Published in final edited form as:

Leuk Res. 2014 March ; 38(3): 316–322. doi:10.1016/j.leukres.2013.12.006.

Phase I evaluation of XL019, an oral, potent, and selective JAK2 inhibitor

Srdan Verstovsek^{a,*}, Constantine S. Tam^{a,1}, Martha Wadleigh^b, Lubomir Sokol^c, Catherine C. Smith^d, Lynne A. Bui^e, Chunyan Song^e, Douglas O. Clary^e, Patrycja Olszynski^d, Jorge Cortes^a, Hagop Kantarjian^a, and Neil P. Shah^d

^aThe University of Texas MD Anderson Cancer Center, Houston, TX, USA

^bDana-Farber Cancer Institute, Boston, MA, USA

^cH. Lee Moffitt Cancer Center & Research Institute, Tampa, FL, USA

^dUniversity of California, San Francisco (UCSF), San Francisco, CA, USA

^eExelixis, Inc., South San Francisco, CA, USA

Abstract

This phase I study evaluated selective JAK2 inhibitor XL019 in 30 patients with myelofibrosis. The initial dose cohorts were 100, 200, and 300 mg orally on days 1–21 of a 28-day cycle. Central and/or peripheral neurotoxicity developed in all patients. Subsequently, patients were treated on lower doses; neurotoxicity was again observed, leading to study termination. Peripheral neuropathy resolved in 50%, and central neurotoxicity in all patients within months after therapy cessation. Myelosuppression was minimal. The terminal half-life of XL019 was approximately 21 h, with steady state reached by Day 8. International Working Group defined responses were seen in three (10%) patients.

Keywords

Myelofibrosis; JAK2; XL019; Mutation; Inhibitor

1. Introduction

Myelofibrosis (MF) is a clonal proliferative disease of hematopoietic stem cells [1]. The incidence of MF has been estimated to be 1.46 per 100,000 in the population [2], and the

© 2013 Published by Elsevier Ltd.

*Corresponding author at: The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 428, Houston, TX 77030, USA. Tel.: +1 713 745 3429; fax: +1 713 745 0930. sverstov@mdanderson.org, kjnewber@mdanderson.org (S. Verstovsek).

¹Current address: Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia.

Contributions. SV, MW, LS, CCS, CS PO, JC, HK and NPS conducted the study, analyzed data and reviewed the paper. SV, CST and NPS analyzed clinical data and wrote the manuscript. LAB, CCS, CS and DC designed the study, analyzed clinical and correlative data, and reviewed the paper.

Conflict-of-interest

SV, MW, LS, JC and NPS received research support from Exelixis for the conduct of clinical studies. CS and DC hold stock in Exelixis, of which DC is an employee. The remaining authors declare no competing financial interests.

disease results in excessive release of cytokines, bone marrow fibrosis and cytopenias, constitutive mobilization of committed progenitor cells into the peripheral blood, and extramedullary hematopoiesis [3]. MF is characterized by a progressive clinical course and a shortened life expectancy, with up to 30% of patients transforming to acute myeloid leukemia (AML) [4].

Until recently, the main treatment goal for most patients with MF was palliation of symptoms related to massive splenomegaly and anemia. Currently, allogeneic stem-cell transplantation is the only potentially curative intervention for patients with primary MF [4]. The range of potential treatments of patients with MF was substantially changed by the discovery that the majority of PMF or post-ET MF, and nearly all of those with MF subsequent to PV, have the janus kinase 2 (JAK2) V617F mutation [5]. While the role of this mutation in MF is not fully understood, hyperactive JAK–STAT intracellular signaling pathway has become an important target for recently approved and emerging MF treatments such as ruxolitinib [6–10]. Ruxolitinib is an inhibitor of JAK1 and JAK2 tyrosine kinases and likely derives its benefit by a combination of anti-proliferative (inhibition of JAK2) and anti-inflammatory (inhibition of both JAK1 and JAK2) activity. In the majority of patients ruxolitinib significantly reduces enlarged spleen and controls MF-related constitutional symptoms [6,7,9]. Recent results suggest that with better control of the signs and symptom in MF patients, ruxolitinib therapy may provide a survival advantage over other therapies used to treat MF [11,12]. Ruxolitinib is not selective for JAK2V617F mutated protein and is equally active in patients with and without JAK2V617F mutation. Whether the inability of ruxolitinib to affect more pronounced disease responses in MF patients with JAK2V617F mutation reflects a non-essential role of JAK2V617F in the maintenance of MF remains unclear. Myelosuppression with ruxolitinib has been dose-limiting, as is the case with most other JAK inhibitors in the development, and is likely related to its inhibition of wild-type JAK2 protein that is essential for normal hematopoiesis [6,7,9].

XL019 is a potent and selective JAK2 inhibitor. XL019 shows 50-fold or greater selectivity for JAK2, versus a panel of over 100 serine/threonine and tyrosine kinases, including other members of the JAK family (Table 1). In assays performed using ATP-dependent luciferase-coupled chemiluminescence, the IC₅₀ of XL019 for JAK2 was 2.3 nM, compared with IC₅₀ of 134 nM for JAK1, JAK3 and TYK2. XL019 is non-selective for JAK2V617F or wild-type JAK2, and potently inhibits STAT3 and STAT5 phosphorylation in cells harboring either JAK2V617F or wild-type JAK2 [13–15]. In rat and dog studies, the major toxicity of XL019 was dose-dependent myelo-suppression; neurological toxicity was not seen. Here we report the phase I experience of XL019 in patients with advanced MF.

2. Materials and methods

2.1. Study design and XL019 dosing

This was a phase 1, multicenter, open-label, dose-escalation study of XL019 administered as a single agent orally in subjects with PMF, post-PV MF, or post-ET MF, irrespective of JAK2 mutation status, whose proportion of blasts in the bone marrow did not meet or exceed 20 percent (registered at www.clinicaltrials.gov as # NCT00595829). The original protocol used data from animal toxicity studies to guide the starting dose (100 mg daily) and

schedule (active drug on days 1–21, rest on days 22–28, repeated in 28 day cycles). Patients were treated in cohorts of three, with dose-escalation if no dose-limiting toxicities (DLT) were observed at the previous dose level. Thus, 9 patients were enrolled at dose levels of 100 mg ($n = 3$), 200 mg ($n = 3$) and 300 mg ($n = 3$). The period for DLT surveillance was initially 4 weeks, but after the emergence of peripheral neuropathy (mainly outside of the DLT window) in most patients, the study was suspended. The protocol was then amended to reduce the starting dose, increase number of patients per cohort, and incorporate prolonged and targeted surveillance for neurotoxicity, but also to modify the schedule (to eliminate resting period during the third week of the 4 week cycle, due to observed loss of a therapy-induced clinical benefit during that week in some of the initial 9 patients). Patients with pre-existing peripheral neuropathy were excluded from participation, and study patients were monitored by specialist neurologists. Thus, the remaining 21 patients were treated in 28-day cycles with XL019 in subsequent order: 25 mg continuously daily ($n = 8$), 25 mg Monday/Wednesday/Friday (25 mg TIW, $n = 8$), and 50 mg continuously daily ($n = 5$). Due to emerging safety data, however, enrollment of further patients was paused in November 2008, and formally closed in May 2009.

2.2. Subjects

Subjects included in the trial were ≥ 18 years of age with PMF, post-PV MF, or post-ET MF that required therapy. Subjects were previously treated with MF-directed therapy and had been refractory or had relapsed; previously untreated subjects were permitted to enroll if they had a symptomatic spleen that was ≥ 10 cm below left costal margin on exam of abdomen, or Lille intermediate- or high-risk disease [16]. Subjects were also required to have an Eastern Cooperative Oncology Group performance status ≤ 2, adequate organ function (serum creatinine ≤ 1.5 times upper limit of normal (ULN), bilirubin ≤ 2.0 mg/dL, and ALT/AST ≤ 2.5 times ULN if no liver involvement or ≤ 5 times ULN if liver involved with MF), and adequate marrow reserve (absolute neutrophil count $\geq 1.0 \times 10^9/L$, platelet count $\geq 50 \times 10^9/L$).

2.3. Endpoints

2.3.1. Safety—Safety was assessed by standard clinical findings, recording of AEs, concomitant medications, electrocardiogram (ECG), and laboratory tests. Following protocol amendment, patients received targeted monitoring for neuropathy every month by treating physicians and by neurologists every three months.

2.3.2. Efficacy—Treatment response was evaluated for spleen and hematologic responses at specified intervals using the MF response criteria developed by the 2006 International Working Group for Myelofibrosis Research and Treatment (IWG-MRT [17]). To meet IWG-MRT criteria, responses must be maintained for ≥ 8 weeks. Exploratory endpoints included monthly monitoring for molecular response (reduction in JAK2V617F allele burden) in peripheral blood samples using PCR assays specific for the wild type and V617F alleles of JAK2, and changes in plasma cytokines assessed by comparing baseline and on-treatment plasma samples (samples from Day 1 (4 h post dose), Day 22, and Day 29 were evaluated) using a multiplex Luminex-based format (Millipore).

2.4. Pharmacokinetics

Pharmacokinetic analysis of plasma samples from 26 subjects dosed in six cohorts (25–300 mg qd, 25 mg qMWF) was performed. This was non-compartmental analysis of plasma concentration-time profile for each subject using WinNonlin version 5.2. Pharmacokinetic sampling time-points were: [A] Days 1 and 22 of Cycle 1: pre-dose; at 30 min and 1, 2, 4, and 8 h post-dose; Day 2: pre-dose (Schedule A) or 24 h post dose (Schedule B); Day 8: pre-dose and 4 h post-dose; Day 15: pre-dose; Day 23: pre-dose; and [B] Days 1 and 15 of Cycle 2 and 3: pre-dose and 4 h post-dose. In addition for cycles 4 and up, Day 1 pre-dose samples were collected.

2.5. Kinase domain sequencing

Samples obtained from two patients who experienced a decrease in bone marrow blasts while on therapy with XL019, were analyzed at the time of disease progression. Both patients gave informed consent according to the Declaration of Helsinki to participate both in the collection of non-protocol-specified samples. For sequencing, Ficoll-purified mononuclear cells obtained from blood or bone marrow were lysed in Trizol (Invitrogen) and RNA was isolated according to manufacturer protocol. cDNA was synthesized using Superscript II (Invitrogen) per manufacturer's protocol. The JAK2 JH1 (kinase domain) and adjacent JH2 (pseudokinase) domain were PCR amplified from cDNA using primers JH2-JH1F (5'-CCAGATGGAACTGTTCGCTCAGA-3') and JH2-JH1R (5'-ACTGGTGGCCTCATGAAGAA-3'). PCR products were cloned using TOPO TA cloning (Invitrogen) and transformed into competent *E. coli*. Individual colonies were plucked, expanded in liquid culture overnight and plasmid DNA for sequencing was isolated using the QIAprep Spin Miniprep kit (Qiagen). Each colony was considered representative of a single mRNA. The primers JH1F (5'-TCACATAAACTTCTGCAGTACACA-3'), JH1R (5'-TTGGTTAACCCAAAATCTCCA-3'), JH2F (5'-TGCTAAACAGTTGGCATGG-3') and JH2R (5'-GGAGGATTCCTGTCTTCCTG-3') were used for bidirectional sequencing. Alignments the wild type JAK2 sequence were performed using Sequencher software (Gene Codes Corporation).

3. Results

3.1. Patients

Thirty patients were enrolled in this study and received study treatment at four sites in the United States. The demographic and clinical characteristics are summarized in Table 2. Median age was 63 years, and 50% of patients were female. The diagnosis was PMF in 17 (57%), post-PV MF in 9 (30%), and post-ET MF in 4 (13%). The median time since initial diagnosis was 2.6 years. The JAK2V617F mutation was present in 22 patients (73%), and one (3%) had the MPLW515L mutation. Ten (33%) patients had clinical hepatomegaly, and 18 (60%) had an enlarged spleen; for patients with clinical splenomegaly, the median extension below left costal margin was 15 cm. Twenty patients (67%) had received prior treatment with a median 3 lines of therapy. The majority of patients (73%) had an ECOG status of 0 or 1.

3.2. Treatment duration and patient disposition

Patients received XL019 for a median of 91 days (range, 4–960 days). All patients have discontinued XL019 therapy. The last patient on the study started treatment in July 2008, achieved and maintained a clinical response on XL019 until March 2011, when treatment discontinuation was necessitated by impending expiration of study drug supply. In the remaining patients, reasons for cessation were: adverse event (38%), investigator decision (21%), patient withdrew consent (14%), death (7%), sponsor decision (7%), disease progression (3%) and other (10%).

3.3. Safety

3.3.1. Neurotoxicity—The study was terminated because of the occurrence of neurotoxicity. The patterns of neurotoxicity can be summarized as (1) early occurrence of central nervous system (CNS) toxicity including dizziness, confusion, word-finding difficulty, slurred speech, and balance disorder; and (2) later onset of classical “glove & stocking” sensory peripheral neuropathy.

In the initial three cohorts (XL019 100–300 mg daily, days 1–21 of 28-day cycle), neurotoxicity occurred in all patients. Transient, grade 1–2 CNS toxicity occurred in 2 of 3 patients in each of cohorts 1 (100 mg) and 2 (200 mg); these effects resolved with continued dosing. Subsequently, all 6 patients who received 100–200 mg per day developed grade 1–2 peripheral neuropathy. In the highest dose cohort (300 mg), two patients developed CNS toxicity (1 each of grades 2 and 3), leading to cessation of XL019 in both. The third patient on 300 mg developed grade 2 peripheral neuropathy, and ceased drug therapy. Subsequent experience with all six cohorts showed that the median time to onset of peripheral neuropathy was 2.4 months; thus, the occurrence of peripheral neurotoxicity was not initially apparent in the first 3 cohorts, which focused on finding DLT within 4 weeks of starting XL019.

Patients on the lower dose cohorts (25 mg daily, 25 mg TIW, and 50 mg daily) received additional and prolonged surveillance for neuropathy. CNS toxicity occurred in 3 of 8 (38%) of patients receiving 25 mg TIW, 4 of 8 (50%) of patients receiving 25 mg daily, and 2 of 5 (40%) of patients receiving 50 mg daily. In general, with the exception of one case (grade 5 encephalopathy and aspiration pneumonia leading to death, see below), CNS toxicities were low grade in nature and in those who stopped therapy would resolve within a month. Five of 8 patients with low-grade CNS toxicity continued XL019 treatment, with complete resolution of toxicity in four. Peripheral neuropathy occurred in 1 (13%) of patients on 25 mg TIW, 3 (38%) of patients on 25 mg daily, and 3 (60%) of patients on 50 mg daily. All were grade 1–2 in severity. Five of 8 (63%) of patients with peripheral neuropathy ceased XL019 on account of neurotoxicity. All patients who developed neurotoxicity have since stopped XL019 treatment. Of the ten patients who stopped XL019 therapy at the time of peripheral neuropathy, 5 (50%) recovered fully within few months, 4 (40%) recovered with sequelae, and 1 (10%) failed to recover.

3.3.2. Other adverse events—Non-neurologic adverse events are summarized in Table 3. The most common adverse events regardless of relationship to XL019 were fatigue

(30%), nausea (30%), peripheral edema (23%), hyperkalemia (20%), abdominal pain (17%), diarrhea (17%), constipation (17%) and pain in extremity (17%). Notably, hematologic events were infrequent: anemia and thrombocytopenia (all grades) attributable to XL019 therapy occurred in 7% and 3% of patients, respectively.

3.3.3. Deaths—Three deaths occurred during the study. One death was judged possibly related to XL019: a 77 year old woman with previously treated PMF was on her fifth cycle of XL019 25 mg daily when she developed grade 2 confusion, evolving into encephalopathy, vomiting, fever, hemorrhagic colitis and multi-organ failure; she eventually died from aspiration pneumonia. The other deaths were judged unrelated to XL019 and were: (1) cardiac arrest (presumed pulmonary embolism) following admission for septic knee, and (2) heparin-induced thrombocytopenia/thrombosis complicating an admission for sepsis.

3.4. Efficacy

In total, clinical activity (defined as reductions in splenomegaly, reductions in peripheral blood and/or bone marrow blasts, or improvements in hemoglobin) was observed in seven (23%) patients: in 6 of 22 (27%) JAK2V617F positive patients, and 1 of 8 (13%) JAK2 wild-type patients ($p = 0.39$). The lone patient with a MPLW515L mutation failed to respond. Three patients (10%) met formal IWG-MRT criteria for clinical improvement. The first patient was assigned 25 mg XL019 daily, and had a sustained improvement in hemoglobin (from baseline of 9.1 g/dL to 11.2 g/dL) fulfilling criteria for “anemia response”. This patient also had reduction in palpable splenomegaly (from 3 cm to 0 cm) and continued treatment for 32 months. The second patient received 25 mg XL019 TIW and had durable improvement in hemoglobin (from 9.6 to 12.7 g/dL) and splenomegaly (from 10 cm to 0 cm) fulfilling criteria for “anemia” and “spleen” response. This patient experienced a reduction in marrow blasts from 10% to 5% (as well as peripheral blood blasts from 8% to 0%) but developed progressive disease with an increase in blasts at 10 months while on therapy. Notably, this was the only patient to show a substantial decrease in JAK2V617F allele burden on therapy (see below). The third responder was the patient who died of encephalopathy and aspiration pneumonia during the fifth cycle of XL019 at 25 mg daily; prior to her death she was in a sustained “spleen” response (reduction from 12 cm to 6 cm), without improvement in cytopenias.

Three patients demonstrated a reduction in splenomegaly of 50% that did not meet IWG-MRT criteria as the response was not sustained for 8 weeks. These patients had reductions in palpable splenomegaly from 16 cm to 7 cm, 12 cm to 5 cm, and 13 cm to 0 cm, respectively. The first two patients received 100 mg and 200 mg XL019 daily, but had to stop early due to peripheral neuropathy, and developed recurrent splenomegaly after drug cessation. The third patient received 50 mg daily and ceased treatment after 1 month due to side effects; this patient had no further follow-up for treatment efficacy.

Finally, one patient with JAK2V617F mutation positive MF whose disease had failed to respond to AML induction chemotherapy prior to study entry had reduction in marrow blasts from 18% to 1% (by flow cytometry) on 25 mg TIW XL019 after 6 months of therapy,

accompanied with an elimination of circulating blasts (8–0%, but without a change in JAK2V617F allele burden); this patient had reduction in clinical splenomegaly insufficient to meet IWG-MRT criteria for response (from 9 cm to 5 cm). The patient subsequently developed progressive disease with an increase in blasts on XL019 therapy at 8 months. We performed DNA sequencing of the JAK2 kinase domain in sample from this patient upon progression and found no evidence for a JAK2-resistant kinase domain mutation.

3.5. Pharmacokinetics

Pharmacokinetic analysis of plasma samples from 26 subjects dosed in six cohorts (25–300 mg qd, 25 mg qTIW) showed that systemic exposure increased approximately linearly with increasing dose. The terminal half-life of XL019 was approximately 21 h. The drug accumulated during daily dosing with steady state reached by Day 8. At that point the accumulation ratio was 2–5 fold for the daily regimen, and 1.8 fold for the qTIW regimen.

3.6. JAK2 allele burden and plasma protein markers

Most patients with JAK2V617F showed no significant change in mutant allele burden in peripheral blood samples. Serial testing in 17 JAK2V617F positive patients showed <10% allelic burden change in 15 (88%), 10–20% reduction in 1 (6%), and 20% reduction in 1 (6%). The latter patient was an IWG-MRT responder with significant reductions in marrow and peripheral blood blasts, as mentioned above, in association with reduction of JAK2V617F allele burden. Eventually, this patient developed disease progression with increasing blast count, despite a persistently suppressed mutant JAK2 allele burden. Sequencing of the JAK2 kinase domain revealed no evidence for an acquired JAK2-resistant mutation.

Plasma protein analysis was performed for two cohorts (25 mg continuously daily and 25 mg qTIW, $n = 6$). Analysis of baseline samples showed upregulation of multiple cytokines in study patients compared to normal controls, including at least a 3-fold elevation in every patient for GCSF, GM-CSF, IL-8, IL-10, sCD40L, sVCAM-1 and TNF- α . Within the first 3 weeks of XL019 treatment, IL-1RA, FGF-2, IFN γ , VEGF & IL-15 were significantly reduced, and MIP-1 β , leptin & EGF increased (Fig. 1 and data not shown).

4. Discussion

Clinical experience with JAK2 inhibitors to date has suggested that the biology of MF is complex, and that JAK2 inhibition in MF is not analogous to BCR-ABL inhibition in chronic myeloid leukemia, although it is important to note that the extent to which potent inhibition of JAK2 is currently achieved clinically is not known. While patients commonly experience rapid and dramatic improvements in splenomegaly and constitutional symptoms, the JAK2V617F allelic burden is overall minimally altered by existing therapies, and the risk of leukemic transformation appears unchanged [6–10,18]. Patients who are negative for JAK2V617F derive as much benefit from JAK2 inhibitor therapies as those carrying the mutation [6–9]. The selectivity for JAK1, JAK2, JAK3 and TYK2 varies between different compounds, and yet the clinical results appear in general similar, providing no consistent relationship between side-effects and the selectivity profile (reviewed in [19]). Furthermore,

while there are occasional patients with JAK2V617F mutation positive MF that experience significant reduction in JAK2V617F allele burden while on JAK inhibitor therapy, the relationship between the clinical benefit (reduction in spleen size or improvement in constitutional symptoms) and the reduction in the JAK2V617F allele burden has not been shown so far [20].

The present report suggests that selective JAK2 inhibition may be sufficient to elicit clinically relevant responses in some MF patients, including improvement in hemoglobin and substantial reductions in bone marrow blast percentage, without causing excessive myelosuppression. Unfortunately this activity was observed in too few patients and not properly evaluated due to emerging neurotoxicity. Still, this is in contrast to published experience with SAR302503 (TG101348), another specific JAK2 inhibitor in the development, which was associated with significant myelo-suppression at doses that provided reductions in splenomegaly and improvement in quality of life [8]. SAR302503 was also associated with gastrointestinal (GI) side effects (nausea and diarrhea) but not with neurotoxicity. Preliminary results of yet another specific JAK2 inhibitor in the development, pacritinib, provided experience similar to that of XL019 described here (i.e. clinical benefit without excessive myelosuppression); this agent was also associated with the GI side effects and not with neurotoxicity. Therefore, overall experience so far with JAK2 inhibitors suggests that other factors apart from target selectivity are at play and contribute to observed side effect profile of JAK2 inhibitors [7,8,21–23].

XL019 demonstrated leukemic blast reducing activity in a couple of patients with advanced MF and excess blasts, an activity not yet reported with other JAK2 inhibitors in development. Interestingly, one of them had a transient control of the JAK2V617F-positive clone with XL019, prior to the disease progressing with leukemic transformation in a second (JAK2V617F-negative) clone. While this observation is common upon transformation [24], it underscores the heterogeneous molecular landscape within individual patients with MF, and suggests an increased reliance upon JAK2V617F kinase activity in some leukemic blasts but not others. In this difficult clinical situation with excess blasts, one has few therapeutic options [24]. Recent reports suggest a role for hypomethylating agents, decitabine and 5-azacitidine, as active therapy in post-MPN AML patients [25,26], and clinical study evaluating the combination therapy of decitabine and JAK inhibitor ruxolitinib is planned for this group of patients.

Similar to the experience with ruxolitinib [7,9], modulation in cytokine levels was observed following XL019, although there are clear differences in cytokine effects between ruxolitinib and XL019. For example, XL019 did not substantially alter levels of erythropoietin, IL-6, IL-8 or TNF- α , which were all cytokines modulated by ruxolitinib [7,9]. The lack of IL-6 and TNF- α modulation was also observed in studies of CEP-701 [10] and SAR302503 (TG101348) [8], and may be related to the lack of JAK1 inhibition.

Unfortunately, XL019 treatment was associated with the unexpected occurrence of neurotoxicity. Recently, another JAK2 inhibitor, CYT387, has been reported as causing peripheral neuropathy in a subset of patients, but not CNS toxicity [27]. Since neurotoxicity – particularly CNS neurotoxicity – has been rare or absent in studies of other JAK2

inhibitors, its almost universal occurrence with XL019 suggests likely an undefined, off-target effect. Alternatively, XL019 may be particularly efficient in crossing the blood–brain barrier in humans (as would be suggested by the observation in this study of early CNS effects), and thereby uncovered the consequences of neurological JAK2 blockade in humans. The role of JAK-STAT signaling in the brain is poorly defined. Erythropoietin is known to be neuroprotective in models of ischemic CNS injury, at least partly through the activation of the JAK-STAT pathway [28], and JAK2 knock-down abrogates the neuroprotective effects of leptin in animal models of Parkinson's disease [29]. More recent studies have identified JAK2 as being highly expressed in the brain, particularly in the postsynaptic density, where it may have a key role in brain synaptic plasticity [29]. Whether long terminal half-life of XL019, of approximately 21 h, contributed to observed toxicity remained unexplained.

Acknowledgments

This work was supported by funding from the Leukemia & Lymphoma Society (to CCS and NPS), by the UCSF T-32 Molecular Mechanisms of Cancer grant (to CCS) and by Art and Alison Kern. We thank Leo Tseng and Linh Nguyen for the pharmacokinetic data.

References

1. Tefferi A, Thiele J, Vardiman JW. The 2008 World Health Organization classification system for myeloproliferative neoplasms: order out of chaos. *Cancer*. 2009; 115:3842–7. [PubMed: 19472396]
2. Mesa RA, Silverstein MN, Jacobsen SJ, Wollan PC, Tefferi A. Population-based incidence and survival figures in essential thrombocythemia and agnogenic myeloid metaplasia: an Olmsted County Study, 1976–1995. *American Journal of Hematology*. 1999; 61:10–5. [PubMed: 10331505]
3. Tefferi A. Myelofibrosis with myeloid metaplasia. *The New England Journal of Medicine*. 2000; 342:1255–65. [PubMed: 10781623]
4. Vannucchi AM. Management of myelofibrosis. Hematology/the Education Program of the American Society of Hematology American Society of Hematology Education Program. 2011; 2011:222–30. [PubMed: 22160038]
5. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature*. 2005; 434:1144–8. [PubMed: 15793561]
6. Harrison C, Kiladjian JJ, Al-Ali HK, Gisslinger H, Waltzman R, Stalbovskaya V, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. *The New England Journal of Medicine*. 2012; 366:787–98. [PubMed: 22375970]
7. Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. *The New England Journal of Medicine*. 2012; 366:799–807. [PubMed: 22375971]
8. Pardanani A, Gotlib JR, Jamieson C, Cortes JE, Talpaz M, Stone RM, et al. Safety and efficacy of TG101348, a selective JAK2 inhibitor, in myelofibrosis. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*. 2011; 29:789–96. [PubMed: 21220608]
9. Verstovsek S, Kantarjian H, Mesa RA, Pardanani AD, Cortes-Franco J, Thomas DA, et al. Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. *The New England Journal of Medicine*. 2010; 363:1117–27. [PubMed: 20843246]
10. Santos FP, Kantarjian HM, Jain N, Manshouri T, Thomas DA, Garcia-Manero G, et al. Phase 2 study of CEP-701, an orally available JAK2 inhibitor, in patients with primary or post-polycythemia vera/essential thrombocythemia myelofibrosis. *Blood*. 2010; 115:1131–6. [PubMed: 20008298]
11. Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF, et al. Long-term outcome of ruxolitinib treatment in patients with myelofibrosis: durable reductions in spleen volume,

- improvements in quality of life, and overall survival advantage in COMFORT-I. Blood: American Society of Hematology. 2012
12. Cervantes, F.; Kiladjian, J-J.; Niederwieser, D.; Sirulnik, A.; Stalbovskaya, V.; McQuity, M., et al. Blood: American Society of Hematology. 2021 L ST NW, SUITE 900, WASHINGTON, DC 20036 USA: 2012. Long-term safety, efficacy, and survival findings from Comfort-II, a phase 3 study comparing ruxolitinib with best available therapy (BAT) for the treatment of myelofibrosis (MF).
 13. Verstovsek, S.; Pardanani, AD.; Shah, NP.; Sokol, L.; Wadleigh, M.; Gilliland, DG., et al. Blood: American Society of Hematology. 170A-A, 1900M STREET. NW SUITE 200, WASHINGTON, DC 20036 USA: 2007. A phase I study of XL019 a selective JAK2 inhibitor, in patients with primary myelofibrosis and, post-polycythemia vera/essential thrombocythemia myelofibrosis.
 14. Shah NP, Olszynski P, Sokol L, Verstovsek S, Hoffman R, List AF. A phase I study of XL019, a selective JAK2 inhibitor, in patients with primary myelofibrosis, post-polycythemia vera, or post-essential thrombocythemia myelofibrosis. Blood. 2008; 112:98.
 15. Paquette, R.; Sokol, L.; Shah, NP.; Silver, RT.; List, AF.; Clary, DO., et al. Blood: American Society of Hematology. Vol. 971. 1900M STREET. NW SUITE 200, WASHINGTON, DC 20036 USA: 2008. A phase I study of XL019, a selective JAK2 inhibitor, in patients with polycythemia vera.
 16. Dupriez B, Morel P, Demory JL, Lai JL, Simon M, Plantier I, et al. Prognostic factors in agnogenic myeloid metaplasia: a report on 195 cases with a new scoring system. Blood. 1996; 88:1013–8. [PubMed: 8704209]
 17. Tefferi A, Barosi G, Mesa RA, Cervantes F, Deeg HJ, Reilly JT, et al. International Working Group (IWG) consensus criteria for treatment response in myelofibrosis with myeloid metaplasia, for the IWG for Myelofibrosis Research and Treatment (IWG-MRT). Blood. 2006; 108:1497–503. [PubMed: 16675707]
 18. Mascarenhas J, Mughal TI, Verstovsek S. Biology and clinical management of myeloproliferative neoplasms and development of the JAK inhibitor ruxolitinib. Current Medicinal Chemistry. 2012; 19:4399–413. [PubMed: 22830345]
 19. Santos FP, Verstovsek S. JAK2 inhibitors for myelofibrosis: why are they effective in patients with and without JAK2V617F mutation? Anti-Cancer Agents in Medicinal Chemistry. 2012; 12:1098–109. [PubMed: 22583424]
 20. Nicolas CS, Peineau S, Amici M, Csaba Z, Fafouri A, Javalet C, et al. The Jak/STAT pathway is involved in synaptic plasticity. Neuron. 2012; 73:374–90. [PubMed: 22284190]
 21. Seymour, J.; Scott, B.; Roberts, A.; To, B.; Odenike, O.; Estrov, Z., et al. Long-term safety and efficacy analysis of the two phase 1 studies of SB1518, a novel oral JAK2 inhibitor, in patients with advanced myeloid malignancies. 16th Congress of the European Hematology Association: Haematologica Journal Office; 2013. p. 378
 22. Tam CS, Verstovsek S. Investigational Janus kinase inhibitors. Expert Opinion on Investigational Drugs. 2013; 22:687–99. [PubMed: 23432430]
 23. Verstovsek, S.; Mesa, RA.; Rhoades, SK.; Giles, JL.; Pitou, C.; Jones, E., et al. Blood: American Society of Hematology. 1900M STREET. NW SUITE 200, WASHINGTON, DC 20036 USA: 2011. Phase I study of the JAK2 V617F inhibitor, LY2784544, in patients with myelofibrosis (MF), polycythemia vera (PV), and essential thrombocythemia (ET); p. 1213–4.
 24. Tam CS, Nussenzveig RM, Popat U, Bueso-Ramos CE, Thomas DA, Cortes JA, et al. The natural history and treatment outcome of blast phase BCR-ABL-myeloproliferative neoplasms. Blood. 2008; 112:1628–37. [PubMed: 18566326]
 25. Mascarenhas J, Navada S, Malone A, Rodriguez A, Najfeld V, Hoffman R. Therapeutic options for patients with myelofibrosis in blast phase. Leukemia Research. 2010; 34:1246–9. [PubMed: 20627294]
 26. Thepot S, Itzykson R, Seegers V, Raffoux E, Quesnel B, Chait Y, et al. Treatment of progression of Philadelphia-negative myeloproliferative neoplasms to myelodysplastic syndrome or acute myeloid leukemia by azacitidine: a report on 54 cases on the behalf of the Groupe Francophone des Myelodysplasies (GFM). Blood. 2010; 116:3735–42. [PubMed: 20664061]

27. Pardanani A, Laborde RR, Lasho TL, Finke C, Begna K, Al-Kali A, et al. Safety and efficacy of CYT387, a JAK1 and JAK2 inhibitor, in myelofibrosis. *Leukemia*. 2013; 27:1322–7. [PubMed: 23459451]
28. Kretz A, Happold CJ, Marticke JK, Isenmann S. Erythropoietin promotes regeneration of adult CNS neurons via Jak2/Stat3 and PI3K/AKT pathway activation. *Molecular and Cellular Neurosciences*. 2005; 29:569–79. [PubMed: 15936213]
29. Weng Z, Signore AP, Gao Y, Wang S, Zhang F, Hastings T, et al. Leptin protects against 6-hydroxydopamine-induced dopaminergic cell death via mitogen-activated protein kinase signaling. *The Journal of Biological Chemistry*. 2007; 282:34479–91. [PubMed: 17895242]

Fig. 1. Change in Peripheral Blood Cytokine Panel after 3 Weeks of XL019 Treatment. Figure represents change between baseline (pre-treatment; panel A) cytokines, and repeat assay after 3 weeks of XL019 treatment (panel B). A1 denotes patients treated with 25 mg daily. B1 denotes patients treated with 25 mg TIW.

Table 1

Discordance between referral diagnoses and final diagnoses at MDACC.

Primary myelofibrosis discordant group, N = 70 (12.5%) Referring diagnosis			
WHO classification	Diagnosis	N	%
MPN	MPN-unclassifiable	18	26
	Polycythemia vera	12	17
	Essential thrombocythemia	7	10
	CML	5	7
MDS/MPN	MDS/MPN unclassified	15	21
	Atypical CML (BCR/ABL negative)	2	3
	CMML	2	3
MDS	RCMD	3	4
	RARS	1	1
	RAEB-1	2	3
	RAEB-2	2	3

Among 560 patients with confirmed diagnosis of primary myelofibrosis, 70 were referred to MD Anderson with different diagnosis, as outlined in this Table.

MPN: myeloproliferative neoplasm; MDS/MPN: myelodysplastic syndrome/myeloproliferative neoplasm; CML: chronic myeloid leukemia; CMML: chronic myelomonocytic leukemia; MDS: myelodysplastic syndrome; RCMD: refractory cytopenia with multilineage dysplasia; RARS: refractory anemia with ring sideroblasts; RAEB: refractory anemia with excess blasts.

Table 2

Comparison of patient characteristics between the discordant and non-discordant groups.

Characteristic	Non-discordant		Discordant		Total N = 560	P value
	N = 490	(%)	N = 70	(%)		
Age (mean)	67 ± 13		65 ± 13			
<65	164	33	31	44	195	0.075
65	326	67	39	56	365	
Gender						
Male	314	64	58	83	372	0.002
Female	176	36	12	17	188	
DIPSS-plus						
Low	10	2	2	3	12	0.37
Intermediate-1	63	13	11	16	74	
Intermediate-2	246	50	26	37	272	
High	152	31	25	36	177	
Not done	19	4	6	8	25	
WBC (10 ⁹ /μL)						
<25	403	82	54	77	457	0.30
25	87	18	16	23	103	
Median LDH (IU/L)	1279		1269			0.81
Median spleen size (cm)	10		8			0.44
Hb (g/dL) 10						
<10	214	44	25	36	239	0.21
10	276	56	45	64	321	
Platelets						
<100	121	25	20	29	141	0.48
100	369	75	50	71	419	
Cytogenetics						
Favorable	309	63	40	57	349	0.38
Unfavorable	162	33	27	39	189	
Not done	19	4	3	4	22	

Characteristic	Non-discordant		Discordant		Total N = 560	P value
	N = 490	(%)	N = 70	(%)		
Peripheral blasts	378	77	36	51	414	<0.001
Bone marrow fibrosis ^a						
Grade 0–1	41	8	12	17	60	0.013
Grade 2–3	424	87	52	74	476	
Not done	25	5	6	9	31	
JAKV617 positive	381	78	46	66	427	0.027

^aEuropean Consensus of grading of bone marrow fibrosis [17].

Table 3

Non-neurological adverse events.

	Toxicity	All grades (%)	Grade 3 (%)
Hematologic	Anemia (all)	5 (17)	4 (13)
	Anemia (related)	2 (7)	1 (3)
	Thrombocytopenia (all)	4 (13)	3 (10)
	Thrombocytopenia (related)	1 (3)	0 (0)
Other (10%)	Fatigue	9 (30)	3 (10)
	Nausea	9 (30)	2 (7)
	Peripheral edema	7 (23)	1 (3)
	Hyperkalemia	6 (20)	3 (10)
	Abdominal pain	5 (17)	0 (0)
	Diarrhea	5 (17)	0 (0)
	Constipation	5 (17)	0 (0)
	Pain in extremity	5 (17)	0 (0)
	Decreased appetite	4 (13)	1 (3)
	Vision blurring	4 (13)	0 (0)
	Pyrexia	4 (13)	0 (0)
	Upper respiratory tract infection	3 (10)	1 (3)
	Chest pain	3 (10)	1 (3)
	Back pain	3 (10)	0 (0)
	Epistaxis	3 (10)	0 (0)