not required before treatment was started. Targeted sequencing of the baseline blood samples for genetic markers was performed using the FoundationOne Heme next-generation sequencing panel (Foundation Medicine Inc, Cambridge, MA) to explore the genetic landscape of PV beyond \textit{JAK2 V617F} and other genes relevant to myeloid diseases.\textsuperscript{29,30}

**Statistical analysis**

No formal hypothesis testing was performed for the study efficacy endpoints. Descriptive statistical analyses were performed for all study outcomes. Summary statistics of absolute scores were calculated for all scales of the MPN-SAF TSS, EORTC QLQ-C30, and PGIC at each assessment time point. Non-compartmental PK analysis was performed using WinNonlin (v5.2 or higher; Certara, Princeton, NJ). Biomarker analyses were summarized using descriptive statistics and \textit{P} value using Mood's Median Test. SAS software (version 9.4; Cary, NC) was used for the biomarker analyses.
Results

Patients

Between February 2018 and March 2020, 27 patients were enrolled (abridged by early study termination), including 20 ruxolitinib-naive and 7 ruxolitinib-resistant/intolerant patients. Baseline characteristics of all patients are shown in Table 1. The median age was 56 years (range, 34-74) and 59% of patients were male. In addition to prior HU treatment, 5 patients had received prior interferon-α therapy and 1 patient had received both ruxolitinib and interferon-α. Baseline splenomegaly was present in 21 patients (78%), with a median spleen volume of 800.0 cm$^3$ (range, 513.0-2602.4). Two patients (7%) had prior thrombotic events.

Safety

At the clinical cut-off date of June 3, 2020, the median duration of follow-up for study treatment was 41.3 weeks (range, 5.7-100.1), and the median number of treatment cycles was 8 (range, 1-22). All patients were evaluable for safety and had ≥1 treatment-emergent AEs of any grade (Table 2; Table S2). A total of 536 AEs were reported in all patients, with gastrointestinal disorders being the most frequently occurring AE (251 of 536). The most commonly reported any-grade treatment-emergent AEs were nausea (n=25 [93%]), diarrhea (n=21 [78%]), vomiting (n=11 [41%]), and fatigue (n=10 [37%]) (Table 2).

The majority of patients (n=17 [63%]) reported AEs of a maximum severity of grade 2. Nine patients (33%) experienced a total of 12 grade 3 AEs, with the most common being nausea (n=3 [11%]) and fatigue (n=2 [7%]). One patient (n=1 [4%]) experienced a grade 4 atrial fibrillation. Four serious AEs (SAEs) were reported in 3 patients (11%); these events were grade 4 atrial fibrillation in 1 patient (4%), 2 events of grade 3 atrial flutter in 1 patient (4%), and grade 3 nausea in 1 patient (4%). All SAEs resolved and, except for the atrial flutter, were determined to be idasanutlin-related. No deaths, transformation to blast phase, progression to post-PV myelofibrosis, or thrombotic events occurred.

Idasanutlin dosage was increased to 200 mg in 2 patients who showed no response at the end of C5. This was later reduced to 150 mg due to nausea/vomiting (C5) or migraine (C8). Dosage
was reduced to 100 mg in 10 patients, predominantly due to persisting grade 1-2 toxicity (nausea; n=5 [19%]). A total of 43 treatment interruptions occurred in 13 patients, most commonly due to grade 1-3 nausea (n=6 [22%]) (Figure S2). Treatment was discontinued early (before week 32) in 11 patients (41%). Overall reasons for discontinuation were patient decision (n=14 [52%]); AEs (n=1 [4%]), investigator decision (n=5 [19%]), and premature study termination by sponsor (n=7 [26%]). Investigation into the high rate of early study discontinuation revealed low-grade gastrointestinal toxicity as a significant factor influencing patient decision to discontinue treatment.

Efficacy

Primary endpoint analysis was performed in patients with a response assessment at week 32 (n=16). Of the evaluable patients with baseline splenomegaly (n=13), one (8%) achieved a composite response at week 32 (Table 3). Of 16 evaluable patients, 9 (56%; 6/11 [55%] ruxolitinib-naive and 3/5 [60%] ruxolitinib-exposed) achieved hematocrit control (Figure 1). Of 13 patients with ≥12 weeks’ follow-up after week 32, 8 had hematocrit control of ≥12 weeks’ duration (62%; 5/9 [56%] ruxolitinib naive and 3/4 [75%] ruxolitinib exposed). At week 32, 8 of 16 evaluable patients (50%) had achieved a CHR. Of 13 patients with ≥12 weeks’ follow-up after week 32, 6 (46%) had a CHR for ≥12 weeks. The overall response rate at week 32 per modified ELN response criteria was 69% (11/16); 69% in patients with baseline splenomegaly (9/13) and 67% in patients without baseline splenomegaly (2/3). Of these patients, 60% (9/15) had a response duration ≥12 weeks beyond week 32.

Idasanutlin treatment resulted in a reduction in spleen volume at any time in 22 of 24 (92%) evaluable patients with baseline splenomegaly; however, only 2 patients attained a >35% reduction in spleen volume at week 32 (Figure 2). The median reduction in spleen volume at week 32 was −7% (range, −60% to +16%), with any degree of reduction observed in 9 of 13 evaluable patients (69%) at week 32.

Median change from baseline at week 32 was −253×10⁹/L (range, −2083 to +175) for platelet levels and −4.8×10⁹/L (range, −25.3 to 3.4) for leukocyte levels (Figure S3). Of the 15 patients with BM evaluation at week 32, two attained histological remission with corresponding hematocrit control, CHR, and PR per modified ELN response (Table 3).
Patient-reported outcomes

Based on a median score of 13.0 (IQR, 5.0 to 37.0) at week 32 for the patient-reported MPN-SAF TSS instrument, the median change from baseline at week 32 was −5.0 (IQR, −12.0 to 0), and the median percentage change from baseline was −25.4 (IQR, −62.5 to −5.1) (Figure 3), with negative scores indicating improvement. However, these changes were not considerable, as evident from the IQR values either approaching or crossing 0 in median change from baseline at each visit (Figure 3A). At week 32, 43% of patients (6/14) had a ≥50% reduction in the TSS (Figure 3C), and 48% of patients had a ≥50% reduction in the TSS at any time point (Table 3). The majority of patients did not experience a large improvement in symptoms during the course of treatment. Patient-reported outcomes per the EORTC QLQ-C30 and PGIC are summarized in the Supplemental results (Figures S4 and S5).

Exploratory exposure-response analyses

Pharmacokinetic properties of idasanutlin in patients with PV were consistent with those in patients with AML and solid tumors, as assessed by a population PK model.\textsuperscript{13} The PK of idasanutlin was linear with dose ($C_{\text{max}}$, and average concentration over 5 days).

Despite some inter-patient variability, a dose-exposure MIC-1–related increase was observed in patients with PV, consistent with previous findings in patients with AML and solid tumors showing that MIC-1 release and idasanutlin exposure on C1D5 were directly proportional (Figure S6).

Molecular response patterns

All patients carried the $JAK2$ V617F mutation, with a median baseline VAF of 66% (range, 7%-96%) (Figure 4; Table 1). No patients had mutations in TP53 at baseline.

A reduction in $JAK2$ V617F VAF was observed as early as the end of C3 (Figure 4), with a median reduction of 39% (n=19). This reduction was sustained at later time points, with median reductions of 58% at the end of C5 (n=17) and 76% at week 32 (n=13). Median reductions in $JAK2$ V617F VAF were significantly higher in patients with CHR and hematocrit control than non-responders on C3D28 (CHR, $P<.01$; hematocrit control, $P=.04$) and C5D28 (CHR, $P<.01$;
hematocrit control, \( P = .03 \), with a similar trend at week 32 (CHR, \( P = .06 \); hematocrit control, \( P = .19 \)) (Figure 4). Change from baseline in \( JAK2 \ V617F \) VAF at C2D1, C3D1, C3D28, C5D28, and week 32 are shown in Figure S7.

**Somatic mutations**

Several mutations were detected by targeted sequencing (26 patient samples) in myeloid-associated and DNA repair genes at baseline (Figure S8). Among the myeloid-associated genes, mutational variants were detected in \( CHEK2 \) and \( TET2 \) in 3 patients (12%) and in \( ASXL1 \) in 2 patients (8%). A VAF of \( \geq 50\% \) was observed in \( ATM \) (n=3; range, 48%-52%), \( BRCA2 \) (n=3; range, 47%-51%), \( PARP1 \) (n=3; range, 52%), \( FANCM \) (n=2; range, 47%-50%), \( MSH3 \) (n=2; range, 49%), \( ATR \) (n=1; range, 49%), \( BLM \) (n=1; range, 48%) and \( PARP4 \) (n=1; range, 49%) genes. However, final confirmation of germline status of these variants was not possible because non-hematopoietic tissue was unavailable for sequencing in this study.

**Discussion**

In this phase 2 study, we observed response per modified ELN criteria in 69% of patients (n=9), and a reduction in spleen volume of >35% in 7% of patients (n=2) at week 32. Of evaluable patients with baseline splenomegaly, 8% of patients (n=1) achieved composite response with idasanutlin at week 32, and 56% (n=9) achieved hematocrit control, including 55% of ruxolitinib-naive patients (n=6) and 60% of ruxolitinib-exposed patients (n=3). A rapid reduction in \( JAK2 \ V617F \) VAF was observed (median reduction, 39%), with greater reduction seen in patients who achieved CHR and hematocrit control vs non-responders, suggesting an association between early molecular response and a higher probability of clinical efficacy. BM histopathological remission at week 32 was also seen in 2 treated patients, further supporting the disease-modifying potential of this agent. However, low-grade gastrointestinal toxicities, limited to the period of study drug administration, frequently led to treatment discontinuation.

The predominant first-line treatment in patients with low-risk PV is therapeutic phlebotomy; in patients with high-risk PV, a cytoreductive agent is added, usually HU or interferon-\( \alpha \). In patients receiving HU, 11% develop resistance and 13% are intolerant due to toxicities. Therapeutic phlebotomy presents challenges, including iron deficiency, fatigue, and intolerance.
in some patients. Although interferon-α has demonstrated clinical efficacy and significant JAK2 allele burden reduction in the first- and second-line settings over prolonged periods of administration, 25% to 40% of patients discontinue treatment due to toxicities. The potent JAK2 inhibitor ruxolitinib has shown meaningful clinical benefit in patients intolerant of or refractory to HU; 21% of patients with imaging-defined splenomegaly achieved composite response and 60% achieved hematocrit control at week 32 in the RESPONSE study.

However, long-term use of ruxolitinib can be complicated by increased risk of infections and skin malignancies. Therefore, there remains an unmet need for a tolerable second-line treatment in PV that facilitates durable hematocrit control and can modify disease course to reduce the risk of progression to myelofibrosis or transformation to AML.

Maintenance of hematocrit <45% is an established PV treatment outcome and linked to a reduced risk of thrombosis. Hematocrit control and composite response at week 32 were thus chosen as the primary efficacy endpoints of this study, aligning with similar studies like those evaluating ruxolitinib in PV, with splenomegaly (RESPONSE-1) and without splenomegaly (RESPONSE-2). The proportion of patients achieving hematocrit control with idasanutlin in this study (56%) was similar to that in RESPONSE-1 (60%) and RESPONSE-2 (62%). Responses with idasanutlin were durable, with 62% (8/13) patients having hematocrit control ≥12 weeks. However, follow-up in our trial was relatively short for a PV study, with a median follow-up of 48.3 weeks. Durable responses were also seen with ruxolitinib, reported in a recent 5-year follow-up of the RESPONSE-1 trial, where the probabilities of maintaining primary composite response and CHR were 74% (95% CI, 51%-88%) and 55% (95% CI, 32%-73%), respectively. However, ruxolitinib requires continuous daily treatment, and durability may be due to the myelosuppressive effects rather than true disease modification in light of lower molecular responses reported. Further highlighting the importance of maintaining response in PV, the incidence of thromboembolic events was numerically lower with long-term follow-up in patients assigned to ruxolitinib. Overall response rate per ELN criteria was a secondary endpoint in this idasanutlin study, with 69% of patients attaining a response, comparable to 60% reported with pegylated interferon-α-2a.

Improving QoL and reducing symptom burden is an important consideration in the development of therapies for PV. Idasanutlin treatment showed a trend toward improvement in patient-reported outcomes, with a ≥50% reduction in symptom burden per MPN-SAF TSS experienced by 43% of patients at week 32. The corresponding proportion of ruxolitinib-treated patients in
RESPONSE-1 was 49%,\textsuperscript{36} whereas it was only 5% in the best-available-therapy arm (including HU or interferon-α). However, the findings in our study should be interpreted with caution due to the small size of the study population and the change in IQR values approaching or crossing 0.

Despite durable clinical responses, a rapid reduction in the \textit{JAK2} V617F VAF, and BM histopathological remission in some patients, gastrointestinal toxicities led to treatment discontinuation in >50% of patients, highlighting the importance of tolerability in therapies for chronic diseases such as PV. Notably, no significant improvement or deterioration in patient-reported outcomes in the EORTC QLQ-C30, which include gastrointestinal symptom scales, was reported (Supplemental results). In contrast, interferon treatment, typically associated with more chronic toxicity than HU, was discontinued by only 8% of patients in the 12-month phase 3 PROUD-PV trial\textsuperscript{44} and by 13.9% receiving pegylated interferon in the phase 2 Myeloproliferative Disorders Research Consortium 111 trial\textsuperscript{43} at 12 months. Longer follow-up would have been required to determine if idasanutlin-related toxicity subsided, at least partially, over time, which is the clinical experience with interferon-α toxicity.\textsuperscript{45} Unfortunately, antiemetic prophylactic treatment (dexamethasone and 5HT3 antagonists) failed to mitigate the gastrointestinal toxic effects, suggesting that the nausea associated with idasanutlin may interfere with other pathways than the ones typically leading to nausea in patients treated with cytostatic therapies.

Remarkably, a rapid reduction in \textit{JAK2} VAF was seen in some patients as early as at the end of 3 treatment cycles, which correlated with clinical response (90.9% median reduction at 32 weeks in CHR responders vs 49% in non-responders). The median reduction in \textit{JAK2} VAF of 76% at 32 weeks compares favorably to the \textasciitilde40% maximum mean reduction in \textit{JAK2} VAF reported with interferon-α and ruxolitinib after longer treatment durations (12 months\textsuperscript{45} and 36 months\textsuperscript{36}). Studies of HU treatment have not shown consistent reductions in \textit{JAK2} VAF.\textsuperscript{46, 47} Reduction in \textit{JAK2} VAF may, from a conceptual standpoint, lead to improved clinical outcomes because a high \textit{JAK2} VAF indicates a greater risk of disease progression and thrombosis in patients with PV.\textsuperscript{48, 49} However, the clinical value of attaining a \textit{JAK2} VAF reduction and the association with risk of thrombosis and transformation have never been confirmed in clinical trials.

Idasanutlin, as an MDM2 inhibitor, had the potential to add a new mechanism of action to therapies for PV. MDM2 inhibition leads to p53 stabilization, which is a key player in cellular...
stress response.\textsuperscript{50} The preclinical mode of action data were further supported by the fact that response to idasanutlin was absent in a patient with an inactivating \textit{TP53} mutation in the prior phase 1 study of idasanutlin in patients with HU-resistant/intolerant PV, highlighting the importance of screening for mutations in the \textit{TP53} gene.\textsuperscript{15} A subsequent analysis of the same phase 1 data found that in 5 of the 12 patients treated with idasanutlin, an expansion of 12 \textit{TP53}-mutated clones was observed during therapy.\textsuperscript{15, 51} Interestingly, the \textit{TP53} mutations were not induced by idasanutlin, as the mutations could be identified prior to start of idasanutlin therapy but at levels that are below the conventional detection threshold.\textsuperscript{51} In 8 of 9 cases, VAF reduced spontaneously after therapy cessation and with no cases of disease progression to AML or MF noted at 32 months of observation. Nevertheless, expanding \textit{TP53} clones would be a concern given the correlation between \textit{TP53} clones and transformation or progression in PV, as well as the therapy-resistance associated with \textit{TP53} mutations in secondary AML.\textsuperscript{52, 53}

Despite promising results in the previous phase 1 study, the benefit-risk profile of idasanutlin treatment in this phase 2 study was compromised by the high treatment-discontinuation rate, owing to treatment-related low-grade gastrointestinal toxicities. The efficacy and safety of another MDM2 antagonist, KRT-232, is currently being compared with ruxolitinib in a phase 2 trial in patients with phlebotomy-dependent PV (NCT03669965).\textsuperscript{54} More individualized dosing regimens could be a solution to the chronic toxicity; allowing each patient to receive the minimally active dose either alone or in combination with other active agents could allow patients to have longer periods off treatment earlier in treatment—when many patients dropped out. As it is unclear whether modification of idasanutlin dosing would substantially improve its toxicity profile, there are no current plans to further explore idasanutlin treatment in patients with PV. The early molecular response seen with idasanutlin therapy is promising, confirms that the MDM2 pathway is important in the pathogenesis of PV, and deserves further clinical evaluation.

\textbf{Data sharing}

Qualified researchers may request access to individual patient level data through the clinical study data request platform (https://vivli.org/). Further details on Roche’s criteria for eligible studies are available here (https://vivli.org/members/ourmembers/). For further details on Roche’s Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see here
(https://www.roche.com/research_and_development/who_we_are/how_we_work/clinical_trials/our_commitment_to_data_sharing.htm).

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Authorship contributions

T. C. E-G, B.H., C.J., B.K., and R.M. conceptualized the study; T. C. E-G, B.H., and B.K. developed the methodology; J.M., K.B., T. C. E-G, B.H., B.K., S.K., and A.Y. validated the experiments and research outputs; J.M., C.J., B.K., S.K., R.M., and A.Y. analyzed the data; J.M., K.B., A.G., B.K., M.M., R.M., A.M.V., and A.Y. conducted the experiments; J.M., T. C. E-G, V.G., B.H., A.M.V., and, A.Y. provided resources; J.M., T. C. E-G, A.G., B.H., and A.Y. curated the data; J.M., T. C. E-G, A.G., B.H., C.J., B.K., and A.Y. visualized the data; J.M., K.B., A.G., B.K., and A.Y. supervised the study; T. C. E-G, A.G., and B.H. coordinated the research activities. All authors contributed to manuscript writing and provided final approval of the manuscript.

Conflict of interest disclosures

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References

1. Mascarenhas J, Higgins B, Anders D, et al. Safety and Efficacy of Idasanutlin in Patients (pts) with Hydroxyurea (HU)-Resistant/Intolerant Polycythemia Vera (PV): Results of an International Phase II Study. Blood. 2020;136(Supplement 1):29-31. doi:10.1182/blood-2020-135868
2. Passamonti F, Burbury K, El-Galaly TC, et al. Molecular Response Patterns in Hydroxyurea (HU)-Resistant or Intolerant Polycythemia Vera (PV) during Treatment with Idasanutlin: Results of an Open-Label, Single-Arm Phase 2 Study. Blood. 2020;136(Supplement 1):38-40. doi:10.1182/blood-2020-136135
3. Griesshammer M, Sadjadian P. The BCR-ABL1-negative myeloproliferative neoplasms: a review of JAK inhibitors in the therapeutic armamentarium. Expert Opin Pharmacother. 2017;18(18):1929-1938. doi:10.1080/14656566.2017.1404574
4. Pardanani A, Lasho TL, Finke C, Hanson CA, Tefferi A. Prevalence and clinicopathologic correlates of JAK2 exon 12 mutations in JAK2V617F-negative polycythemia vera. *Leukemia*. 2007;21(9):1960-3. doi:10.1038/sj.leu.2404810

5. Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2021 update on diagnosis, risk-stratification and management. *Am J Hematol*. 2020;95(12):1599-1613. doi:10.1002/ajh.26008

6. Vannucchi AM, Antonioli E, Guglielmelli P, Pardanani A, Tefferi A. Clinical correlates of JAK2V617F presence or allele burden in myeloproliferative neoplasms: a critical reappraisal. *Leukemia*. 2008;22(7):1299-307. doi:10.1038/leu.2008.113

7. Accurso V, Santoro M, Raso S, et al. Splenomegaly impacts prognosis in essential thrombocythemia and polycythemia vera: A single center study. *Hematol Rep*. 2019;11(4):8281. doi:10.4081/hr.2019.8281

8. Radia D, Geyer HL. Management of symptoms in polycythemia vera and essential thrombocythemia patients. *Hematology Am Soc Hematol Educ Program*. 2015;2015:340-8. doi:10.1182/asheducation-2015.1.340

9. Mesa RA, Schwager S, Radia D, et al. The Myelofibrosis Symptom Assessment Form (MFSAF): an evidence-based brief inventory to measure quality of life and symptomatic response to treatment in myelofibrosis. *Leuk Res*. 2009;33(9):1199-203. doi:10.1016/j.leukres.2009.01.035

10. Tefferi A, Rumi E, Finazzi G, et al. Survival and prognosis among 1545 patients with contemporary polycythemia vera: an international study. *Leukemia*. 2013;27(9):1874-81. doi:10.1038/leu.2013.163

11. Konopleva M, Martinelli G, Daver N, et al. MDM2 inhibition: an important step forward in cancer therapy. *Leukemia*. 2020;34(11):2858-2874. doi:10.1038/s41375-020-0949-z

12. Momand J, Jung D, Wilczynski S, Niland J. The MDM2 gene amplification database. *Nucleic Acids Res*. 1998;26(15):3453-9. doi:10.1093/nar/26.15.3453

13. Yee K, Papayannidis C, Vey N, et al. Murine double minute 2 inhibition alone or with cytarabine in acute myeloid leukemia: Results from an idasanutlin phase 1/1b study small star, filled. *Leuk Res*. 2021;100:106489. doi:10.1016/j.leukres.2020.106489

14. Lu M, Wang X, Li Y, et al. Combination treatment in vitro with Nutlin, a small-molecule antagonist of MDM2, and pegylated interferon-alpha 2a specifically targets JAK2V617F-positive polycythemia vera cells. *Blood*. 2012;120(15):3098-105. doi:10.1182/blood-2012-02-410712

15. Mascarenhas J, Lu M, Kosiorek H, et al. Oral idasanutlin in patients with polycythemia vera. *Blood*. 2019;134(6):525-533. doi:10.1182/blood.2018893545
16. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood.* 2016;127(20):2391-405. doi:10.1182/blood-2016-03-643544

17. Barosi G, Birgegard G, Finazzi G, et al. A unified definition of clinical resistance and intolerance to hydroxycarbamide in polycythaemia vera and primary myelofibrosis: results of a European LeukemiaNet (ELN) consensus process. *Br J Haematol.* 2010;148(6):961-3. doi:10.1111/j.1365-2141.2009.08019.x

18. Barbui T, Barosi G, Birgegard G, et al. Philadelphia-negative classical myeloproliferative neoplasms: critical concepts and management recommendations from European LeukemiaNet. *J Clin Oncol.* 2011;29(6):761-70. doi:10.1200/JCO.2010.31.8436

19. Barosi G, Birgegard G, Finazzi G, et al. Response criteria for essential thrombocythemia and polycythemia vera: result of a European LeukemiaNet consensus conference. *Blood.* 2009;113(20):4829-33. doi:10.1182/blood-2008-09-176818

20. Felip E, Altorki N, Zhou C, et al. Adjuvant atezolizumab after adjuvant chemotherapy in resected stage IB-IIIA non-small-cell lung cancer (IMpower010): a randomised, multicentre, open-label, phase 3 trial. *Lancet.* 2021;398(10308):1344-1357. doi:10.1016/S0140-6736(21)02098-5

21. Aaronson NK, Ahmedzai S, Bergman B, et al. The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. *J Natl Cancer Inst.* 1993;85(5):365-76. doi:10.1093/jnci/85.5.365

22. Barosi G, Mesa R, Finazzi G, et al. Revised response criteria for polycythemia vera and essential thrombocythemia: an ELN and IWG-MRT consensus project. *Blood.* 2013;121(23):4778-81. doi:10.1182/blood-2013-01-478891

23. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature.* 2014;515(7528):563-7. doi:10.1038/nature14011

24. Abelsson J, Andreasson B, Samuelsson J, et al. Patients with polycythemia vera have worst impairment of quality of life among patients with newly diagnosed myeloproliferative neoplasms. *Leuk Lymphoma.* 2013;54(10):2226-30. doi:10.3109/10428194.2013.766732

25. Emanuel RM, Dueck AC, Geyer HL, et al. Myeloproliferative neoplasm (MPN) symptom assessment form total symptom score: prospective international assessment of an abbreviated symptom burden scoring system among patients with MPNs. *J Clin Oncol.* 2012;30(33):4098-103. doi:10.1200/JCO.2012.42.3863
26. Scherber R, Dueck AC, Johansson P, et al. The Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF): international prospective validation and reliability trial in 402 patients. *Blood*. 2011;118(2):401-8. doi:10.1182/blood-2011-01-328955

27. Fitzsimmons D, Johnson CD, George S, et al. Development of a disease specific quality of life (QoL) questionnaire module to supplement the EORTC core cancer QoL questionnaire, the QLQ-C30 in patients with pancreatic cancer. EORTC Study Group on Quality of Life. *Eur J Cancer*. 1999;35(6):939-41. doi:10.1016/s0959-8049(99)00047-7

28. Mesa R, Verstovsek S, Kiladjian JJ, et al. Changes in quality of life and disease-related symptoms in patients with polycythemia vera receiving ruxolitinib or standard therapy. *Eur J Haematol*. 2016;97(2):192-200. doi:10.1111/ejh.12707

29. FoundationOne®Heme Technical Information. https://assetsctfassetsnet/w98cd481qypp0/42r1cTE8VR4137CaHrsaen/baf91080cb3d78a52ada10c6358fa130/FoundationOne_Heme_Technical_Specificationspdf. 2019;Accessed 15 June 2021

30. Sun JX, He Y, Sanford E, et al. A computational approach to distinguish somatic vs. germline origin of genomic alterations from deep sequencing of cancer specimens without a matched normal. *PLoS Comput Biol*. 2018;14(2):e1005965. doi:10.1371/journal.pcbi.1005965

31. National Cancer Center Network. Myeloproliferative Neoplasms (Version 1.2020). Accessed March 8, 2021, https://www.nccn.org/professionals/physician_gls/pdf/mpn.pdf

32. Alvarez-Larran A, Pereira A, Cervantes F, et al. Assessment and prognostic value of the European LeukemiaNet criteria for clinicohematologic response, resistance, and intolerance to hydroxyurea in polycythemia vera. *Blood*. 2012;119(6):1363-9. doi:10.1182/blood-2011-10-387787

33. Assi TB, Baz E. Current applications of therapeutic phlebotomy. *Blood Transfus*. 2014;12 Suppl 1:s75-83. doi:10.2450/2013.0299-12

34. Kiladjian JJ, Chomienne C, Fenaux P. Interferon-alpha therapy in bcr-abl-negative myeloproliferative neoplasms. *Leukemia*. 2008;22(11):1990-8. doi:10.1038/leu.2008.280

35. Sever M, Newberry KJ, Verstovsek S. Therapeutic options for patients with polycythemia vera and essential thrombocythemia refractory/resistant to hydroxyurea. *Leuk Lymphoma*. 2014;55(12):2685-90. doi:10.3109/10428194.2014.893310

36. Vannucchi AM, Kiladjian JJ, Griesshammer M, et al. Ruxolitinib versus standard therapy for the treatment of polycythemia vera. *N Engl J Med*. 2015;372(5):426-35. doi:10.1056/NEJMoa1409002
37. Nazha A, Khoury JD, Verstovsek S, Daver N. Second line therapies in polycythemia vera: What is the optimal strategy after hydroxyurea failure? *Crit Rev Oncol Hematol*. 2016;105:112-7. doi:10.1016/j.critrevonc.2016.06.013

38. Marchioli R, Finazzi G, Specchia G, et al. Cardiovascular events and intensity of treatment in polycythemia vera. *N Engl J Med*. 2013;368(1):22-33. doi:10.1056/NEJMoa1208500

39. Passamonti F, Griesshammer M, Palandri F, et al. Ruxolitinib for the treatment of inadequately controlled polycythemia vera without splenomegaly (RESPONSE-2): a randomised, open-label, phase 3b study. *Lancet Oncol*. 2017;18(1):88-99. doi:10.1016/S1470-2045(16)30558-7

40. Ronner L, Podoltsev N, Gotlib J, et al. Persistent leukocytosis in polycythemia vera is associated with disease evolution but not thrombosis. *Blood*. 2020;135(19):1696-1703. doi:10.1182/blood.2019003347

41. Colafigli G, Scalzulli E, Pepe S, et al. The advantages and risks of ruxolitinib for the treatment of polycythemia vera. *Expert Rev Hematol*. 2020;13(10):1067-1072. doi:10.1080/17474086.2020.1816819

42. Kiladjian JJ, Zachee P, Hino M, et al. Long-term efficacy and safety of ruxolitinib versus best available therapy in polycythemia vera (RESPONSE): 5-year follow up of a phase 3 study. *Lancet Haematol*. 2020;7(3):e226-e237. doi:10.1016/S2352-3026(19)30207-8

43. Yacoub A, Mascarenhas J, Kosiorek H, et al. Pegylated interferon alfa-2a for polycythemia vera or essential thrombocythemia resistant or intolerant to hydroxyurea. *Blood*. 2019;134(18):1498-1509. doi:10.1182/blood.2019000428

44. Gisslinger H, Klade C, Georgiev P, et al. Ropeginterferon alfa-2b versus standard therapy for polycythemia vera (PROUD-PV and CONTINUATION-PV): a randomised, non-inferiority, phase 3 trial and its extension study. *Lancet Haematol*. 2020;7(3):e196-e208. doi:10.1016/S2352-3026(20)30034-4

45. Masarova L, Patel KP, Newberry KJ, et al. Pegylated interferon alfa-2a in patients with essential thrombocythaemia or polycythemia vera: a post-hoc, median 83 month follow-up of an open-label, phase 2 trial. *Lancet Haematol*. 2017;4(4):e165-e175. doi:10.1016/S2352-3026(17)30030-3

46. Vannucchi AM, Pieri L, Guglielmelli P. JAK2 Allele Burden in the Myeloproliferative Neoplasms: Effects on Phenotype, Prognosis and Change with Treatment. *Ther Adv Hematol*. 2011;2(1):21-32. doi:10.1177/2040620710394474
47. Antonioli E, Carobbio A, Pieri L, et al. Hydroxyurea does not appreciably reduce JAK2 V617F allele burden in patients with polycythemia vera or essential thrombocythemia. *Haematologica*. 2010;95(8):1435-8. doi:10.3324/haematol.2009.021444

48. Vannucci AM, Antonioli E, Guglielmelli P, et al. Prospective identification of high-risk polycythemia vera patients based on JAK2(V617F) allele burden. *Leukemia*. 2007;21(9):1952-9. doi:10.1038/sj.leu.2404854

49. Alimam S, Harrison C. Experience with ruxolitinib in the treatment of polycythemia vera. *Ther Adv Hematol*. 2017;8(4):139-151. doi:10.1177/2040620717693972

50. Shadfan M, Lopez-Pajares V, Yuan ZM. MDM2 and MDMX: Alone and together in regulation of p53. *Transl Cancer Res*. 2012;1(2):88-89.

51. Marcellino BK, Farnoud N, Cassinat B, et al. Transient expansion of TP53 mutated clones in polycythemia vera patients treated with idasanutlin. *Blood Adv*. 2020;4(22):5735-5744. doi:10.1182/bloodadvances.2020002379

52. Grinfeld J, Nangalia J, Baxter EJ, et al. Classification and Personalized Prognosis in Myeloproliferative Neoplasms. *N Engl J Med*. 2018;379(15):1416-1430. doi:10.1056/NEJMoa1716614

53. Sallman DA, McLemore AF, Aldrich AL, et al. TP53 mutations in myelodysplastic syndromes and secondary AML confer an immunosuppressive phenotype. *Blood*. 2020;136(24):2812-2823. doi:10.1182/blood.2020006158

54. Gotlib J, Gabrail N, O'Connell CL, et al. A Randomized, Open-Label, Multicenter, Phase 2 Study to Evaluate the Efficacy, Safety, and Pharmacokinetics of KRT-232 Compared with Ruxolitinib in Patients with Phlebotomy-Dependent Polycythemia Vera. *Blood*. 2019;134(Supplement_1):4168-4168. doi:10.1182/blood-2019-123546
## Tables

**Table 1.** Baseline demographics and characteristics.

| Baseline characteristics | All patients (N = 27) |
|--------------------------|-----------------------|
| Median age (range), y    | 56.0 (34-74)          |
| Male, n (%)              | 16 (59)               |
| Median time since PV diagnosis (range), y | 6.3 (0.6-27.5) |
| Prior thrombosis, n (%)  | 2 (7)                 |
| Median baseline TSS (IQR)* | 31 (15-40)           |
| Median baseline score of GHS/QoL and EORTC QLQ-C30 (IQR)† | 66.7 (50.0-66.7) |
| **Reason for HU discontinuation, n (%)** |                     |
| Intolerance              | 24 (89)               |
| Resistance               | 5 (19)                |
| Intolerance and resistance | 2 (7)            |
| **Prior cytoreductive therapies‡, n (%)** |                     |
| Ruxolitinib              | 7 (26)                |
| Interferon-α             | 5 (19)                |
| Ruxolitinib and interferon-α | 1 (4)            |
| None                     | 14 (52)               |
| Median JAK2 V617F VAF (range), % | 65.7 (7-96)          |
| Cytogenetic abnormalities, n (%) | 2 (7)                |
| Splenomegaly§, n (%)     | 21 (78)               |
| Median spleen volume in patients with splenomegaly, (range), cm³ | 800.0 (513.0-2602.4) |
| Median baseline hematocrit (range), % | 43 (40-50)          |
| Median baseline WBC count (range), × 10⁹/L | 13.7 (5-44)         |
| Median baseline platelet count (range), × 10⁹/L | 576.0 (176-2314)     |

GHS, global health status; IQR, interquartile range; WBC, white blood cell.
* Data available for n = 25 patients.
† The overall GHS/QoL scale was calculated from a combination of both the GHS & QoL items; therefore, the baseline score reported here is composed of the score of both items for n = 27.
‡ Excluding HU.
§ Spleen volume > 450 cm$^3$ as determined by imaging.
Table 2. Treatment-emergent AEs occurring in ≥ 5 patients (15%) regardless of attribution.

| n (%)     | All grades (N = 27) | Grade ≥ 3* |
|-----------|---------------------|------------|
| Any AE    | 27 (100)            | 10 (37)    |
| Nausea    | 25 (93)             | 3 (11)     |
| Diarrhea  | 21 (78)             | 0          |
| Vomiting  | 11 (41)             | 1 (4)      |
| Fatigue   | 10 (37)             | 2 (7)      |
| Constipation | 9 (33)            | 0          |
| Headache  | 8 (30)              | 0          |
| Dizziness | 7 (26)              | 0          |
| Abdominal pain | 6 (22)       | 0          |
| Taste disorder | 6 (22)       | 0          |
| Decreased appetite | 6 (22)   | 0          |
| Insomnia  | 6 (22)              | 1 (4)      |
| Anemia    | 5 (19)              | 0          |
| Thrombocytopenia† | 5 (19)    | 0          |

One grade 4 event (atrial fibrillation; n = 1) and no grade 5 events occurred during the study.† Includes the terms “thrombocytopenia” and “platelet count decreased” as defined by the Medical Dictionary for Regulatory Activities.
Table 3. Treatment response summary.

|                          | All patients (N = 27) |
|--------------------------|-----------------------|
| **Response at week 32, n (%)** |                       |
| Composite response†      | 1 (8)                 |
| Hematocrit control‡      | 9 (56)                |
| Spleen volume reduction >35% at any time point, n (%)§ | 7 (33) |
| Hematocrit control, n (%) |                       |
| At C3D28‖                | 19 (73)               |
| At C5D28§                | 15 (68)               |
| CHR at week 32, n (%)‡    | 8 (50)                |
| CHR at any time point, n (%) |                      |
| ELN response at week 32, n (%)‡ |                 |
| CR                       | 3 (19)                |
| PR                       | 8 (50)                |
| PD                       | 0                     |
| No response              | 5 (31)                |
| ELN response (CR or PR) at any time point, n (%) | 21 (78) |
| Bone marrow histologic remission at week 32, n (%)#   | 2 (13) |
| Median TSS reduction from baseline at week 32 (IQR) ** | -25.4 (-62.5 to -5.1) |
| TSS reduction ≥50% from baseline at week 32, n (%)**   | 6 (43)                |
| TSS reduction ≥50% from baseline at any time point, n (%)†† | 12 (48) |

CR, complete remission; PD, progressive disease; PR, partial remission.

* The response-evaluable population included 16 patients who had undergone response assessment at week 32 or had withdrawn prior to week 32, due to lack of response or PD. Composite response was the primary endpoint at week 32 in patients with splenomegaly. For all other patients, the primary endpoint was hematocrit control at week 32. Hematocrit control was defined as ≤1 phlebotomy between start of the study and week 8 and no phlebotomies after week 8. Protocol-defined indications for phlebotomy were hematocrit >45%, which was 3% higher than the screening value, or hematocrit >48%, regardless of screening value. † Evaluable patients, n = 13. ‡ Evaluable patients, n = 16. § Evaluable patients, n = 21. ‖ Evaluable patients, n = 26. ¶ Evaluable patients, n = 22. # Evaluable patients, n = 15. †† Evaluable patients, n = 14. ** Evaluable patients, n = 25.
Figure legends

Figure 1. Clinical response in evaluable patients at C3D28, C5D28, week 32, and C11D28. (A) Percentage of patients with hematocrit (Hct) control. (B) Percentage of patients who showed response according to the ELN hematologic response criteria. (C) Percentage of patients with CHR. (D) Percentage of patients with composite response.

Figure 2. Change in spleen volume by ruxolitinib exposure. (A) Percentage change in spleen volume from baseline at any time in evaluable patients. (B) Percentage change in spleen volume at week 32 in evaluable patients. (C) Percentage change in spleen volume at C3D28, C5D28, and week 32 in each patient evaluable at the assessment points.

Figure 3. Patient-reported outcomes per MPN-SAF TSS in evaluable patients. (A) Median change from baseline in MPN-SAF TSS at key assessment timepoints. (B) Median percentage change from baseline in patient-reported scores at C2D1, C3D28, C5D28, and week 32. Error bars represent IQR. (C) Patients with a ≥50% reduction from baseline MPN-SAF TSS.

Figure 4. JAK2 allele burden in evaluable patients. (A) JAK2 allele burden at baseline in patients with or without splenomegaly. (B) Percentage change from baseline in JAK2 allele burden in patients with (responders) or without CHR (non-responders) at C3D28, C5D28, and week 32. (C) Comparison of reduction in JAK2 allele burden between patients with (responders) or without hematocrit control (non-responders). (D) Comparison of reduction in JAK2 allele burden between patients with (responders) or without CHR (non-responders). Black stars represent the mean.
Figure 1

A. Hct control vs. No Hct control

B. CR vs. PR vs. No response

C. CHR vs. No CHR

D. Response vs. No response
Change from baseline in spleen volume (%)

Figure 2

A

B

C

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Figure 3

A

Median change from baseline (%)

C2D1 (n = 22)
C3D28 (n = 21)
C5D28 (n = 18)
C11D28 (n = 7)
C12D28 (n = 1)
C14D28 (n = 8)
C17D28 (n = 6)
C20D28 (n = 2)
Final visit (n = 18)

Improvement

B

Median change from baseline (%)

C2D1 (n = 21)
C3D28 (n = 20)
C5D28 (n = 17)
Week 32 (n = 14)

Median -17.78 -26.97 -36.36 -25.38

Improvement

C

Patients, n (%)

C2D1 (n = 21)
C3D28 (n = 20)
C5D28 (n = 17)
Week 32 (n = 14)

≥ 50% reduction
< 50% reduction
| Baseline JAK2 allele burden (%) | With splenomegaly | Without splenomegaly |
|-------------------------------|-------------------|----------------------|
| Mean                          | 62.89             | 65.65                |
| Median                        | 60.00             | 60.00                |
| 25th percentile               | 49.00             | 87.10                |
| 75th percentile               | 7.10              | 81.80                |
| Minimum                       | 7.10              | 95.80                |
| Maximum                       | 95.80             | 95.80                |

**With splenomegaly:**
- Mean: 62.89
- Median: 65.65
- 25th percentile: 49.00
- 75th percentile: 87.10
- Minimum: 7.10
- Maximum: 95.80

**Without splenomegaly:**
- Mean: 65.65
- Median: 60.00
- 25th percentile: 87.10
- 75th percentile: 81.80
- Minimum: 7.10
- Maximum: 95.80

**Responders (Hct control):**

| Week 32 | Responders | Non-responders |
|---------|------------|----------------|
| **C3D28** | Median (range) reduction: **-75.9% (-96.6% to 1.5%)** | Median (range) reduction: **-57.8% (-97.6% to 4.2%)** |
| **C5D28** | Median (range) reduction: **-75.9% (-96.6% to 1.5%)** | Median (range) reduction: **-57.8% (-97.6% to 4.2%)** |

| Responders | n | Median (IQR) change from baseline in JAK2 allele burden, % |
|------------|---|---------------------------------------------------------|
|            | 13 | (-75.9 to -10.3)                                        |
|            | 11 | (-75.9 to -10.3)                                        |
|            | 7  | (-75.9 to -10.3)                                        |

| Non-responders | n | Median (IQR) change from baseline in JAK2 allele burden, % |
|----------------|---|---------------------------------------------------------|
|                | 6 | (-8.8 to -10.3)                                         |
|                | 6 | (-10.3 to -6.1)                                         |
|                | 6 | (-54.0 to -6.1)                                         |

**P value:**
- Responders: 0.04
- Non-responders: 0.19

**Responders (CHR):**

| Week 32 | Responders | Non-responders |
|---------|------------|----------------|
| **C3D28** | Median (range) reduction: **-72.4% (-83.8% to 6.4%)** | Median (range) reduction: **-72.4% (-83.8% to 6.4%)** |
| **C5D28** | Median (range) reduction: **-72.4% (-83.8% to 6.4%)** | Median (range) reduction: **-72.4% (-83.8% to 6.4%)** |

| Responders | n | Median (IQR) change from baseline in JAK2 allele burden, % |
|------------|---|---------------------------------------------------------|
|            | 6 | (-72.4 to -8.8)                                         |
|            | 6 | (-72.4 to -8.8)                                         |
|            | 6 | (-72.4 to -8.8)                                         |

| Non-responders | n | Median (IQR) change from baseline in JAK2 allele burden, % |
|----------------|---|---------------------------------------------------------|
|                | 13| (-10.2 to -5.6)                                         |
|                | 11| (-10.2 to -5.6)                                         |
|                | 7 | (-10.2 to -5.6)                                         |

**P value:**
- Responders: 0.008
- Non-responders: 0.005

**Non-responders:**

- Median (IQR) change from baseline in JAK2 allele burden, %
- Minimum: 7.10
- Maximum: 95.80

**P value:**
- Responders: 0.006
- Non-responders: 0.005

**Responders:**

- Median (IQR) change from baseline in JAK2 allele burden, %
- Minimum: 7.10
- Maximum: 95.80

**P value:**
- Responders: 0.005
- Non-responders: 0.005