The Nonclinical Toxicology Profile of Pacritinib, a JAK2/FLT3 Inhibitor with no Dose-Limiting Clinical Myelosuppression

Abstract #5076

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ABSTRACT

Pacritinib is an orally bioavailable kinase inhibitor being developed for the treatment of myelofibrosis (MF). Pacritinib is a potent inhibitor of wild-type and mutant isoforms of JAK2 and FLT3 (IC_{50} values < 15 nM), and it does not suppress JAK1 at clinically relevant concentrations. Additional kinases targeted by pacritinib and evaluated in a comprehensive kinome profile by Reaction Biology Corp. include IRAK1 (IC_{50} = 13.6 nM) and c-fms (or CSF1R; IC_{50} = 39.5 nM). Pacritinib is pharmacologically active in nonclinical tumor models driven by JAK2 or FLT3 overexpression or overactivity, and clinical trials have demonstrated efficacy of pacritinib in MF patients. A key differentiating feature of pacritinib over currently available JAK2 inhibitors is the lack of dose-limiting clinical myelosuppression, allowing for treatment of patients with cytopenias. The comparatively reduced myelosuppressive activity of pacritinib is thought to be due to its lack of pharmacological activity on JAK1, and/or its inhibition of IRAK1 and c-fms, which may contribute to anti-inflammatory activity. Nonclinical pacritinib data from completed rodent and canine studies were evaluated in comparison to publicly available information for other JAK2 inhibitors to determine if differences in nonclinical endpoints might correlate with the observed clinical differences in myelosuppressive effects. The qualitative nonclinical findings seen in rodents were similar for all JAK2 inhibitors, and included decreases in leukocytes, red cell mass, and reticulocytes; lymphoid depletion in the spleen, thymus, and/or lymph nodes; and hypocellularity of the bone marrow. Interestingly, platelet decreases were not observed in rodent or canine nonclinical models, even with JAK2 inhibitors associated with clinically-significant thrombocytopenia AEs. However, in the dog, pacritinib is unique for its minimal myelosuppressive effects in the pivotal 30-day and 39-week studies. With pacritinib, non-adverse lymphoid reduction was seen in the spleen, lymph nodes, Peyer's patches, and/or thymus; but there were no clinical pathology findings indicative of marked myelosuppression, nor were there any treatment-related findings in detailed bone marrow evaluations. Overall, these comparisons suggest that nonclinical bone marrow data and clinical pathology in the dog may be informative endpoints for estimating the myelosuppressive potential of JAK2 inhibitors in the clinic.

BACKGROUND

- Pacritinib is a promising drug currently developed by CTI BioPharma Corp. and Baxalta Inc. for the treatment of myelofibrosis (MF). Pacritinib lacks dose-limiting myelosuppression; thus, it can potentially be particularly beneficial to patients with cytopenias which are commonly seen in MF patients.
- Nonclinical study data for pacritinib were reviewed to assess the concordance of the observed nonclinical toxicities to potential adverse effects in the clinic. These data were compared to publically available nonclinical data (from European Medicines Agency [EMA] , Australian Public Assessment Reports [AusPAR] and Center for Drug Evaluation and Research [CDER] Pharmacology reviews) for ruxolitinib and tofacitinib, two currently approved JAK inhibitors.
  - Ruxolitinib: JAK1/JAK2 inhibitor approved for treatment of MF and polycythemia vera (PV)
    CDER application 20292Orig1s000, 2011; AusPAR PM-2012-01504-3-4, 2014; EMA report 465846, 2012
  - Tofacitinib: JAK1/JAK3 inhibitor approved for treatment of rheumatoid arthritis
    CDER application 203214Orig1s000, 2011; AusPAR PM-2012-00788-3-3, 2015; EMA report 425279, 2013
- For pacritinib, ruxolitinib, and tofacitinib, pivotal nonclinical studies were performed in one rodent model and one non-rodent large animal model. The goal of these studies was to identify a no adverse effect level (NOAEL), if possible, and to characterize toxicities anticipated above that dose.
- Consistent with JAK inhibition, the hematopoietic system (spleen, lymph nodes, thymus, bone marrow) was a toxicity target with all three JAK inhibitors. However, there were differences in the nonclinical myelosuppression and immunosuppression profiles.

Pivotal Nonclinical Toxicology Studies

Drug	Rodent	Large Animal
Pacritinib	Repeat dose toxicity studies for up to 6 mo. in the BALB/C mouse	Repeat dose toxicity studies for up to 9 mo. in the Beagle dog
Ruxolitinib	Repeat dose toxicity studies for up to 6 mo. in the Sprague-Dawley rat	Repeat dose toxicity studies for up to 12 mo.in the Beagle dog
Tofacitinib	Repeat dose toxicity studies for up to 6 mo. in the Sprague-Dawley rat	Repeat dose toxicity studies for up to 9 mo. in the Cynomolgus monkey

NONCLINICAL SUMMARY

I. Dose-Limiting Toxicities

Dose-limiting toxicities for pacritinib, ruxolitinib, and tofacitinib are presented below. In rodent models, dose-limiting toxicities are fairly similar for pacritinib and ruxolitinib; for tofacitinib, effects secondary to immunosuppression contributed to dose limiting toxicities. In large animal models, adverse GI effects were dose limiting with pacritinib, while infections secondary to immunosuppression were seen with ruxolitinib and tofacitinib.

Nonclinical Dose Limiting Toxicities

JAK Inhibitor	Rodent	Large Animal
Pacritinib	• Poor clinical appearance (ruffled coat, ungroomed) Decreased body weight and food consumption • Mortality at high doses	• GI-related effects: Emesis, abnormal feces, diarrhea • Decreased body weight and food consumption • Moribund status due to severe lymphadenopathy
Ruxolitinib	• Poor clinical appearance • Decreased body weight and food consumption • Mortality at high doses	• Adverse clinical observations • Early deaths consistent with opportunistic infections (secondary bacterial skin infections and pyrogranulomatous inflammation associated with intrafollicular mites)
Tofacitinib	• Poor clinical appearance • Decreased body weight and food consumption • Bacterial infections of the kidney and lung • Mortality at high doses	• Bacterial and viral infections • Lymphomas secondary to treatment-related immunosuppression.

II. Myelosuppression

- Clinical Pathology Data
  - All the JAK inhibitors presented with reductions in red cell mass (red blood cells, hemoglobin, and/or hematocrit) and leukocyte populations.
  - In the rodent, the observed reductions in red cell mass were mild, and not considered adverse. Decreased red cell mass was more pronounced in the large animal studies with ruxolitinib and tofacitinib.
  - Changes in leukocyte populations were most pronounced with ruxolitinib and tofacitinib compared to pacritinib.
- Histopathology Data
  - Rodent findings for all JAK inhibitors consisted of lymphoid depletion of the spleen, thymus and lymph nodes, indicative of mild myelosuppression.
  - In the large animal studies, myelosuppression was most pronounced with tofacitinib and least pronounced with pacritinib
- The observed clinical pathology and histopathology alterations associated with the JAK inhibitors were generally reversible.
- Results from the large animal studies were more predictive of clinical performance compared to results from the rodent studies.

Nonclinical Rodent Hematology in Pivotal Studies*

	Pacritinib: 6 mo. Mouse Study[a]		Ruxolitinib: 6 mo. Rat Study[b]		Tofacitinib: 6 mo. Rat Study[c]	
Sex	M	F	M	F	M	F
Doses (mg/kg/day)	42.7, 142.2, 213.4		5, 15, 30, 60		1, 10, 100	
Red blood cells	up to 5.2%↓	up to 6.6%↓	NA	NA	1-13% ↓	1-15% ↓
Hemoglobin	-	-	-	-	up to 10% ↓	1-10% ↓
Hematocrit	-	up to 6.5%↓	-		up to 12% ↓	up to 12% ↓
Reticulocytes	-	-	NA	NA	NA	NA
White blood cells	-	up to 39%↓	5-32%↓	10-38%↓	19-62% ↓	19-60% ↓
Neutrophils	up to 100%↑	-	6-12%↑	-	22-203%↑	31-298%↑
Lymphocytes	up to 35%↓	up to 56%↓	5-43%↓	18-46%↓	4-38% ↓	5-45% ↓
Monocytes	-	-	up to 14%↓	10-40%↓	up to 56%↑	12-134%↑
Eosinophils	-	-	-	11-44%↓	NA	NA
Basophils	-	-	up to 57%↓	-	NA	NA

* Results reflect the last hematology sample time point prior to the end of the dosing phase of the study. -: No notable changes.
[a] NOAEL was 71.1 mg/kg/day pacritinib due to mortality and reduced body weight & food consumption at higher dose level.
[b] A NOAEL was not provided, but no adverse effects were seen in females up to 60 mg/kg and in males up to 30 mg/kg (males at 60 mg/kg had reduced body weight gain)
[c] the NOAEL in this study is <100 mg/kg/day, and exact NOAEL was not provided due to the absence of certain histopathology data.

Nonclinical Large Animal Hematology in Pivotal Studies*

	Pacritinib: 9 mo. Dog Study[a]		Ruxolitinib: 6 mo. Dog Study[b]		Ruxolitinib: 12 mo. Dog Study[c]		Tofacitinib: 9 mo. Monkey Study[d]	
Sex	M	F	M	F	M	F	M	F
Doses (mg/kg/day)	4.2, 14.4, & 28.8		5 & 10		0.75, 1.5, 3, & 6		0.5, 2, & 10	
Red blood cells	up to 10% ↓	-	14-27%↓	8-14%↓	up to 17%↓		5-20% ↓	4-10% ↓
Hemoglobin	up to 15% ↓	-	16-33%↓	9-19%↓	up to 22%↓		4-17% ↓	6-11% ↓
Hematocrit	up to 12%↓	-	15-30%↓	9-17%↓	up to 18%↓		4-19% ↓	5-13% ↓
Reticulocytes	-	-	15-22%↑	32-47%↑	compensatory ↑ not observed		8-67%↑	up to 51%↑
White blood cells	30%↓ to 25%↑	up to up to 32%↑	up to 50%↑	83-117%↑	NA		1-30% ↓	3-30% ↓
Neutrophils	-	-	9-64%↑	up to 10%↑	up to 40%↑		NA	NA
Lymphocytes	up to 20%↓	up to 61%↑	14%↓ to 28%↑	-	up to 35%↓		21-43% ↓	26-40% ↓
Monocytes	-	-	31-102%↑	20-76%↑	up to 40%↑		NA	NA
Eosinophils	-	-	36-59%↓	73-80%↓	up to 55%↓		NA	NA
Basophils				up to 50%↑			NA	NA

* Results reflect the last hematology sample time point prior to the end of the dosing phase of the study. -: No notable changes.
[a] NOAEL was 14.4 mg/kg/day due to gastrointestinal toxicities observed above that dose.
[b] A NOAEL was not provided, but above 2.5 mg/kg/day, immunological conditions were observed. Only hematology data for the 5 and 10 mg/kg/day dose levels were available.
[c] A NOAEL was not provided, but above 1.5 mg/kg/day, generalized demodicosis was observed
[d] A NOAEL could be determined in this study due to lymph node lymphocyte hyperplasia at the low dose, which was considered adverse.

Key Nonclinical Histopathology Findings*

Drug	Rodent	Large Animal
Pacritinib	• **Hypocellularity of the bone marrow at the high doses** • **Lymphoid depletion in spleen, thymus, and lymph node** • **Slight decrease in spleen weights**	• **Minimal lymphoid depletion in the Peyer's patches and spleen, lymphoid hyperplasia of the thymic medulla.** • **No treatment-related changes in the myeloid and erythroid populations in the bone marrow.** • **Slight decrease in spleen and thymus weights**
Ruxolitinib	• **Minimal to mild lymphoid depletion in the spleen and lymph nodes** • **Significant reduction in spleen and adrenal gland weights** • Minimal cortical atrophy of the adrenal glands.	• **Marked lymphoid depletion in the spleen and lymph nodes** • **Bone marrow hypocellularity in some early death animals** • Granulomatous inflammation with parasitic mites in the lymph nodes, acute and/or subacute inflammation of the lung, tissue pyrogranulomatous inflammation of the skin and footpad secondary to infection
Tofacitinib	• **Bone marrow erythroid and lymphoid depletion** • **Lymphoid depletion in the spleen, thymus, and lymph node** • Enlarged stomach with multifocal necrosis • **Reduced spleen and thymus weights**	• **Lymphoid depletion of the lymph nodes, spleen, and/or gut associated lymphoid tissue** • **Bone marrow depletion, with reduction in hematopoietic cells and relative increase in adipose tissue and/or decrease in the myeloid: erythroid ratio** • **Reductions in thymic, spleen, and reproductive organ weights**

* Findings related to myelosuppression are in **boldface**.

III. Immunosuppression

- Adverse effects of immunosuppression were primarily evident in the large animal studies.
- With pacritinib, one death of a high dose animal was possibly exacerbated by treatment-related immunosuppression.
- With ruxolitinib and tofacitinib, secondary infections were clearly treatment-related and prevalent in large numbers of dogs and monkeys, respectively.

Nonclinical Immunosuppression

JAK Inhibitor	Large Animal Study Findings
Pacritinib	In the 30 day dog study, there was one high dose animal in which death an infection secondary to lymphadenopathy might have been exacerbated by treatment-related immunosuppression. Immunosuppression was not seen in the 9 month study.
Ruxolitinib	In the dog, significant immunosuppression was observed. This resulted in demodectic mange-associated bacterial infections of the skin, and parasitic mites.
Tofacitinib	In the monkey, significant immunosuppression was observed. In shorter term studies, this manifested in secondary infections of open wounds and gastrointestinal erosions or ulcers, in a longer term study, this resulted in lymphomas associated with lymphocryptovirus infection.

DISCUSSION & CONCLUSIONS

- As illustrated below, major clinical adverse events associated with pacritinib, ruxolitinib, and tofacitinib are generally consistent with preclinical study findings.

Clinical Adverse Events and Preclinical Toxicities

JAK Inhibitor	Major Human Clinical Adverse Event(s)	Preclinical Animal Model Correlates
Pacritinib	Diarrhea, nausea	GI findings were reported in the rodent (thick abdominal structures, intestinal dilatation) and vomiting and diarrhea were observed in the dog.
Ruxolitinib	Anemia, thrombocytopenia, neutropenia, immunosuppression	The anemia and neutropenia were predictable based on the preclinical hematology (particularly in the dog). Immunosuppression was clear in the dog. Thrombocytopenia was not seen in the preclinical studies.
Tofacitinib	Susceptibility to opportunistic infections, neutropenia, lymphopenia, hematologic malignancies, liver damage, lipid profile changes	Opportunistic infection susceptibility, neutropenia, and lymphopenia are consistent with preclinical observations, particularly in the monkey.

- In comparison to other JAK inhibitors, pacritinib had mild myelosuppressive effects in preclinical studies, and no remarkable immunosuppression.
- Similarly, in the clinic pacritinib is not associated with dose-limiting myelosuppression or immunosuppression.
- Pacritinib's unique nonclinical and clinical profiles may be due to its lack of JAK1 inhibition at relevant concentrations. JAK1 inhibition blocks IL2 and interferon signaling required for a healthy immune response to infection.
- Further investigations comparing effects of JAK inhibitors on specific targets can potentially help determine which specific pathways to target in order to optimize benefit-risk .

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