

JAK2 Inhibition: Current Roles in Myelofibrosis and Initial Lessons Learned from Mexico

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RESUMEN

Las neoplasias mieloproliferativas BCR-ABL negativas incluyen a la trombocitosis esencial o primaria, a la policitemia vera y a la mielofibrosis primaria. Este último padecimiento destaca por ser el de mayor morbilidad y mortalidad entre las subclases. El complejo biogénico subyacente a estas hemopatías clonales ha tenido, históricamente, avances notables en terapias contra genes específicos e inhibición de las citocinas proinflamatorias fundamentales para el inicio de los síntomas.

El descubrimiento de la mutación JAK2V617F condujo a la integración de biomarcadores genéticos para estrategias diagnósticas y terapéuticas. La década pasada quedó marcada por el desarrollo rápido de terapias con inhibidores de JAK2 capaces de reducir la esplenomegalia, las citopenias y los síntomas constitucionales con mínima mielosupresión y toxicidad secundaria.

El ruxolitinib fue el primer inhibidor JAK2 aprobado por la FDA en 2011 para riesgo alto e intermedio de mielofibrosis. El año pasado se realizaron avances notables en el entendimiento de eficacia, tolerabilidad y repercusiones en la esperanza de vida, incluido el análisis *post-hoc* de los ensayos COMFORT-I y COMFORT-II, la repercusión del fármaco a pesar de las trombocitopenias de base y efectos del estado mutacional de JAK2V617F. Sin embargo, la integración de los avances científicos en la práctica clínica permanece como un reto para todo el personal de salud que participa en el tratamiento de pacientes con mielofibrosis primaria.

El desarrollo reciente de los programas de abastecimiento individual de pacientes (Individual Patient Supply Programs) y los programas de uso compasivo (Compassionate Use Programs) se han extendido significativamente y las terapias eficaces a pacientes, que de otro modo no serían accesibles fuera del entorno de ensayos clínicos. Mientras se examina el futuro de los tratamientos de las neoplasias mieloproliferativas, está claro que la integración de los avances científicos debe permanecer paralela al tiempo-sensibilidad de la aplicación del paciente, para ofrecerle mayor alivio a quienes padecen alguna clase de neoplasia mieloproliferativa.

Palabras clave: trombocitopenia esencial, policitemia vera, mielofibrosis, mielofibrosis primaria, neoplasias mieloproliferativas, inhibidor JAK2, leucemia aguda.

ABSTRACT

BCR-ABL negative myeloproliferative neoplasms (MPN's) include essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF), the latter notable for harboring the greatest morbidity and mortality amongst the subclass. The complex biogenetic underscoring of these clonal hemopathies has historically stifled meaningful advances in gene-targeted therapies and compromised inhibition of the pro-inflammatory cytokines foundational to symptom development. The paramount discovery of the JAK2V617F mutation led to the integration of genetic biomarkers into diagnostic and treatment strategies. This past decade has since been marked by the rapid development of JAK2 inhibitor therapies capable of reducing splenomegaly, cytopenias and constitutional symptoms with minimal myelosuppression and secondary toxicities. Ruxolitinib was the first among JAK inhibitors to achieve FDA approval in 2011 for intermediate- and high-risk myelofibrosis. Notable advances in our understanding of this therapies efficacy, tolerability and impact on life-expectancy have been made within the past year, including post-hoc analysis of the COMFORT-I and COMFORT-II trials, drug impact despite baseline thrombocytopenias and effects on JAK2V617F mutational status. Integrating scientific advancement into clinical practice remains a challenge for all MF providers. The recent development of Individual Patient Supply Programs (IPSP's) and Compassionate Use Programs (CUP's) has extended meaningful and efficacious therapies to patients that otherwise may not have access outside the clinical trial setting. As we survey the future of MPN treatments, it is clear that integration of scientific advancements must parallel time-sensitive patient application so as to offer the greatest relief to those afflicted by the MPN disease burden.

Key words: essential thrombocythemia; polycythemia vera; myelofibrosis; primary myelofibrosis; myeloproliferative neoplasms; JAK2 inhibitor; acute leukemia

In 1951, William Dameshek was the first to describe a unique set of clonal hemopathies subsequently entitled, 'myeloproliferative disorders' (MPD's).¹ This clustering initially included chronic myelogenous leukemia (CML), primary myelofibrosis (PMF), polycythemia vera (PV), essential thrombocythemia (ET) and erythroleukemia. Identification of the BCR-ABL gene mutation defining CML in 1976 lead to further realization that microarchitecture deregulation may play a substantial role in disease development and course.² In 2005, the landmark discovery of the JAK2V617F mutation identified a common genetic aberrancy researchers had suspected was contributory to disease development. In 2008, the World Health Association (WHO) employed this molecular marker to categorize myeloproliferative diseases, her forth entitled myeloproliferative neoplasms (MPN's).³ Within the BCR-ABL negative MPNs, the JAK2V617F mutation has been observed in PV(96%), PMF(65%) and ET(55%).^{4,5}

Myelofibrosis has traditionally carried the greatest symptomatic burden with an expected prognosis of 5-7 years. Cause of death is typically attributed to leukemic progression, infection or cytopenias.⁶⁻⁸ Incidence is estimated to be 1.7 to 2.4 per 100,000 patients.⁹ The disease is characterized by clonal myeloproliferation with successive marrow fibrosis, heightened cytokine activity, ineffective erythropoiesis, constitutional symptoms and hepatosplenomegaly. Marrow fibrosis may result in secondary osteosclerosis, painful extramedullary hematopoiesis and pancytopenias. Hepatosplenomegaly is frequent and may result in abdominal discomfort, portal hypertension, ascites, pleural effusions and variceal bleeding. Constitutional symptoms include profound fatigue, concentration

difficulties, early satiety, inactivity, night sweats, pruritus, bone pain, abdominal discomfort, weight loss and fevers.¹⁰ The source of marrow fibrosis remains unknown. It has been postulated that cytokines involved in megakaryocytes and platelet function, including transforming growth factor-B, are contributory but not obligatory to fibrosis development.¹¹

DIAGNOSIS

Diagnosis is based on the 2008 WHO recommendations in conjunction with the International Working Group for MPN Research and Treatment (IWG-MRT).^{3,12} As described, myelofibrosis may arise de novo, or secondary to post-PV/ET. Diagnosis for PMF requires meeting all 3 major criteria and two minor criteria. Major criteria include 1) megakaryocyte proliferation and atypical with either reticulin and/or collagen fibrosis; 2) not meeting WHO criteria for CML, PV, myelodysplastic syndrome (MDS) or other myeloid neoplasm; 3) demonstration of JAK2V617F or other clonal marker or no evidence of reactive marrow fibrosis. Minor criteria include 1) Leukoerythroblastosis; 2) increased serum LDH levels; 3) anemia; 4) palpable splenomegaly. The diagnosis of post-ET/PV MF is applied in patients meeting IWG-MRT criteria. Diagnosis requires a previous diagnosis of PV/ET and bone marrow fibrosis (grade 2-3 or 3-4) and meeting two additional sub-criteria from each category. Generalized myelofibrosis may be acquired from a variety of other sources including malignant and benign pathologies (Table I).^{13,14} Chronic myeloid leukemia, MDS, acute lymphoblastic leukemia, systemic mastocytosis, Hodgkin and non-hodgkin lymphomas and metastatic tumors are all have MF as a recognized complication. Myelodysplastic syndrome, which may be challenging to distinguish from MPN's in its early stages, has a notably reduced survival in the form of leukemic evolution and non-leukemic causes of death when associated with moderate to severe fibrosis.¹⁵ Similarly, CML displays myelofibrosis in up to 40% of cases and has been associated with a worse prognosis. It is differentiated from MPN-related MF via genetic marker testing for the BCR-ABL aberration.¹⁶ Differentiation between PMF and MDS with fibrosis is performed through molecular marker review. In addition, MF has been identified in benign conditions including rheumatological disorders (systemic lupus erythema-

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Table 1. Differential diagnosis of bone marrow fibrosis

<i>Disease</i>	<i>Genomics</i>	<i>Bone Marrow Biopsy</i>	<i>Other</i>
Primary myelofibrosis	JAK2V617F mutation	Diffuse, coarse reticulin fiber network; areas of collagenization	Anemia, thrombocytopenia, splenomegaly constitutional symptoms
Post-ET myelofibrosis	JAK2V617F mutation, MPL mutations	Diffuse, coarse reticulin fiber network; areas of collagenization	Anemia thrombocytopenia, thrombocytosis, splenomegaly constitutional symptoms
Post-PV myelofibrosis	JAK2V617F mutation, Exon 12 mutation	Diffuse, coarse reticulin fiber network; areas of collagenization	Anemia, erythrocytosis, thrombocytopenia, splenomegaly, constitutional symptoms
Chronic myeloid leukemia	Philadelphia chromosome (9:22 translocation)	Diffuse, coarse reticulin fiber network; areas of collagenization	Hypercellularity, with expansion of the myeloid cell line (neutrophils, eosinophils, basophils) and its progenitor cells
Myelodysplastic syndrome	-7/del(7q); -5/del(5q); del(13q); del(11q); del(12p); t(12p); del(9q); others	Diffuse, coarse reticulin fiber network; areas of collagenization	Cellular dysplasia w/ cytopenias, absence of splenomegaly
Hairy cell leukemia	BRAF V600E	Increased reticulin fibrosis, but not collagen fibrosis.	Diffuse, focal or interstitial hairy cell infiltration
Metastatic malignant infiltration -	-	Diffuse, coarse reticulin fiber network; areas of collagenization	Evidence of hypercellular marrow with external malignancy
Tuberculosis	-	Diffuse, coarse reticulin fiber network; areas of collagenization	Positive skin tuberculin test (PPD), positive culture, positive tuberculin blood test
Autoimmune Disorders	-	Diffuse, coarse reticulin fiber network; areas of collagenization	Positive serological testing (ANA, ENA, RF, anti-CCP, anti-Ro/SS-A and anti-La/SS-B, anti-SCL70, others)
HIV infection	-	Increased reticulin fibrosis, but not collagen fibrosis.	Western Blot, ELISA, HIV RNA detection

ELISA, enzyme linked immunoassay; ET, essential thrombocythemia; HIV, human immunodeficiency virus; PV, polycythemia vera RNA; ribonucleic acid

tosus, systemic sclerosis, Sjogren syndrome), primary/secondary hyperparathyroidism, nutritional deficiencies, tuberculosis, osteomalacia and primary hypertrophic osteoarthropathy. Focal marrow fibrosis may include osteomyelitis, Paget's disease, previous bone irradiation, previous biopsy or a healing fracture.

Prognostic Scoring

To better understand the risk factors contributing to PMF progression, the IWG-MRT developed a 5-factor prognostic scoring system applicable at the time of diagnosis. The International Prognostic Scoring System (IPSS) incorporates age over 65 years, hemoglobin level <10 g/dL, white blood cell count >25 x 10(9)/L, peripheral blood blasts >=1 and the presence of constitutional symptoms.⁶ The presence of 0 (low risk), 1 (intermediate-1 risk), 2

(intermediate-2 risk), and 3 (high risk) points carries median survivals of 11.3, 7.9, 4.0 and 2.3 years, respectively. A revised model, the Dynamic International Prognostic Scoring System (DIPSS), was developed to accommodate risk assessment at any point during the disease course and includes similar risk factors to IPSS scoring, but different point weights.¹⁷ The Dynamic International Prognostic Scoring System Plus (DIPSS-Plus) incorporates independent prognostic factors including platelet count <100 x 10(9)/L, the need for red blood cell transfusions and unfavorable karyotype (including +8, -7/7q-, i(17q), inv(3), -5/5q-, 12p-, 11q23) in addition to the factors used in DIPSS.¹⁸ DIPSS-Plus risk scoring has identified median survival in low risk (180 months), intermediate-1 risk (80 months), intermediate-2 risk (35 months) and high risk (16 months) populations.

JAK Signaling and Mutations

The most prevalent mutation in BCR-ABL negative MPNs, JAK2V617F, results from a somatic G-to-T mutation involving Janus Kinase-2 on exon 14.¹⁹ This leads to the substitution of valine for phenylalanine, thereby disrupting the noncatalytic pseudokinase domain leading to constitutive STAT 5, STAT 2, MAPK and PI3K/AKT activation. JAK2 disruption prevents regulation of hematopoietic progenitor cell lines including leukocytes, monocytes, erythrocytes and thrombocytes.²⁰ Overproduction of STAT 3 and STAT 5 have been shown to increase gene expression involved in angiogenesis (VEGF), lead to apoptosis resistance (BCL2L1, BIRC5, MLC1) and induce cellular proliferation (CCND1).²¹ The final result is a compromise in auto-inhibitory activity and induction of persistent cellular activation with cytokine-independent growth and proliferation.^{22,23} It should be noted that the JAK2V617F mutation does not appear to be disease initiating, but rather reflects the complex structural mutations inherent to the disease.²⁴ Uniquely, a lower mutant allele burden has been associated with poorer survival.²⁵ Homozygous V617F mutations have been associated with higher white blood cell and hemoglobin counts, older age, larger spleen size, greater need for cytoreductive therapies and splenomegaly.²⁶ The role of JAK2V617F in the development of acute leukemia or overall survival remains controversial.^{6,25,27,28}

JAK Inhibitors: Ruxolitinib

COMFORT I:

The discovery of the JAK2V617F mutation led to global emphasis on developing novel gene-specific therapies capable of inhibiting the cytokine-JAK-STAT signaling pathways. Ruxolitinib (JAKAFI®, Wilmington, DE, USA) was the first and only JAK inhibitor to receive FDA approval in November 2011 for intermediate- and high-risk MF. A potent JAK kinase modulator, ruxolitinib has demonstrated activity against JAK1, JAK2, JAK3 and mutant JAKV617F-induced extramedullary hematopoiesis and pro-inflammatory cascades.^{29,30} Evaluation of the efficacy and toxicity of ruxolitinib was conducted via two randomized, double-blind studies, the Controlled Myelofibrosis Study with Oral JAK Inhibitor Treatment Trials (COMFORT-I and COMFORT-II Trials).^{31,32} The COMFORT-I trial involved 309 prospectively enrolled post-PV, post-ET and PMF patients. Patients received 15

mg or 20 mg oral ruxolitinib twice daily with a primary end-point of a >35% reduction in spleen volume at 24 weeks. The Myelofibrosis Symptom Assessment Form (MF-SAF) was used to evaluate symptom response. Upon completion of the study, the primary objective was attained in 41.9% of patients receiving ruxolitinib vs 0.7% in the placebo arm. Improvements in abdominal discomfort, night-sweats, inactivity, splenic pain, early satiety, bone/muscle pain and itching were all described. Patients receiving ruxolitinib had notable improvements in their total symptom scores (45.9%) vs placebo (5.3%). These findings remained consistent irrespective of the presence or absence of the JAK2V617F mutation, symptom severity, MF disease subtype, IPSS risk group, age or initial splenic size. A survival benefit was also noted with 13 deaths in the ruxolitinib group and 24 deaths in the placebo group (hazard ratio of 0.499) at study completion. In a multivariate comparison analysis, ruxolitinib was deemed to be an independent factor in providing survival advantage with death occurring in 30.8% of the ruxolitinib group vs 60.9% of the control group at 32 and 22 months, respectively. In a follow-up analysis, COMFORT-1 patients were analyzed for symptoms, tolerability and survival advantage one year beyond completion of the COMFORT-1 study.³³ All patients receiving placebo crossed over to ruxolitinib within three months of COMFORT-I study completion. Patients that had achieved >35% reduction in splenic volume had a durable response at 108 weeks. Symptom improvements previously noted persisted throughout duration of therapy. A survival benefit continued to be observed with deaths occurring in 27 vs 41 originally randomized to ruxolitinib vs placebo respectively. Regardless of baseline risk status and hemoglobin levels, overall survival favored ruxolitinib use across all subgroups. The proportion of patients receiving blood cell transfusions decreased to the level seen with placebo patients by week 36 and remained stable thereafter. No reports of withdrawal syndrome were documented following ruxolitinib discontinuation. An additional post-hoc analysis demonstrated ruxolitinib's efficacy in promoting weight gain and improving total cholesterol with an improvement in overall survival in patients experiencing these effects.³⁴

COMFORT-II

In COMFORT-II, ruxolitinib was compared to best available therapy (BAT) in 219 post-ET, post-PV and PMF

patients. The primary endpoint was a >35% reduction in splenic volume from baseline by week 48. Starting doses were 15mg or 20mg BID. At week 48, the primary endpoint had been achieved in 28% of patients receiving ruxolitinib vs 0% on BAT. Similarly, mean palpable spleen length was improved by 56% for patients receiving ruxolitinib while spleen length had increased on BAT. Improved quality of life and role functioning was noted in patients receiving ruxolitinib, including reductions in myelofibrosis-associated symptoms such as dyspnea, appetite-loss, fatigue, pain and insomnia. Worsening of these symptoms was documented in BAT patients. Non-hematological side effects included diarrhea (23%), peripheral edema (22%), asthenia (18%) and dyspnea (16%) while hematological side effects included anemia (40.4%) and thrombocytopenia (44.5%). Grade 3/4 adverse events were rare and included pneumonia, dyspnea, anemia and thrombocytopenia. Efficacy was independent of baseline splenic size, IPSS risk category, MF subtype, presence of the JAK2V617F mutation or starting dose. In a post-hoc analysis, patients receiving ruxolitinib also had a larger reduction in JAK2V617F allele burden in comparison to those receiving BAT.³⁵ Reductions in %V617F mutations were associated with spleen responses suggesting that ruxolitinib may alter disease course through reduction in the burden of JAK2V617F mutated cell populations. In a follow-up evaluation of COMFORT-II patients, within a median follow-up of 112 weeks, 48.3% of patients receiving ruxolitinib achieved >35% reduction from baseline in spleen volume at any time throughout the study and 97.1% of patients experienced some degree of splenic size reduction.³⁶ Approximately 61.6% of patients in the BAT arm entered the extension phase to receive ruxolitinib. Patients randomized to ruxolitinib had improved survival in comparison to those randomized to BAT. Similar to follow-up results from COMFORT-1, mean hemoglobin levels decreased over the first 12 weeks of treatment, then resolved to levels comparable to BAT from week 24 onward.

UPDATES ON RUXOLITINIB

An abstract presented at the 2012 European Hematology Association included a retrospective analysis of 28 post-ET, post-PV and PMF patients receiving ruxolitinib in clinical practice following FDA approval.³⁷ Patients were

initiated on 20 mg BID or lower if concomitant cytopenias were present. Overall, ruxolitinib was well tolerated with high response rates. Of the patients seen in follow-up, 64% had experienced a reduction in spleen size and 36% experienced complete resolution of palpable splenomegaly. Significant improvements were noted in baseline symptoms. These included fatigue (75%), early satiety (71%), night sweats (53%), abdominal pain (71%), pruritus (56%), weight loss (69%), bone pain (33%) and fevers (33%). Non-hematological toxicities were infrequent. Hematological toxicities included anemia and thrombocytopenia, primarily affecting those with baseline cytopenias. Dose reductions were required in four patients and therapy was discontinued in three patients. For patients with baseline platelet counts <100 x 10⁹/L, no significant decline in platelet levels were noted during therapy. Overall, the results of this study reinforced the safety and efficacy of ruxolitinib as seen in the COMFORT I and II trials. A similar study presented at the 2012 American Society of Hematology (ASH) conference evaluated 23 patients with PMF (15), post-PV MF (7) and post-ET MF (1) receiving ruxolitinib in their clinical practice post-FDA approval.³⁸ Among the 20 patients evaluable for reduction of palpable splenomegaly, the rate of IWG-defined clinical improvement was 15% at >8 weeks. Forty percent of patients experienced a >50% reduction in splenomegaly at any point in time. A >50% reduction in the 8-point MPN-SAF TSS was observed in 96% of patients. Four patients underwent dose reductions for cytopenias or grade 3 hepatotoxicity. The author's concluded that efficacy and safety results were consistent with those observed in the COMFORT trials.

Similarly, a study presented at the 2012 American Society of Clinical Oncology explored the safety and efficacy of ruxolitinib in MF patients with platelet counts between 50-100 x 10⁹/L.³⁹ All patients were intermediate-1 to high-risk MF and were started on 5 mg BID. Symptom assessment was performed using the Myelofibrosis Symptom Assessment Form Total Symptom Score (MF-SAF TSS). By weeks 4 and 8, TSS had improved 14% and 23% in patients receiving ruxolitinib, respectively. Mean spleen length had similarly improved at weeks 4 and 8 by 22% and 27% respectively. No Grade 4 platelet counts, holds for adverse events or drug discontinuations were observed. The authors concluded that initial dosing strategies of 5 mg BID with subsequent dose optimization was

beneficial and well tolerated in MF patients with baseline thrombocytopenia.

Emerging Treatments and Combination Therapy

Ruxolitinib is one of more than a dozen JAK agents currently under pre-clinical or clinical development. Other JAK inhibitors such as SB1518, SAR302503, LY2784544, CEP701, CYT-387, XL-019, AZD1480, BMS-91143 and NS018 inhibit mutant and native JAK with varying degrees of selectivity, along with several other kinases (Table II). The in vivo complexities of JAK-STAT signaling and plethora of molecular drivers that foster MPN transformation have provided the rationale for combination therapies that address parallel pathways and/or novel sites of intervention.

Inhibition of the Flt3/MAPK pathway is a potential target given the constitutive phosphorylation of AKT and ERK present in deviant JAK2 signaling.^{40,41} Evidence supports the theory that p38-MAPK contributes to alterations in differentiation, proliferation and migration processes within PMF, leading to inflammatory cytokine release. Inhibition of the FL/flt3/p38 axis may work in a synergistic manner to JAK-STAT inhibition in controlling cytokine levels. Similarly, histone deacetylase inhibitors (HDAC's) may be applied to decrease STAT3 dimerization thereby reducing intracellular signaling. Studies have identified elevated HDAC enzyme activity within PMF CD34+ cells and HDAC activity correlates with the degree of splenomegaly.^{42,43} Evaluation of JAK2 inhibitors in combination with HDAC's has provided pro-

Table 2. JAK2 therapy trials for myelofibrosis (Continued on next page)

Drug	Inhibition/ Trial	Study	Disease	N	Improvements	Grade 3+ Toxicities	Toxicities
Ruxolitinib/ INCB018424	JAK1, JAK2, JAK3 and JAK2V617F	Verstovsek 53	Primary or secondary MF	153	Improved splenomegaly (44%), constitutional symptoms, reduced inflammatory cytokines	Thrombocytopenia (20%)	Anemia, fatigue, GI
		COMFORT-1 Trial	Verstovsek 54	Primary or secondary MF	309	Reduction in splenomegaly (42%), reduction in anemia (45%), thrombocytopenia (12.9%), constitutional symptoms	-
	COMFORT-1 Trial	Verstovsek 33	Primary or secondary MF	309	Durable reductions of splenic size at 102 weeks of therapy	Anemia, thrombocytopenia	Fatigue, GI
	COMFORT-1 Trial	Mesa34	Primary or secondary MF	309	Improved weight gain and total cholesterol, survival advantage	-	-
	COMFORT-2 Trial	Harrison 32	Primary or secondary MF	219	Reduction in spleen size (28%), survival advantage, improved QOL	Thrombocytopenia, anemia, dyspnea	GI, peripheral edema, anemia, thrombocytopenia
	COMFORT-2 Trial	Vannucchi35	Primary or secondary MF	219	Larger reductions in V617F allele burden corresponding to splenic size reduction compared to BAT	-	-

Table 2. JAK2 therapy trials for myelofibrosis (Continued on next page)

<i>Drug</i>	<i>Inhibition/ Trial</i>	<i>Study</i>	<i>Disease</i>	<i>N</i>	<i>Improvements</i>	<i>Grade 3+ Toxicities</i>	<i>Toxicities</i>
CEP-701	COMFORT-2 Trial FLT3, JAK2, JAK2V617F	Cervantes36	Primary or secondary MF	219	Improved splenic size in 97.1% of pts at 112 weeks of therapy, survival advantage	Anemia (40.4%), lymphopenia (22.6%), thrombocytopenia (9.6%)	GI, peripheral edema, anemia
		Hexner 55	Primary or secondary MF	26	Reduction in spleen size (23%), no impact on transfusion dependency	Diarrhea (4%), thrombocytopenia (9%), neutropenia (4%)	GI
		Santos 56	Primary or secondary MF	22	Reduction in splenomegaly (14%), transfusion independence (n=2)	Anemia (14%); thrombocytopenia (23%); diarrhea (9%),	GI
SB1518/Pa-citinib	JAK2V617F, JAK2, FLT3	Verstovsek 57	AML, MF	43	Reduction in splenomegaly (41%)	Diarrhea (4%), thrombocytopenia (4%)	GI
		Seymour- 58	MF	20	Reduction in splenomegaly (88%), transfusion independence	Diarrhea (6%)	GI, abdominal pain, dysgeusia, rash
		Deeg 559	Primary or secondary MF	33	Reduction in splenomegaly (96%), reduction in MF related symptoms (40-65%)	Diarrhea (6%)	GI, fatigue
		Komrokji 50	Primary or secondary MF	34	Reduction of splenomegaly (88%), 40% of pts experienced >50% reduction; improved symptoms, improved anemia	GI (2%)	50% discontinuation event due to: AE's, disease progression, lack of response
XL019	JAK-WT, JAK2V617F	Shah61	Primary or secondary MF	30	Reduction in spleen size (100% in pts with JAK2V617F mutation), improvement in leukocytosis, reduced circulating blasts	Fatigue	Neuropathy
TG101348	JAK2V617, FLT3, RET	Pardanini 62	Primary or secondary MF	59	Reduction in splenomegaly (47%), normalization of leukocytosis, sea	Anemia (35%), thrombocytopenia (24%), neutrophilic leukocytosis (3%)	GI, hematologic

Table 2. JAK2 therapy trials for myelofibrosis (Continuation)

Drug	Inhibition/ Trial	Study	Disease	N	Improvements	Grade 3+ Toxicities	Toxicities
LY2784544	JAK2	Verstovsek 63	Primary or secondary MF	19	Reduction in splenomegaly (76%), reduced bone marrow fibrosis, improvement in symptoms (59%)	Tumor lysis syndrome, hyperuricemia	Hyperuricemia, creatinine increase hyperkalemia, GI
CYT387	JAK1, JAK2, MAPK8, PRKCN, ROCK2, CDK2/cyclinA, PRKD1, TYK2, TBK1	Pardanini 64	Primary or secondary MF	166	Reduction in splenomegaly (48%), anemia (59%), constitutional symptoms	Thrombocytopenia (25%), hyperlipasemia, headache	Thrombocytopenia, peripheral neuropathy, dizziness, diarrhea, nausea, headache

GI, gastrointestinal; JAK, Janus Kinase; Pts, patients; QOL, quality of life

misgiving results with reductions in splenomegaly, pruritus and JAK2V617F allele burden.⁴⁴⁻⁴⁶ DNA methyltransferase inhibitors such as decitabine and 5-azacytidine are intriguing targets given the aberrant methylation patterns observed in MF, and less prominently in PV and ET.⁴⁷ Hypermethylation of SOCS-1 and -3 results in JAK signaling and proliferation in both mutant and wild-type JAK.⁴⁸ The use of these agents in combination treatment is still under investigation. ENREF_46, ENREF_40. Aurora kinase (AURK) inhibitors have also been investigated as potential interventions. MK0457 was the first to be investigated in MPN patients harboring JAK2V617F. Administration resulted in induction of apoptosis and reduction of JAK2 allele levels.⁴⁹ All of the aforementioned therapeutic concepts remain in early stages of investigation and additional information is required before application in the clinical practice setting.

Mexican compassionate use program

As with its global counterparts, Mexico has faced challenges in providing efficacious treatment options to MF patients. Given ruxolitinib's demonstration of rapid and durable reductions in MF symptoms, splenomegaly and improved quality of life, an individual patient supply pro-

gram (IPSP) was developed to provide ruxolitinib to more than 48 countries including Europe, Latin America, the Middle East and Asia.⁵⁰ As of December, 2012, approximately 1339 requests had been received from more than 800 physicians treating MF populations. Similarly, in 2012, a Compassionate Use Program (CUP's) was developed in Mexico for MF patients refractory to available therapy. Results were submitted to the 2012 ASH conference and included 40 primary and secondary MF patients with IPSS low (10%), Intermediate-1 (53.3%), Intermediate-2 (26.7%) and high (20%) risk scores.⁵¹ Nineteen percent of patients were positive for the JAK2 mutation and 35% did not undergo mutational analysis. By week 20 of therapy, the average spleen size had improved from 9.58 cm to 4.5 cm (53% reduction). Mean hemoglobin and platelet counts decreased 22.4% and 21.9%, respectively. Grade 2 and 3 anemia was noted and resolved with transfusion support. Grade 2 thrombocytopenia was transitory and resolved following two weeks of treatment interruption. The authors concluded that ruxolitinib provided therapeutic benefit including reduction in splenomegaly and improvements in MF-related symptoms to Mexican patients outside of the clinical trial setting. The effect of ruxolitinib in the splenomegaly of a patient with chronic myelomonocytic

leukemia – associated MF has been shown in a Mexican patient who was given the drug also through the CUP.⁵²

CONCLUSION

Myeloproliferative neoplasms including polycythemia vera, essential thrombocytopenia and primary myelofibrosis, have entered a new era whereby molecular classification has become the foundation for aligning disease pathogenesis with tailored treatment approaches. The advancements made this past decade from dedicated MPN-based investigations have provided unprecedented treatment opportunities in the field of MPN research and solidified the need for coordinated care between pathologists and hematologists. Beginning with the discovery of the JAK2V617F gene mutation, the rapid pace of discovery of additional mutations and further insight into the pathogenic disease process has been critical in directing medicine from an era of empirically derived therapies to meaningful and effective treatment strategies. The 2011 FDA approval of the JAK-2 inhibitor, ruxolitinib, marked the first therapy capable of abating the debilitating MF symptoms and is an exciting breakthrough for patients. Much has yet to be learned about these new pharmacologic tools so early in their investigational process. Our knowledge regarding ideal dosing and treatment cycle strategies has made substantial steps forward this past year, but continues to warrant further review. Techniques such as weight-based dosing and disease-burden dosing or induction-maintenance dosing have yet to be applied. The next surge of MPN research will likely focus on the development of biomarkers and other surrogate measures of clinical outcomes in addition to the investigation of novel agents or combination strategies incorporating JAK2 inhibitor therapy. As we employ this growing knowledge base in a clinically relevant manner, it is apparent that we must remain cognizant of the MF patients who may benefit from its therapeutic application in the present. Mexico's Compassionate Use Program is one such manifestation of scientific generosity that offers real-time solutions in the midst of long-term investigations. As we survey the future of MPN research we anticipate that the critical advances in our understanding of hematology's foundational sciences will translate into demonstrable and sustainable clinical efficacy.

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