Published in final edited form as:

Leuk Res. 2012 September ; 36(9): 1124–1127. doi:10.1016/j.leukres.2012.03.003.

THERAPY WITH THE HISTONE DEACETYLASE INHIBITOR PRACINOSTAT FOR PATIENTS WITH MYELOFIBROSIS

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Abstract

Approximately half of the patients with myelofibrosis (MF) carry mutant JAK2^{V617F} proteins. JAK2^{V617F} has been recently shown to translocate to the nucleus and modify specific histones, thus regulating transcription. We report on a phase II study testing the activity and tolerability of the histone deacetylase inhibitor pracinostat given at 60mg every other day for three weeks per month in 22 patients with intermediate or high risk MF. Eight (36%) patients experienced clinical benefit, with 6 (27%) experiencing reductions in splenomegaly (median 3cm, range 1–4cm). According to International Working Group criteria, 2 (9%) patients had clinical improvement (anemia response in both cases). The most frequent side effect associated to pracinostat therapy was fatigue, which occurred in 20 (91%) patients (grade 2 in 3 patients). Grade 3–4 neutropenia, anemia, and thrombocytopenia occurred in 13%, 0%, and 21%, respectively. Twenty-one patients permanently discontinued pracinostat, mainly due to lack of efficacy. In conclusion, pracinostat at the dose tested is reasonably tolerated and has modest activity in patients with MF.

Keywords

myelofibrosis; pracinostat; SB939; histone deacetylase inhibitor; JAK2

Introduction

Primary myelofibrosis (MF) is a myeloproliferative neoplasia (MPN) that results from the malignant transformation of a hematopoietic stem cell.¹ Of all the BCR-ABL1-negative MPNs, MF is the most debilitating and associated with a worse prognosis. In a recent analysis from the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT), the median survival of patients with MF was 69 months.² The Janus kinases

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Contributions: AQ-C and SV designed research, analyzed data, and wrote the manuscript. HK, ZE, GB, and JC accrued patients to the study and approved the manuscript.

Conflict of interest: AQ-C and SV have received research funding from SBIO. All other authors have no conflict of interest.

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(JAK) JAK1, JAK2, TYK2, and JAK3 are cytoplasmic protein tyrosine kinases that engage with cytokine receptors and convey signals to the cell nucleus via phosphorylation of downstream transcription factors such as signal transducer and activator of transcription 5 (STAT5).^{3,4,5} A recurrent G-to-T mutation at nucleotide 1849 of the JAK2 gene (JAK2^{V617F}) is present in approximately half of patients with MF.^{3,6,7,8,9} The discovery of the JAK2^{V617F} mutation has fueled the development of small molecule inhibitors to target the activity of this mutant enzyme. Several JAK2 inhibitors have shown remarkable activity in clinical studies for patients with MF and some are currently being tested in multicenter phase III studies, demonstrating significant improvement in response rates, particularly reduction in splenomegaly and resolution of constitutional symptoms, over standard therapy.^{10,11} However, several clinical manifestations of MF are not significantly affected by JAK2 inhibitor therapy, including improvement of cytopenias, reduction of bone marrow fibrosis, or reduction of the JAK2^{V617F} allele burden.¹² These limitations of JAK2 inhibitor therapy suggest that these agents may not address all activities of the mutated JAK2^{V617F} protein and that combinatorial approaches may be necessary to therapeutically encompass all facets of the pathogenesis of MF.

It has been recently shown that, in addition to activating STAT transcription factors, JAK2 kinase can directly activate transcription.¹³ Both, wild-type JAK2 as well as JAK2^{V617F} kinases translocate to the nucleus of human leukemic cells and primary CD34⁺ hematopoietic progenitors and phosphorylate histone H3 at tyrosine 41 (H3Y41). There, JAK2 appears to be the only kinase responsible for H3Y41 phosphorylation as treatment with JAK2 inhibitors abrogates the phosphorylation of nuclear H3Y41.¹³ In *Drosophila*, JAK2 kinase activation disrupts the binding of the transcriptional repressor heterochromatin protein 1 α (HP1 α) from chromatin. Interestingly, the affinity of HP1 α for histone H3 depends on the phosphorylation status of H3Y41. Indeed, H3Y41 phosphorylation reduces the affinity of H3 to HP1 α . JAK2 inhibitors ablate H3Y41 phosphorylation and increase chromatin-bound HP1 α in cells, thus repressing HP1 α -regulated genes. Of note, most JAK2-regulated genes do not contain a predicted STAT5 binding site, suggesting that these genes are regulated by signals other than JAK2-STAT5 pathway. Of special interest among these genes is *lmo2*, which is involved in normal hematopoiesis and in leukemogenesis.¹⁴ JAK2 inhibitors decrease H3Y41 phosphorylation and increase HP1 α binding at the *lmo2* transcriptional start site, thereby resulting in downregulation of *lmo2* expression. Thus, the JAK2-H3Y41-HP1 α pathway links JAK2 kinase activity to histone phosphorylation, aberrant gene expression and genome instability, and provides the rationale for the use of histone modifying agents, ideally in combination with JAK2 inhibitors, for the treatment of JAK2-driven malignancies, in an attempt to suppress all pro-tumorigenic activities emanating from JAK2^{V617F} kinase.

Pracinostat (formerly SB939, SBI-0, Singapur) is a novel orally bioavailable dialkyl benzimidazole competitive histone deacetylase inhibitor (HDACi) that has over 1000-fold selectivity for HDAC class 1 and 2 *versus* class 3. Pracinostat has shown antitumor activity in xenograft models of acute myeloid leukemia (MV4-11) and B-cell lymphoma (Ramos), as well as in solid tumors.¹⁵ We tested the activity and tolerability of pracinostat in a phase II study for patients with MF.

Patients and methods

Eligibility criteria

Patients with a diagnosis of MF (primary, post-essential thrombocythemia, or post-polycythemia vera) with intermediate-1, intermediate-2 or high risk disease according to the International Working Group (IWG) prognostic scoring system, or with low risk and symptomatic splenomegaly measuring at least 5 cm below left costal margin were eligible. Patients were required to have an Eastern Cooperative Oncology Group performance status 2, serum creatinine less than 2.0 mg/dL, serum bilirubin 2.0 times the upper limit of the normal range, and normal cardiac function. Eligible patients were not allowed to receive chemotherapy (including hydroxyurea) for at least 2 weeks before entering the study. All patients signed an informed consent approved by the M. D. Anderson Cancer Center Institutional Review Board.

Patient assessment and treatment schedule

Screening studies included a complete physical examination, complete blood cell (CBC) count, comprehensive biochemistry panel (including liver function tests), pregnancy test (in female patients), and bone marrow (BM) aspiration and biopsy with cytogenetics and *JAK2*^{V617F} testing and quantitation. Follow-up evaluations included complete physical examination, 12-lead ECG, CBC, and biochemistry panel every 2 weeks during the first cycle (28-day cycles), every cycle during cycles 2 and 3, and every 3 cycles subsequently. *JAK2*^{V617F} allele burden was quantified in peripheral blood every 3 months.¹⁶ Toxicity was evaluated using the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0). Response to pracinostat was assessed using the IWG criteria.¹⁷

The initial dose schedule of pracinostat was 60mg every other day three times weekly for three weeks. The starting dose could be modified according to tolerance, with stepwise 10mg dose reductions to 50mg and 40mg, respectively. Pracinostat was administered for as long as the patient obtained clinical benefit. The dose was escalated to 80mg in the absence of clinical benefit and significant toxicity for 4 weeks. Therapy was continued, if reasonably tolerated, in the event of grade 1 or 2 toxicity. In the event of persistent significant drug-related grade 2 toxicity or any grade 3 or 4 toxicity, therapy was interrupted until resolution of the toxicity to grade 0 or 1 and resumed at the immediate lower dose level.

Statistical considerations

This was a prospective, open-label single-center, phase II study with an implemented minimum value/maximum value (MinMax) two-stage design. The primary end-point was objective clinical response. The initial target response rate was 35%. A response rate of 20% was considered unacceptable and pracinostat therapy was to be discontinued. Given the response rates stated above, if the probability of inappropriately accepting a poor therapy was 10%, a total sample size of 41 patients resulted in 80% power. The statistical analysis for response rates was determined on an intention-to-treat basis. In the first stage of the design, a total of 22 patients were enrolled. If four or fewer patients responded to the pracinostat after 6 months of therapy, the study was to be terminated and the therapy would be declared ineffective but as soon as five or more patients were found to respond to

pracinostat, enrollment was to be resumed. However, after accrual of the first 22 patients to the study, the sponsor decided to stop accrual for financial reasons.

Results

Patient characteristics

Twenty-three patients (17 male) were accrued but only 22 received therapy and therefore are evaluable for response and toxicity assessment (Table 1). Of them, 18 (82%) carried the JAK2^{V617F} mutation, with a median baseline allele burden of 59.66% (range, 19.85% to 94.95%). Seven (33%) patients had abnormal cytogenetics at study entry, including 2 patients who had a complex karyotype. Most patients (n=20) were symptomatic at study entry with a performance status of 0 (n=1 [5%]), 1 (n=16 [73%]), or 2 (n=5 [22%]). The majority of patients entered the study having splenomegaly (n=21 [95%]), with a median spleen size of 13cm beneath the left costal margin (range, 0 to 29cm).

Response to pracinostat

The current median follow-up is 5 months (range, 1 to 11.3 months). Overall, 8 (36%) patients experienced clinical benefit from pracinostat therapy. Although when analyzed as a whole, the cohort of patients did not experience a significant reduction in spleen size, 6 (27%) patients experienced a reduction in splenomegaly (median reduction 3cm, range 1 to 4cm) (Figure 1). However, no patient had a response that could be classified as splenomegaly clinical improvement by IWG criteria (i.e. spleen reduction of at least 50% from baseline). When only responders were considered, the reductions in spleen size from baseline were significant (p=0.02). Two patients had anemia clinical improvement by IWG criteria, experiencing increments in their hemoglobin level from 9.1 g/dL at baseline to 11.1 g/dL and from 7.9 g/dL to 15.3 g/dL at last follow-up, respectively. Five (50%) of 10 patients with hepatomegaly reduced their liver size by a median of 3cm (range, 1cm to 6cm). Three (17%) of 18 JAK2^{V617F}-positive patients had reductions of allele burden (median 11.4%). However, no significant differences in JAK2^{V617F} allele burden reduction were observed when the entire cohort of JAK2^{V617F}-positive patients was analyzed as a whole (Figure 2). In summary, although a significant number of patients exhibited signs of clinical activity to pracinostat, only 2 patients achieved a response according to IWG criteria (2 anemia responses as described).

Adverse effects during pracinostat therapy

While all patients started pracinostat at 60mg daily, 8 of them required at least one dose reduction to 50mg and 1 patient required two dose reductions to 40mg daily. Conversely, 6 patients had their dose of pracinostat increased to 80mg daily due to lack of efficacy. Eight patients continued therapy at 60mg daily. The most frequent side effect associated to pracinostat therapy was fatigue, which occurred in 20 (91%) patients, which was grade 1 in 17 and grade 2 in 3 patients. Other toxicities included pain (n=5), peripheral edema (n=4), and diarrhea (n=3), all grade 1. Rates of grade 3–4 neutropenia, anemia, and thrombocytopenia were 13%, 0%, and 21%, respectively. No patient has died during the conduction of the study and 1 remains on the study receiving pracinostat at 50mg daily. Twenty-one patients are off study due to lack of response (n=9), disease progression (n=6),

patient's request (n=2), unrelated medical problems (n=3: surgery for aortic aneurism, prostate cancer, infection), fatigue (n=1).

Discussion

We report on the activity and tolerability of the HDACi pracinostat in a phase II study for patients with MF. A phase I study of pracinostat in patients with advanced hematologic malignancies established the maximum tolerated dose as 120 mg every other day three times per week. A dose of 100 mg every other day three times per week was declared the recommended phase II dose.¹⁵ Because fatigue was frequently observed during pracinostat therapy (44% overall, 16% grade 3–4),¹⁵ we selected a dose of 60 mg for our phase II study in patients with MF, whom frequently present with constitutional symptoms including fatigue. Overall, pracinostat therapy was safe but even at an initial dose of 60 mg, a significant number of patients required dose reductions, interruptions, and eventually a high number of patients required treatment discontinuation due to lack of efficacy or toxicity, with fatigue being reported in most patients. However, a number of patients clearly benefited from single agent pracinostat therapy, either in terms of decrease spleen size or regarding improvements in anemia.

The rationale for the use of histone modifiers in MF relies on the fact that both JAK2 as well as JAK2^{V617F} can translocate to the cell nucleus and phosphorylate specific lysine residues at histone H3 (H3Y41).¹³ The affinity of the transcriptional repressor heterochromatin protein 1α (HP1α) for histone H3 depends on the phosphorylation status of H3Y41. Phosphorylation of H3Y41 decreases the affinity of H3 to HP1α. JAK2 inhibitors such as TG101209 or AT9283 abrogate nuclear H3Y41 phosphorylation, which increases chromatin-bound HP1α in cells, thus leading to repression of HP1α-regulated genes such as *lmo2*.¹³ Of note, *lmo2* is involved in leukemogenesis.¹⁴ Thus, the JAK2-H3Y41-HP1α pathway links JAK2 kinase activity to histone modifications, aberrant gene expression and leukemogenesis, thus providing the rationale for the use of agents that target histone modifications in combination with JAK2 inhibitors to more thoroughly suppress JAK2^{V617F} signaling in JAK2-driven malignancies.

As single agents, the activity of other HDACi has been tested in MF. Givinostat (ITF2357) was tested in a phase II study that included patients with PV (n=12), ET (n=1), and MF (n=16) carrying the JAK2^{V617F} mutation.¹⁸ Patients received givinostat at the starting dose of 50 mg twice daily for a median of 20 weeks. Dose reductions and treatment interruptions were frequent and 10 patients required treatment termination. Responses consisted mainly in reduction of peripheral blood counts, symptom improvement, and/or spleen reductions. Indeed, pruritus resolved in the majority of patients affected and a reduction in splenomegaly size was documented in 75% of patients with ET or PV and in 38% of those with MF. Reductions in JAK2^{V617F} allele burden were modest.¹⁸ The potent pan-deacetylase inhibitor panobinostat has been tested in a phase II study for patients with MF and an IPSS score of 2 or 3 and symptomatic splenomegaly larger than 10cm below the left costal margin or anemia.¹⁹ The initial panobinostat dose schedule was 40 mg three times a week. Preliminary results indicate that most of the 31 patients enrolled (24 carrying the JAK2^{V617F} mutation) required dose reductions. Therapy with panobinostat depleted

phospho-STAT3, phospho-STAT5, phospho-AKT, phospho-ERK1/2, and phospho-PIM proteins. Interestingly, panobinostat induced a reduction of Mcl-1 and Bcl-x_L and reductions in JAK2^{V617F} allele burden ranging from 10% to 90% were observed in 10 patients, suggesting a direct effect on the malignant clones.¹⁹ The final results of a separate phase I study of panobinostat in 18 patients with intermediate or high risk MF have been reported.¹⁹ The recommended phase II dose was 25 mg orally three times per week. Five patients entered an extension phase of the study and received panobinostat for at least 6 months. Three of the patients had clinical improvement by IWG criteria. The mean reduction in palpable splenomegaly at 6 months was 83% and 2 patients had durable improvement in anemia, one of which also exhibited significant reduction of reticulin and collagen fibrosis.¹⁹ These data suggest that a lower dose of panobinostat (25 mg three times weekly or lower) may result in longer and safer panobinostat exposure in patients with MF, leading to amelioration of symptoms and signs of the disease and perhaps even reversal of marrow fibrosis over time.

Given the activity of single agent HDACi therapy, it is provocative to think that a combination of these agents with JAK2 inhibitors might be synergistic in patients with MF. Preliminary data from a Scid-beige mouse model in which animals are injected with Ba/F3-EpoR JAK2^{V617F}-luciferase cells to induce an MPN syndrome appear to support this hypothesis.²⁰ The combination of the JAK2 inhibitor ruxolitinib and the HDACi panobinostat produced a greater reduction in tumor burden assessed by bioluminescence compared to the administration of each agent alone.²⁰ A clinical trial with these two agents in patients with MF is currently underway.

In conclusion, pracinostat has modest activity as single agent in patients with MF. Most responses consist of reductions in spleen and/or liver size. Improvements in anemia occur in a minority of patients. A dose of pracinostat lower than 60 mg is recommended, particularly if used in combination with other agents.

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Figure 1.

Dynamics of splenomegaly reduction during pracinostat therapy for all patients with splenomegaly (n=21) and for those that responded to pracinostat therapy (n=6).

Figure 2.
Dynamics of JAK2^{V617F} allele burden during pracinostat therapy (n=18)

Table 1

Patient and disease characteristics

Characteristic	Median (range)	No.	Percentage
Age (median)	67 (51–74)		
Sex			
Male		17	77
Female		5	23
Race			
Caucasian		20	91
Other		2	9
Median hemoglobin (range)	9.4 (8.4–14.3)		
Median WBC (range)	12.25 (2–46.4)		
Median platelet count (range)	146 (21–572)		
No. JAK2 ^{V617F} -carriers (%)		18	82
Median % JAK2 ^{V617F} allele burden (range)		59.66 (19.85–94.95)	
Symptoms	Yes	20	91
	No	2	9
Splenomegaly (%)	Yes	21	95
	No	1	5
Spleen median (range) size (cm)	13 (5–29)		
	0	1	5
Performance status	1	16	73
	2	5	22
	Diploid	14	64
Cytogenetics (%)	Abnormal	7	32
	Insufficient metaphases	1	4