







## RESEARCH ARTICLE

# Janus kinase inhibitor ruxolitinib in combination with nilotinib and prednisone in patients with myelofibrosis (RuNiC study): A phase Ib, multicenter study

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## Abstract

This phase Ib, non-randomized, open-label study evaluates the safety and tolerability of ruxolitinib in combination with nilotinib and prednisone in patients with naïve or ruxolitinib-resistant myelofibrosis (MF). A total of 15 patients with primary or secondary MF received the study treatment; 13 patients had received prior ruxolitinib treatment (86.7%). Eight patients completed seven cycles (53.3%) and six patients completed twelve cycles of treatment (40%). All the patients experienced at least one adverse event (AE) during the study (the most common AEs were hyperglycemia, asthenia, and thrombocytopenia), and 14 patients registered at least one treatment-related AE (the most common treatment-related AEs were hyperglycemia (22.2%; three grade 3 cases). Five treatment-related serious AEs (SAEs) were reported in two patients (13.3%). No deaths were registered throughout the study. No dose-limiting toxicity was observed. Four out of fifteen (27%) patients experienced a 100% spleen size reduction at Cycle 7, and two additional patients achieved a >50% spleen size reduction, representing an overall response rate of 40% at Cycle 7. In conclusion, the

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tolerability of this combination was acceptable, and hyperglycemia was the most frequent treatment-related AE. Ruxolitinib in combination with nilotinib and prednisone showed relevant clinical activity in patients with MF. This trial was registered with EudraCT Number 2016-005214-21.

#### KEYWORDS

combination therapy, myelofibrosis, nilotinib, prednisone, ruxolitinib

## 1 | INTRODUCTION

Myelofibrosis (MF) is characterized by the clonal expansion of myeloid cells due to the acquisition of driver mutations in the *JAK2*, *CALR*, or *MPL* genes, and/or other mutations such as those in *ASXL1*, *SRSF2*, and *U2AF1* [1, 2].

Ruxolitinib is a reversible and selective inhibitor of Janus kinase-1 (*JAK1*) and *JAK2*/signal transducer and activator of transcription (*STAT*) signaling that has been approved in the European Union for the treatment of disease-related splenomegaly or symptoms in adult patients with de novo MF, MF arising from previous polycythemia vera, or essential thrombocythemia (MF) [3–11]. Ruxolitinib achieves spleen reduction and symptom control in a significant proportion of patients, leading to improved quality of life and possible survival benefits. However, the median response duration for ruxolitinib of 3 years, its lack of disease-modifying effect, and treatment discontinuation due to adverse events (AEs) highlight the need to incorporate new treatment options for patients with MF [5–11].

A recent *in vitro* study investigated the administration of ruxolitinib in combination with nilotinib and prednisone to treat patients with MF [12] and showed that ruxolitinib had a synergistic effect with nilotinib and prednisone in MF patient samples and cell lines, partly mediated through significant inhibition of the *STAT5* and protein kinase B (*AKT/PKB*) pathways. Moreover, the combination of ruxolitinib and nilotinib has proven efficacy in chronic myeloid leukemia, and nilotinib has shown antifibrotic effects in the liver and lung in animal models [13–16].

The present phase Ib clinical trial was a two-arm study designed to obtain and assess the maximum tolerated dose (MTD) and/or recommended phase III dose (RP3D) of ruxolitinib in combination with nilotinib and prednisone in two MF separate populations: patients naïve to *JAK*-inhibitor treatment and patients not responding to or relapsed after *JAK*-inhibitor treatment. The study was also designed to provide preliminary efficacy and safety data for ruxolitinib when administered in combination with nilotinib and prednisone in MF patients.

## 2 | METHODS

### 2.1 | Patient population

This study consisted of two cohorts: patients naïve to *JAK*-inhibitor treatment and patients who did not respond or relapsed after *JAK*-

inhibitor treatment. Patients not responding to or who relapsed after 12 weeks of *JAK*-inhibitor treatment were defined according to the international Working group for Myelofibrosis Research and Treatment criteria (IWG-MRT) (ref Tefferi A, Blood 2013;122): patients who did not achieved a splenic response measured by palpation (<50% reduction) or symptoms measured by modified MF-SAF (<50% reduction) or had > 50% of spleen size increase from best response or >50% increase in symptoms assessment from best response. A detailed explanation of the key inclusion and exclusion criteria is included in the Supplementary Material.

### 2.2 | Study design

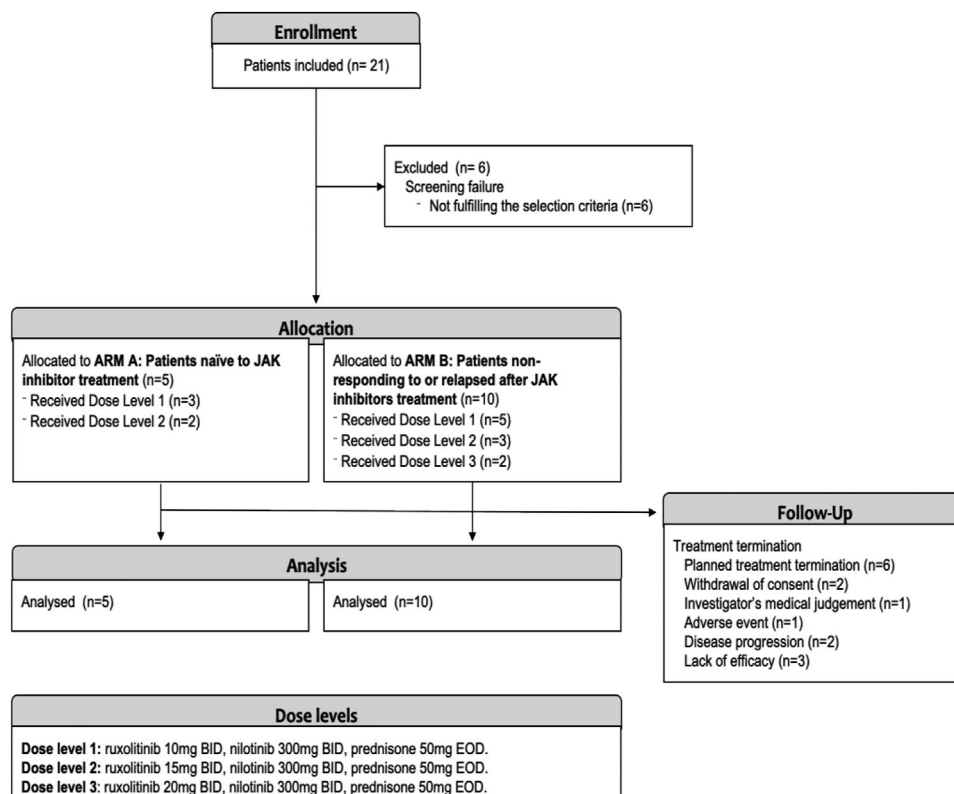
RuNiC is a phase Ib/II, open-label, multicenter, dose-finding study on ruxolitinib in combination with nilotinib (300 mg twice a day (BID)) and prednisone (50 mg every other day) for patients with MF [17–19]. The dose-escalation phase (first six cycles, 28-day cycles) initially planned with ruxolitinib starting from 10 mg BID was based on phase II studies [13], according to the classic (non-Bayesian) model [20, 21]. Next, a dose-expansion phase has been planned (12–24 cycles, 1–2 years), whereby an additional 10 patients in each arm (20 patients in total) will be treated according to their respective arm (Figure 1). For patients with prior ruxolitinib, no washout phase was needed.

A dose-limiting toxicity (DLT) was defined as any treatment-related toxicity occurring within the first 28 days (from Cycle 1 on Day 1 to Cycle 1 on Day 28) of treatment with ruxolitinib, nilotinib, and prednisone and that met any of the DLT criteria (Table S1).

Dose Modifications and dose delay of combination therapy: Hematologic toxicity is performed with ruxolitinib dose adjustment. This study does not contemplate a reduction in doses of nilotinib and prednisone except in cases of toxicities. In such cases, doses of nilotinib and prednisone will be reduced to 200 mg BID and 25 mg every other day (EOD), respectively. But for all patients participating in the study dose reductions of nilotinib are required for platelet count reductions to specified levels considered related (possibly, probably, definitely) to nilotinib.

### 2.3 | Assessments

The primary objective was to determine the MTD and the RP3D of ruxolitinib in combination with nilotinib and prednisone. The



**FIGURE 1** Study flow chart. BID, twice daily; EOD, every other day; JAK, Janus kinase.

secondary objectives included evaluating the clinical activity of ruxolitinib in combination with nilotinib and prednisone and the safety profile (the frequency and severity of AEs as graded by the National Cancer Institute CTCAE version 4.0) of this combination. The clinical activity was evaluated using the Eastern Oncology Cooperative Group (ECOG) performance status at Cycle 4 on Day 1, at Cycle 7 on Day 1, and at Cycle 12 on Day 28; the palpable spleen length reduction (the proportion of patients achieving a  $\geq 50\%$  reduction in palpable measurement), recorded from baseline to Cycle 7 on Day 1 and to Cycle 12 on Day 28; and the TSS and individual item scores in the modified MF-symptom assessment form (SAF) [22], recorded at Cycle 4 on Day 1, at Cycle 7 on Day 1, and at Cycle 12 on Day 28. We have used the IWG-MRT but using palpation assessment (which is referred to as 50%). A symptomatic response was defined as a reduction of 20% or more in TSS.

Chemosensitivity profiling was performed using PharmaFlow, as explained in Supplementary Methods. The mutational profiles were also determined by DNA from total bone marrow or peripheral blood samples. Targeted deep sequencing was performed as previously described using the Ion Torrent technology; we used a custom next generation sequencing (NGS) panel consisting of 43 genes recurrently mutated in myeloid diseases (Table S2) [23].

## 2.4 | Statistical analysis

Quantitative variables are described with measures of central tendency and dispersion: mean, median, SD (standard deviation), Q1 (first

quartile) and Q3 (third quartile), and minimum and maximum. Qualitative variables are described using absolute and relative frequencies (N, %). The description only concerned the descriptive analysis of the data. No statistical modeling procedures were performed. The analysis was performed using the IBM SPSS Statistics software, Version 22.0 (IBM Corp. Armonk, NY).

## 2.5 | Ethical approvals

This study was approved by the institutional review board of the Hospital 12 de Octubre de Madrid (CEIm number 17/182) and the boards of the respective institutions before patient enrolment. The study was conducted following the principles of the Declaration of Helsinki. All the patients provided written informed consent. The trial has been registered at EudraCT: Number 2016-005214-21.

## 3 | RESULTS

### 3.1 | Study population

From November 2017 to June 2020, 21 patients were included in the study at six Spanish sites. Six patients failed the screening for participation and did not receive treatment; these patients were not included in the intention-to-treat or the per-protocol populations. A total of 15 patients received at least one dose of ruxolitinib (Figure 1). Six

**TABLE 1** Baseline characteristics of patients.

Variable	Value
Age, median (range)—years	66.5 (53.4–70.8)
Gender— <i>n</i> (%)	
Male	9 (60)
Female	6 (40)
Ethnicity— <i>n</i> (%)	
Caucasian	14 (93.3)
Arabic	1 (6.7)
Main comorbidities at baseline (> 10%)— <i>n</i> (%)	
Cardiovascular diseases	6 (40)
Musculoskeletal disorders	5 (33.3)
Neurologic or psychiatric disorders	3 (20)
Gastrointestinal disorders	2 (13.3)
Endocrine disorders	2 (13.3)
Other	8 (53.3)
Patients with transfusion 12 weeks prior screening— <i>n</i> (%)	4 (26.7)
Median peripheral-blood blast count (range)—%	4 (2–9)
Hematology, median (range)	
Hemoglobin—g/dL	10.8 (9.2–11.7)
Platelet count— $\times 10^9$ /L	230 (92–467)
Absolute neutrophil count— $\times 10^9$ /L	7.6 (6.2–13.8)
White-cell count— $\times 10^9$ /L	10.6 (4.61–13.5)
Median spleen length (range)—cm	8 (6–20)
ECOG performance status— <i>n</i> (%): 0/1/2	5 (33.3)/7 (46.7)/7 (46.7)
Time from diagnosis, median (range)—years	2.91 (0.59–3.98)
Type of myelofibrosis— <i>n</i> (%) <sup>a</sup>	
Primary MF	6 (40)
Post-polycythemia vera MF	4 (26.7)
Post-essential thrombocythemia MF	4 (26.7)
IPSS risk category— <i>n</i> (%) <sup>b</sup>	
Low	1 (6.7)
Intermediate 1	5 (33.3)
Intermediate 2	4 (26.7)
High	1 (6.7)
DIPSS risk category— <i>n</i> (%) <sup>c</sup>	
Intermediate 1	7 (46.7)
Intermediate 2	6 (40)
High	5 (33.3)
DIPSS-Plus risk category— <i>n</i> (%) <sup>d</sup>	
Intermediate 1	5 (33.3)
Intermediate 2	4 (26.7)
Mutations at diagnosis— <i>n</i> (%)	
JAK2 V617F	9 (60)
CALR Type 1	4 (26.7)
MPL W515L/K	1 (6.7)

(Continues)

**TABLE 1** (Continued)

Variable	Value
Patients with prior antineoplastic therapy— <i>n</i> (%)	13 (86.7)
Antineoplastic therapy type— <i>n</i> (%)	
Hydroxyurea	11 (84.6)
Ruxolitinib	10 (76.9)
Other	5 (38.5)
Alpha- Interferon	2 (15.4)
Anagrelide	1 (7.7)
BKM120	1 (7.7)
Imetelstat	1 (7.7)
Melphalan	1 (7.7)
Patients with prior antineoplastic radiotherapy— <i>n</i> (%)	1 (6.7)

Note: <sup>a</sup>Data were not available for one patient (6.7%). <sup>b</sup>Data were not available for four patients (26.7%). <sup>c</sup>Data were not available for three patients (20%). <sup>d</sup>Data were not available for 6 patients (40%). DIPSS = Dynamic International Prognostic Scoring System; DIPSS-Plus = Dynamic International Prognostic Scoring System Plus; ECOG = Eastern Cooperative Oncology Group. CALR = Calreticulin. <sup>e</sup>Data were not available for seven patients (46.7%). <sup>f</sup>Data were not available for 6 patients (40%); <sup>g</sup>Data were not available for 10 patients (66.7%). IPSS = International Prognostic Scoring System; JAK2 = Janus Kinase 2; MF = Myelofibrosis; MPL = Myeloproliferative leukemia virus gene; PV/TE = Polycythemia vera / Essential thrombocythemia; smPCR = single-molecule PCR TET2 = Tet methylcytosine dioxygenase 2.

patients completed the planned treatment (40%), and nine were withdrawn from the study because of disease progression, a lack of efficacy or no clinical benefit ( $n = 5$ ), the withdrawal of consent ( $n = 2$ ), an AE (pulmonary thromboembolism,  $n = 1$ ), or the investigator's medical judgment ( $n = 1$ ). Nine patients completed the escalation phase and seven patients the extension phase (Cycle 12): four patients continued with treatment after cycle 12.

### 3.1.1 | Demographic, clinical, and MF baseline characteristics

The demographic and clinical characteristics of the patients are described in Table 1.

Most of the patients ( $n = 13$ , 86.7%) had received prior therapy for MF, including hydroxyurea and ruxolitinib in 11 patients (84.6%) and 10 patients (76.9%), respectively.

### 3.1.2 | Study treatment dosing

The maximum tolerated doses were 20 mg twice a day (BID) of ruxolitinib, 300 mg BID of nilotinib, and 50 mg EOD of prednisone, at Cycle 12.

The dose and duration for each treatment were analyzed for study Cycles 4, 7, and 12. A descriptive analysis of the different drug doses

for the total number of patients in each cycle is provided in Tables S2 and S3. Dose reduction was performed in the following cases: for prednisone, in 6/12 cases to Cycle 4, 5/8 cases to Cycle 7, and 4/7 cases to Cycle 12; for nilotinib, in 4/12 cases to Cycle 4, 3/8 cases to Cycle 7, and 2/7 cases to Cycle 12; for ruxolitinib, in 4/12 cases to Cycle 4, 3/8 cases to Cycle 7, and 3/7 cases to Cycle 12.

The median (range) follow-up duration, from the first treatment dose to the last monitoring data collected, was 401 (59–727) days.

### 3.2 | Safety

All the patients experienced at least one AE throughout the study. Fourteen patients (93.3%) registered at least one AE related to any of the study treatments.

A summary of the main all-grade AEs and grade 3–4 AEs is shown in Table 2. The most common AEs were asthenia ( $n = 10$  patients, 66.7%); hyperglycemia ( $n = 5$ , 33.3%); thrombocytopenia ( $n = 4$ , 26.7%); anemia ( $n = 3$ , 20%); dyspnea ( $n = 3$ , 20%); insomnia ( $n = 3$ , 20%); and upper respiratory infection ( $n = 3$ , 20%). Grade 3–4 toxicity in  $\geq 10\%$  of the patients was only observed for hyperglycemia ( $n = 2$ , 13.3%); thrombocytopenia ( $n = 2$ , 13.3%); and anemia ( $n = 2$ , 13.3%).

Overall, 251 AEs were reported, among which 90 were treatment-related AEs (Table S4). The most common treatment-related AEs ( $\geq 5\%$  of the total) were hyperglycemia (22.2%), thrombocytopenia (13.3%), anemia (8.9%), and elevated ALT (6.7%). Temporary interruption of treatment occurred for five (33.3%) patients; dose adjustment was reported for one (6.7%) patient, and permanent discontinuation of the treatment was registered for three (20%) patients.

From the start of the study, a total of five treatment-related SAEs were reported by two patients (13.3%): one patient had pericardial effusion and bilateral pleural effusion (both conditions related to nilotinib), and one patient had congestive heart failure, pleural effusion, and pulmonary hypertension (all conditions related to nilotinib). No deaths were registered throughout the study.

For the patients who discontinued due to nilotinib toxicity, both passed away 9 and 5 months later due to progression of the disease and splenic radiotherapy complications.

### 3.3 | Clinical activity of ruxolitinib in combination with nilotinib and prednisone

The clinical activity is shown in Figure 2. Different distributions of patients were determined among the several ECOG performance status scores at each of the time points analyzed (Figure 2A). At screening, most patients were distributed between ECOG performance status scores of 1 and 2, while at Cycles 4, 7, and 12, most patients were distributed between ECOG performance status scores of 0 and 1. An improvement in ECOG performance status was observed at Cycle 4 in two of the five patients without pretreatment and in three of the ten patients who were resistant to pretreatment.

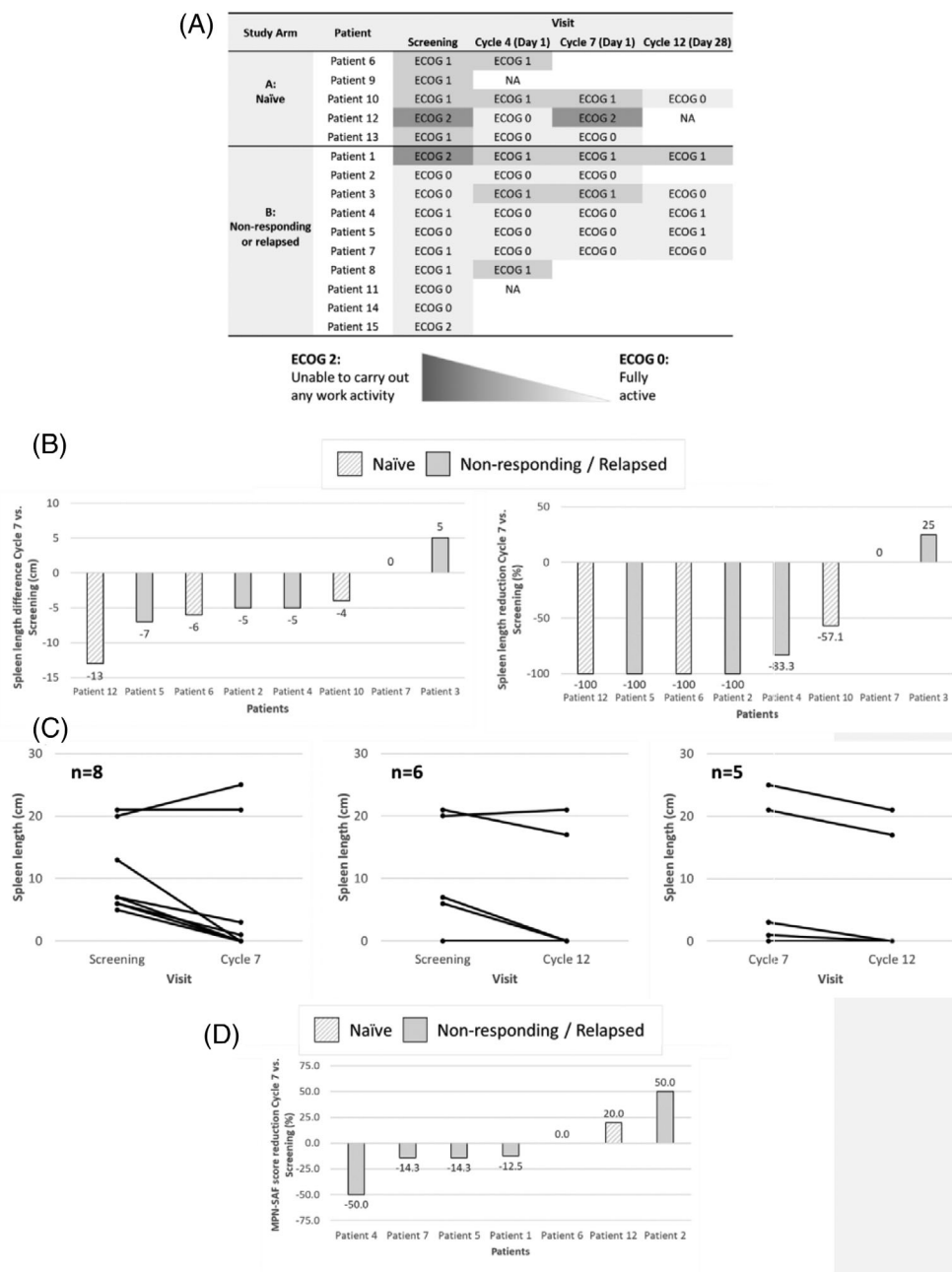
**TABLE 2** Adverse events.

AE	Any grade, n (%)	Grade $\geq 3$ , n (%)
Asthenia	10 (66.7)	1 (6.7)
Hyperglycemia	5 (33.3)	2 (13.3)
Thrombocytopenia	4 (26.7)	2 (13.3)
Anemia	3 (20.0)	2 (13.3)
Dyspnea	3 (20.0)	0 (0.0)
Insomnia	3 (20.0)	0 (0.0)
Nausea	3 (20.0)	0 (0.0)
Upper respiratory infection	3 (20.0)	0 (0.0)
ALT increased	2 (13.3)	1 (6.7)
Anxiety	2 (13.3)	0 (0.0)
AST increased	2 (13.3)	0 (0.0)
Constipation	2 (13.3)	0 (0.0)
Diarrhea	2 (13.3)	0 (0.0)
Dizziness	2 (13.3)	0 (0.0)
Edemas	2 (13.3)	1 (6.7)
Fever	2 (13.3)	0 (0.0)
Hyporexia	2 (13.3)	0 (0.0)
Impaired renal function	2 (13.3)	0 (0.0)
Lower back pain	2 (13.3)	0 (0.0)
Slight fever	2 (13.3)	0 (0.0)
Vomit	2 (13.3)	0 (0.0)
Worsening of anemia	2 (13.3)	1 (6.7)
Bilateral pleural effusion	1 (6.7)	1 (6.7)
Cholestasis	1 (6.7)	1 (6.7)
Congestive heart failure	1 (6.7)	1 (6.7)
Decompensation of biventricular heart failure of multifactorial etiology	1 (6.7)	1 (6.7)
GGT increased	1 (6.7)	1 (6.7)
Lipase increased	1 (6.7)	1 (6.7)
Pericardial effusion	1 (6.7)	1 (6.7)
Pleural effusion	1 (6.7)	1 (6.7)
Pulmonary hypertension	1 (6.7)	1 (6.7)
Pulmonary tromboembolism	1 (6.7)	1 (6.7)
Worsening of thrombocytopenia	1 (6.7)	1 (6.7)

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase.

#### 3.3.1 | Spleen length reduction

The spleen reduction is shown in Figure 2B,C. The Figure 2B,C includes separately the splenic response and the response to symptoms based on naive cases or after failure to ruxolitinib. Of the initial 15 patients, only eight had spleen lengths recorded at screening and Cycle 7. Four of 15 (27%) patients experienced a 100% spleen reduction at Cycle 7, and two additional patients achieved a  $>50\%$  spleen



**FIGURE 2** Clinical activity of ruxolitinib in combination with nilotinib and prednisone: (A) distribution of patients' ECOG performance status scores, with all the visits considered; (B) change in spleen volume from baseline to end of Cycle 7; (C) comparison of the proportion of patients with each percentage decrease in palpable spleen length from screening to the end of Cycle 12; (D) change in MF-SAF total symptom score from baseline to the end of Cycle 7. ECOG, Eastern Oncology Cooperative Group; MF-SAF, Myelofibrosis Symptom Assessment Form; NA, not available.

reduction, accounting for an overall response rate of 40% at Cycle 7, assuming that the patients with no spleen measurements were non-responders. Only five of the eight cases evaluated at Cycle 7 were evaluated for spleen length at Cycle 12, and among these, three patients experienced a 5% reduction in spleen length between Cycles 7 and 12. The mean (SD) and median (range) duration of response, estimated taking into consideration the spleen length reduction, were determined to be 8.4 (3.05) months and 8.38 (5.47, 11.53) months, respectively.

### 3.3.2 | MF-SAF

The descriptive analysis of each MF-SAF individual item and total score at screening and Cycles 4, 7, and 12, are shown in Table 3. As observed, the individual symptom values of the MF-SAF median scores improved from screening to Cycles 4, 7, and 12, for most of the items. An increase in symptoms related to concentration difficulties and itching was observed at Cycle 12. From the initial 15 patients, only six had symptoms evaluated at screening and Cycle 7 (Figure 2D).

**TABLE 3** Median MPN-SAF TSS items and total score, at screening and cycles 4, 7 and 12.

MPN-SAF- TSS (Items)	Median (range)			
	Screening (n = 15)	Cycle 4 (Day 1) (n = 9)	Cycle 7 (Day 1) (n = 8)	Cycle 12 (Day 28) (n = 5)
Fatigue	7.0 (5.0–9.0)	6.0 (5.0–8.0)	6.0 (5.0–7.0)	5.0 (3.5–8.5)
Early satiety	5.0 (0.0–7.0)	4.0 (0.0–5.0)	4.0 (0.8–4.8)	4.0 (1.0–4.5)
Abdominal discomfort	5.0 (0.0–8.0)	0.0 (0.0–4.0)	0.5 (0.0–4.3)	1.0 (0.0–4.5)
Inactivity	5.0 (1.0–8.0)	3.0 (0.0–6.0)	1.0 (0.0–2.8)	1.0 (0.5–6.0)
Concentration difficulties	2.0 (0.0–8.0)	3.0 (0.0–5.0)	1.0 (0.0–2.8)	2.0 (0.0–5.5)
Night sweats	5.0 (0.0–8.0)	0.0 (0.0–4.0)	3.0 (0.5–5.0)	1.0 (0.0–2.0)
Itching	4.0 (0.0–7.0)	2.0 (0.0–5.0)	1.0 (0.0–6.8)	3.0 (1.0–4.0)
Bone pain	4.0 (1.0–8.0)	2.5 (0.0–6.0)	3.0 (0.0–5.8)	1.0 (0.0–9.0)
Fever (>37°C)	0.0 (0.0–1.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
Unintentional weight loss	1.0 (0.0–6.0)	0.0 (0.0–2.0)	0.0 (0.0–0.0)	0.0 (0.0–0.5)
Total score	40.0 (21.0–49.0)	24.0 (14.0–33.8)	23.0 (21.0–25.5)	17.0 (10.0–44.0)

Abbreviations: MFSAF-TSS, myelofibrosis symptom assessment form total symptom score; SD, standard deviation.

Among these six patients, four patients experienced a total symptom score reduction (one patient reported a 50% reduction, two patients reported 14.3% reductions, and one patient reported a 12.5% reduction) from baseline to the end of Cycle 7. By contrast, one patient had the same total symptom score, and two patients reported increases (20% and 50%, respectively).

### 3.3.3 | Ex vivo assays pharmaflow chemosensitivity profiling

We analyzed the correlations between the treatment with ruxolitinib in combination with nilotinib and prednisone by an ex vivo PharmaFlow study in samples from nine treated patients, and the patients' clinical responses (Figure S1). This was feasible in eight out of the fifteen cases evaluated (53.3%). The ex vivo classification correlated with the clinical response to Cycle 7, since 75% of the samples from clinical responders were classified as sensitive. By contrast, 50% of the samples from patients who did not clinically respond to Cycle 7 were classified ex vivo resistant.

### 3.3.4 | Mutational profile by NGS

Mutational profiling was performed by NGS with a custom panel (Table S5) in 10 cases. Driver mutations were detected in the *JAK2* (seven cases, 46.7%), *calreticulin (CALR)* (two cases, 13.3%), and *thrombopoietin (MPL)* genes (one case, 6.7%). The mutational landscapes of the responders and nonresponders in Cycle 7 are shown in Figure S2. Among the seven cases with mutated *JAK2*, five cases were responders; both cases with mutated *CALR* were responders; and the only case with mutated *MPL* was a non-responder. The most commonly co-mutated genes were the *JAK2* gene with *BCORL1* (two cases) and *TET2* (two cases) and the *CALR* gene with *DNMT3A* (two cases) and *TET2*

(three cases). The *MPL* gene co-mutated with *BCORL1*, *DNMT3A*, *PHF6*, and *SH2B3* (one case). The distribution of the most frequent driver mutations in relation to the Cycle 7 response was as follows: among the four cases with mutated *TET2*, three were responders to Cycle 7, and among the four cases with mutated *BCORL1*, two were responders to Cycle 7.

## 4 | DISCUSSION

The main objectives of this phase Ib/II trial were to determine the MTD, toxicity, clinical efficacy, and biological activity of ruxolitinib in combination with nilotinib and prednisone in MF patients, naïve or previously treated with JAK inhibitors. This combination was safe and tolerable with relevant clinical activity in these patients.

In phase III ruxolitinib clinical trials, the rate of discontinuation of ruxolitinib treatment because of AEs was 11% (as compared with 10.6% in the placebo group) [9], with anemia (43%) and thrombocytopenia (17%) being the most common AEs. In our study, 14 patients registered at least one treatment-related AE (the most common were hyperglycemia [22.2%], thrombocytopenia [13.3%], and anemia [8.9%]) but grade 3–4 toxicity was only seen for hyperglycemia ( $n = 2$ , 13.3%); thrombocytopenia ( $n = 2$ , 13.3%); and anemia ( $n = 2$ , 13.3%). These results led to the permanent discontinuation of the treatment in three (20%) patients. In general, the doses of the different drugs in the combination received were well adjusted to what was expected, and there were no important dose losses due to delays, interruptions, or reductions. Thus, the results show that the AEs with this combination were manageable and in line with those reported for ruxolitinib.

The preliminary efficacy data show that most of the patients shifted from ECOG performance status scores of 1 and 2 at the screening to ECOG performance status scores of 0 and 1 at Cycles 4, 7, and 12. Most of the patients had a  $\geq 25\%$  reduction in palpable spleen size while receiving the study treatment. In addition, in most of the MPN-SAF

score items, the median individual symptom values improved from screening to Cycles 4, 7, and 12.

The so-called “add-on” approach (by adding a targeted agent to ruxolitinib for synergy) is currently attracting interest for patients showing insufficient responses to ruxolitinib monotherapy [24–28]. Since MF is a complex disease in which tyrosine-kinase-mediated signaling pathways are involved in the production of hematopoietic clones and with the fibrosis of the bone marrow, the use of drugs in combination against different targets may be beneficial. Previous data have shown that ruxolitinib combined with prednisone and nilotinib exhibits synergistic effects in human cell lines and primary cells from myeloproliferative neoplasms (MPN) [29]. Ruxolitinib showed the ability to diminish or stabilize fibrosis. Ruxolitinib plus nilotinib showed a synergistic behavior that blocked colony formation in patients’ primary cells and inhibited the phosphorylation of STAT5 and ERK1/2. In addition, the combination with prednisone increased this synergy and inhibited the synthesis of collagen in bone marrow mesenchymal cells [29].

Moreover, ex vivo PharmaFlow analyses in samples of patients treated with the combination showed a trend of correlations between the sensitive ex vivo classification and clinical responses, but a larger sample is needed to confirm these results. Regarding the influence of the mutational profile and the response at Cycle 7, five of the seven cases with a mutated *JAK2* gene and the two cases with a mutated *CALR* gene were determined to be responders. On the contrary, the only case with a mutated *MPL* gene was a non-responder. Although the series is small, the mutated genes associated especially with the nonresponder group were *PHF6*, *SRSF2*, *SH2B3*, and *ZRSR2*, and we observed no difference in response between *JAK2* and *CALR* patients.

The responses to ruxolitinib treatment are known to be transitory. Ruxolitinib rechallenge has shown limited effectiveness after a period of treatment interruption [30, 31]. *JAK2-V617F* leukemia cell lines could be re-sensitized to ruxolitinib after a period of withdrawal [31]. Nine of thirteen MF patients retreated with ruxolitinib after the loss of the initial response or an inadequate response registered significant spleen size reductions, and twelve of thirteen patients reported symptom improvement [30]. In this case series, ruxolitinib was ultimately discontinued in seven patients due to a loss of response, inclusion in another clinical trial, or intolerance to the treatment [30].

The main limitation of our study is that, due to the number of patients finally enrolled, it was not possible to comply with the evaluation characteristics of the MTD, and therefore, no data regarding the main objective of the trial were obtained. Another limitation is that seven intermediate-1-risk patients (46.7%) were included, making it difficult to assess the efficacy as compared with other studies. No differences in response between naïve and pretreated patients could be observed, probably due to the low number of patients. However, our study provides relevant information on the efficacy and safety of the combination, its correlations with ex vivo data, and the mutational landscape of the patients.

In conclusion, the current results provide evidence that ruxolitinib in combination with nilotinib and prednisone has therapeutic activity in MF, with a good safety profile.

## AUTHOR CONTRIBUTIONS

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## CONFLICT OF INTEREST STATEMENT

J.M.-L. declares honoraria for lectures; membership on advisory boards with Janssen, BMS, Sanofi, Novartis, Incyte, Roche, and Amgen; and membership on the boards of directors of Hosea and Altum Sequencing. R.A. is an advisory board member for Novartis and Incyte; received speaker honoraria from Astellas, Celgene, and Novartis; and is a member on the board of directors of Altum Sequencing. The remaining authors declare no competing financial interests.

## DATA AVAILABILITY STATEMENT

Questions regarding data sharing should be addressed to the corresponding author. For original data and study protocol, please contact Prof. Joaquín Martínez-Lopez at [jmarti01@med.ucm.es](mailto:jmarti01@med.ucm.es). Deidentified individual participant data that underlie the reported results will be made available 3 months after publication for a period of 5 years after the publication date.

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## SUPPORTING INFORMATION

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