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Brief Report

A phase I trial of pevonedistat in combination with ruxolitinib for the treatment of myelofibrosis

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***Abstract*:** Janus kinase 2 (JAK2) inhibitors such as ruxolitinib have become standard-of-care therapy for patients with myeloproliferative neoplasms (MPNs); however, activation of alternate oncogenic pathways including nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) has limited durable response as single-agent therapy. With the rationale of targeting both pathways, we conducted a phase I dose escalation trial of pevonedistat in combination with ruxolitinib for the treatment of patients with myelofibrosis (NCT03386214). The primary objective was to assess the safety and tolerability of combination therapy with additional objectives of treatment efficacy and alterations of biomarkers. There were no dose-limiting toxicities observed with most adverse events being limited to grades 1/2. In secondary measures, anemia response was observed in two patients. Pro-inflammatory cytokines and iron parameters were longitudinally assessed, which revealed suppression of interleukin-6 and interferon-gamma in a dose-dependent manner across a subset of patients. These results suggest that combination therapy targeting both JAK2 and NFκB may hold clinical merit for MPN patients.

Keywords: clinical trial, myeloproliferative neoplasms, MLN4924, NFκB, JAK2, pevonedistat, ruxolitinib

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Myeloproliferative neoplasms (MPNs) are driven by gain-of-function *JAK2*, *CALR*, or *MPL* mutations that lead to hyperactivation of Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling and propagation of proliferation cascades.[1,2] Despite demonstrating efficacy in the reduction of constitutional symptom burden and splenomegaly, JAK inhibition by ruxolitinib and other agents fails to eradicate underlying disease.[3] Furthermore, aberrant NFκB signaling mediates a pro-inflammatory microenvironment that contributes to disease progression and transformation in MPN.[4–7]

The NEDD8-activating enzyme inhibitor pevonedistat (MLN4924) has demonstrated capacity in NFκB pathway inhibition by preventing the degradation of IκBα.[8] Preclinical activity of pevonedistat has been demonstrated in myeloid neoplasms including acute myeloid leukemia (AML) and in lymphoid malignancies including chronic lymphocytic leukemia and B-cell non-Hodgkin lymphoma.[9–12] We previously showed that pevonedistat reduced disease burden across *ex vivo* primary samples and *in vivo* models of MPN, alone and in combination with ruxolitnib.[4,13] Based on our preclinical data, the combination of pevonedistat and ruxolitinib may provide greater clinical responses in patients with myelofibrosis (MF) compared to ruxolitinib monotherapy *via* inhibition of NFκB in addition to JAK-STAT signaling.

Clinically, the phase III PANTHER study evaluated the combination of pevonedistat plus azacitidine *versus* azacitidine alone as first-line treatment

for patients with higher-risk myelodysplastic syndromes (MDS), chronic myelomonocytic leukemia, and low-blast AML.[14] However, failure to achieve pre-defined statistical significance for the primary endpoint of event-free survival resulted in the termination of the development of pevonedistat for myeloid malignancies in 2021, although *post hoc* analysis revealed significant improvement in median overall survival in the higher-risk MDS cohort that received greater than three cycles of pevonedistat plus azacitidine.[14]

During this period from April 2018 to December 2021, we conducted a phase I dose escalation trial of pevonedistat in combination with ruxolitinib for the treatment of patients with myelofibrosis (NCT03386214). The primary objective was to assess the safety and tolerability of combination therapy. Additional secondary and exploratory objectives include evaluating spleen response, improvement of constitutional symptoms, and alterations of biomarkers following therapy. Inclusion criteria included patients with a confirmed diagnosis of primary or post-MPN myelofibrosis classified as high risk, intermediate-2 risk, or intermediate-1 risk by the International Prostate Symptom Score. All patients at enrollment were required to have received at least 12 weeks of prior ruxolitinib, been on a stable dose for at least 8 weeks, and have not achieved complete response. For all patients with anemia, and especially for patients with hemoglobin values $\leqslant$8 g/dL at screening or during the conduct of the study, RBC transfusions were considered before pevonedistat dosing. Additional inclusion and exclusion criteria are described in the Supplemental Material.

Pevonedistat was administered by intravenous infusion on days 1, 3, and 5 of each 28-day cycle. Consent was obtained from all patients and the trial was performed in accordance with its design as approved by the Institutional Review Board at Washington University School of Medicine and in accordance with the Declaration of Helsinki. Eight patients were enrolled with the study terminated in 2021 following discontinuation of pevonedistat development.

Patients received ruxolitinib as per their standard of care prior to enrollment in combination with 5 mg/m^2 pevonedistat (n = 3), 10 mg/m^2 (n = 3), and 20 mg/m^2 [n = 2; Figure 1(a), Supplemental Table 1]. On average, 7.4 (3–13) treatment cycles were completed. The median age of MF patients at enrollment was 64 years (range, 52–77); 75% (6) were male and 25% (2) were female (Supplemental Table 2). The average Dynamic International Prognostic Scoring System score was 3 (range, 1–5; Supplemental Table 3).

There were no dose-limiting toxicities observed. Most adverse events (AEs) were limited to grades 1 and 2 (Table 1). Constitutional AEs were observed in seven out of eight patients, most commonly nausea (37.5%), weight loss/anorexia (37.5%), and myalgia/weakness (50%), and overall were limited to grades 2 and below. Grade 3 and 4 hematological events included reduced platelets (25%), lymphocytes (12.5%), increased blasts (12.5%), and disease progression (12.5%; Supplemental Table 4). Gastrointestinal, respiratory, urinary tract, and blood infections grade 3 and above were seen in a minority of patients. There were seven severe AEs, including viral and bacterial infections, dyspnea, and disease progression to AML, all of which were deemed not to be related to treatment.

In secondary outcome measures, we first assessed anemia response, applicable for patients with a baseline hemoglobin level less than 10 g/dL for 8 weeks or more and required either $\geqslant$2 g/dL increase in hemoglobin level or becoming transfusion-independent defined as no RBC transfusions in the past 1 month. Of the best responses, anemia response was observed in two patients (25%). Symptom response, defined as $>$50% reduction in total symptom score, was also observed in one patient (12.5%). One patient (01-007) receiving the 20 mg/m^2 dose had spleen volume response greater than 35% reduction (SVR_{35}) by ultrasound [Figure 1(b)].

We also performed biomarker profiling in peripheral blood mononuclear cells and plasma. Genetic analysis of 40 genes recurrently mutated in myeloid malignancies using the MyeloSeq platform revealed stable variant allele frequencies throughout treatment in four out of five patients, with the other patient (01-005) demonstrating clonal evolution concurrent with accelerated MF disease progression [Figure 1(c)]. Across a pro-inflammatory cytokine panel, the addition of pevonedistat to ruxolitinib sustained suppression of interleukin (IL)-6 and interferon-gamma (IFN-γ) in a dose-dependent manner [Figure 1(d)].

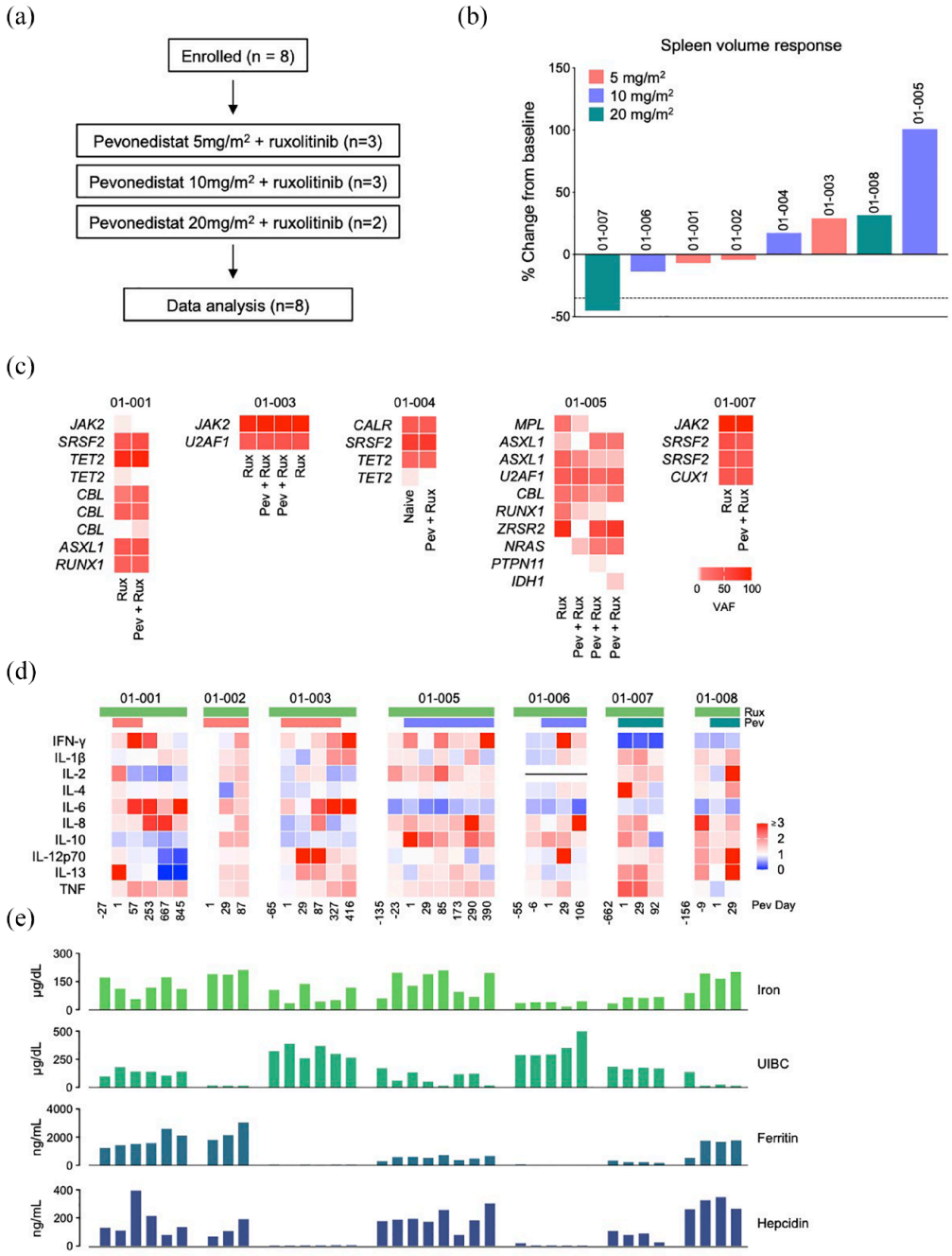

Figure 1. Outcome measures to study drug combination. (a) Flow chart of patient enrollment and for outcome measure analysis. (b) Spleen volume responses at study end-point relative to baseline assessed by ultrasound. (c) Variant allele frequencies by sequencing panel of peripheral blood mononuclear cells throughout treatment. (d) Plasma levels of pro-inflammatory cytokines throughout drug treatment. (e) Iron parameters in plasma throughout drug treatment.

IL-6, in particular, has been associated with adverse prognosis and remains elevated with ruxolitinib treatment,[15,16] which was also observed here in patients who received a low pevonedistat dose. Lastly, iron hemostasis has been found to be dysregulated in MPN, where factors including chronic inflammation upregulate the key iron regulator hepcidin.[14] Elevated

Table 1. All-grade AEs to study drugs.

AE (unique patients)	Grade			
	1	2	3	4
Hematological ($n=5$)				
Decreased platelet count			1	2
Decreased lymph count			1	1
Increased blast count				1
Anemia		1		
Disease progression				1
Gastrointestinal ($n=7$)				
Nausea	3			
Vomiting	1			
Diarrhea		2		
Infection/ gastroenteritis			2	
Abdominal pain		2		
Reflux	1			
Diverticulitis flare		1		
Dyspepsia		1		
Black stool	2			
Constipation	1			
Cardiac ($n=1$)				
Palpitations	1			
Constitutional ($n=7$)				
Dizziness		1		
Headache	1			
Tingling, numbness	1			
Fever	2			
Chills	2			
Fatigue		1	1	
Night sweats		1		
Loss of appetite, early satiety	1	1		
Weight loss, anorexia	3	1		
Myalgia, weakness	4	1		
Insomnia		1		
Skin ($n=4$)				
Pruritis		1		
Allergic reaction		1		
Alopecia	1			
Mouth sores	1			
Flushing	1			
Edema	2			
Infusion site extravasation		1		
Respiratory and infection ($n=5$)				
Dyspnea	1		2	
Pharyngeal mucositis	1			
Sore throat		1		
Nasal congestion		1		
Sinusitis	1	1		
COVID-19 pneumonia			1	
Urinary tract infection			1	
Positive blood culture			1	
Tooth infection		1		
Metabolic ($n=3$)				
Hypokalemia		1		
Hyperkalemia	1			
Hyperuricemia	2			
Hypophosphatemia			1	
Dehydration	1			
Increased creatinine	1			
Renal ($n=2$)				
Calculi			1	
Urinary frequency	1			

AE, adverse event.

hepcidin in MF patients leads to reduced iron availability and restricts erythropoiesis to cause anemia.[17] In evaluating iron homeostasis, two patients (01-006 and 01-007) receiving higher pevonedistat doses demonstrated a sustained reduction in plasma hepcidin through treatment [Figure 1(e)].

In summary, a combinatory regimen of ruxolitinib and pevonedistat was well tolerated with no dose-limiting toxicities in this phase I trial. While the study was terminated prematurely due to discontinuation of pevonedistat development, therapeutic efficacy was observed in a subset of patients including reduction of canonical MPN symptoms and pathological biomarkers. As such, targeting NFκB in MPN and other hematological malignancies by other means (e.g. Bromodomain and Extra-Terminal motif inhibitors) warrants further investigation.

Declarations

Ethics approval and consent to participate

Consent was obtained from all patients and the trial was performed in accordance with its design as approved by the Institutional Review Board at Washington University School of Medicine.

Consent for publication

Not Applicable.

Author contributions

Tim Kong: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing – original draft; Writing – review & editing.

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Competing interests

STO has served as a consultant for Kartos Therapeutics, CTI BioPharma, Celgene/Bristol Myers Squibb, Disc Medicine, Protagonist, Blueprint Medicines, Cogent, PharmaEssentia, Constellation, Geron, Abbvie, Sierra Oncology, and Incyte.

Availability of data and materials

Data are available from the corresponding author upon reasonable request.

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Supplemental material

Supplemental material for this article is available online.

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