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Population Pharmacokinetic Analysis of Orally-Administered Ruxolitinib (INCB018424 Phosphate) in Patients With Primary Myelofibrosis (PMF), Post-Polycythemia Vera Myelofibrosis (PPV-MF) or Post-Essential Thrombocythemia Myelofibrosis (PET MF)

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Abstract

Ruxolitinib is a selective inhibitor of Janus kinase 1 and 2, which is approved to treat intermediate or high-risk myelofibrosis. The population pharmacokinetics for ruxolitinib were characterized by a modeling dataset of 272 subjects from a Phase 2 and a Phase 3 study and validated by an external validation dataset of 142 subjects from a second Phase 3 study. The PK of ruxolitinib was adequately described by a two-compartment disposition model with first-order absorption and linear elimination. All model parameters were estimated with good precision. Gender and body weight were identified as covariates for oral clearance (CL/F) and volume of distribution for central compartment (Vc/F), respectively. Apparent oral clearance was 22.1 and 17.7 L/h for a typical male and female subject, respectively, with 39.1% unexplained inter-individual variability (IIV). The typical V_c/F for a subject with a median weight of 72.9 kg was estimated to be 58.6 L, with 28% unexplained IIV. The model predictive performance was validated by visual predictive check (VPC) and the external validation dataset. This analysis suggests that effects of gender and body weight on ruxolitinib PK are not clinically significant and hence no dose adjustment is needed based on gender and weight.

Keywords

ruxolitinib, population pharmacokinetics, myelofibrosis

Ruxolitinib (INCB018424, Jakafi® is a novel, potent, and selective inhibitor of Janus kinase 1 and 2 (JAK1 &2)[1] that is the first drug approved by the FDA for the treatment of intermediate- or high-risk myelofibrosis (MF).[2,3] Ruxolitinib is a class 1 drug according to the Biopharmaceutical classification system (BCS) with excellent permeability, solubility, and dissolution characteristics. Following a single oral dose in healthy adult volunteers, absorption of ruxolitinib is nearly complete.[4] Dose-proportional exposure has been demonstrated over a dose range of 5–200 mg administered as single doses. Ruxolitinib is eliminated almost completely by oxidative metabolism, with metabolites eliminated by renal and fecal excretion. The terminal elimination half-life of ruxolitinib is approximately 3 hours with no appreciable accumulation of either parent or metabolites with twice daily dosing. Renal excretion of parent molecule is negligible. The oxidative metabolites of ruxolitinib retain the pharmacological activity of the parent to varying degrees.

The pharmacodynamics (PD) effect of Ruxolitinib[5] has been characterized using an ex vivo whole blood assay that quantifies IL-6 stimulated STAT3 phosphorylation (pSTAT3). The total of all active metabolites contributes to approximately 18% of the observed PD effect in the cytokine-induced pSTAT3 ex vivo assay.

In vitro metabolism studies strongly suggest that CYP3A4 is the predominant human CYP isozyme responsible for the metabolism of ruxolitinib. When administering ruxolitinib with strong CYP3A4 inhibitors, the total daily dose should be reduced by approximately 50%.[6] No dose adjustment is necessary when co-administering ruxolitinib with weak or moderate inhibitors or with CYP3A4 inducers.[6]

Accumulation of active metabolites in patients with moderate (creatinine clearance (CrCl) 30–59 mL/min) or severe renal impairment[7] (CrCl <30 mL/min) indicates

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that the recommended starting dose based on platelet count should be reduced by ⅓–½, considering the tablet strength availability. Patients on hemodialysis should initiate dosing with a single dose of 15 or 20 mg, based on platelet counts, with doses on the day of dialysis. Subsequent dose should be titrated based on monitoring of individual safety and efficacy. Ruxolitinib should be avoided in patients with end stage renal disease (CrCl less than 15 mL/min) not requiring dialysis and in patients with moderate or severe renal impairment with platelet counts less than 100×10^9/L.

Increased exposure in patients with hepatic impairment[7] indicates that the recommended starting dose based on platelet count should be reduced by ⅓–½, considering the tablet strength availability with subsequent dose titration based on individual safety and efficacy. Ruxolitinib should be avoided in patients with hepatic impairment with platelet counts less than 100×10^9/L.

The purpose of this analysis was to develop a model of the population pharmacokinetics (PK) for ruxolitinib, based on data from one Phase 2 trial and two Phase 3 clinical trials, in order to better characterize the PK of ruxolitinib in subjects with primary myelofibrosis (PMF), post-polycythemia vera myelofibrosis (PPV-MF) or post-essential thrombocythemia myelofibrosis (PET MF). Covariate analysis was performed to support assessment of the PK of ruxolitinib in specific sub-populations.

Method

Data

The primary objective of the population PK analysis is to assess PK in patients with PMF, PPV-MF, and PET-MF. To that end, only data from three studies in patients with PMF, PPV-MF, and PET-MF were included in this analysis. Data from studies 1 and 2 were used as modeling data and data from study 3 for external validation data. All studies were conducted with subjects who had a diagnosis of PMF, PPV-MF, or PET-MF. The study design and blood sample collection schedule are provided in Table 1.

Briefly, study 1 was an open-label study exploring the safety, tolerability, and efficacy of ruxolitinib, administered orally to patients with PMF, PPV-MF, and PET-MF. The study was comprised of three parts: Part 1—dose escalation and expansion; bid dosing; Part 2—alternative dosing schedules; and Part 3—three independent patient groups. Eight dose regimens were evaluated in the three parts of the study. Part 1 evaluated two dose levels of 25 and 50 mg bid, Part 2 studied five dose regimens of 10, 25 mg bid, 25, 50, and 100 mg qd and Part 3 assessed six dose regimens of 10, 15, 25 mg bid, 50, 100, and 200 mg qd. A total of 154 subjects were enrolled, 32 subjects were in Part 1, 29 subjects were in Part 2 and 93 subjects were in Part 3. Study 2 was a randomized, double-blind, placebo-controlled study comparing the efficacy and safety of ruxolitinib to placebo in subjects with PMF, PPV-MF, or PET-MF. Subjects were randomized to receive ruxolitinib or matching placebo tablets. A total of 309 subjects were enrolled, 155 subjects were on ruxolitinib and 154 subjects were on placebo. Study 3 was an open label, randomized study comparing the efficacy and safety of ruxolitinib tablets versus best-available therapy, as selected by the investigator. Subjects were randomized to receive ruxolitinib or best available therapy (BAT) in a ratio of 2:1. A total of 219 subjects were enrolled, 146 subjects were on Ruxolitinib and 73 subjects were on BAT. For Studies 2 and 3, the starting dose of ruxolitinib was determined based on baseline platelet count; the maximum dose on study did not exceed 25 mg bid (twice daily).

Three clinical studies were conducted in full accordance with the Declaration of Helsinki, principles of good clinical practices (GCP), and local laws and regulations regarding the protection of the rights and welfare of human participants in biomedical research. The protocols were approved by an independent institutional review board (IRB), and informed consents for all participants were obtained prior to screening.

Analytical Methods

Blood samples to analyze ruxolitinib plasma concentrations were collected using 4 mL lavender-top (K_2EDTA) Vacutainer collection tubes. Tubes were immediately placed in an ice water bath, and within 45 minutes of collection, were centrifuged at approximately 2,000*g* for 15 minutes at approximately 5°C. Plasma aliquots were stored in a freezer at –20 to −80°C, then shipped to Incyte Corporation or a designated laboratory with a 2-day supply of dry ice. The plasma samples were assayed by a validated, Good Laboratory Practices (GLP), LC/MS/MS method with a linear range of 1–1,000 nM and a limit of quantitation of 1 nM. Accuracy of the assay was between 98.1% and 101% for ruxolitinib and precision (CV%) of the assay ranged from 2.0% to 8.1%. Plasma concentrations of ruxolitinib were expressed in molar units.

Covariates

Subject demographic assessments (age, weight, body mass index, sex, and race), disease-related evaluations (baseline platelet count, tumor type), and clinical laboratory measurements (creatinine clearance (CrCl), albumin (ALB), total bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase) were explored as time-independent predictors of PK variability. Concomitant medications (CYP3A4 inhibitors and inducers, cytoreductive therapies, warfarin, digoxin, and prednisone) were treated as time-dependent categorical variables. The analysis for any specific drug interaction was only performed if the subjects on the concomitant

Table 1. Summary of Studies Included in the Population PK Analysis

Study	Phase	N	Population	Dose	PK sampling schedule
1	Open-label study phase ½	154 on Ruxolitinib	Subjects with PMF or PPV-MF, or PET-MF	Part 1: 25 and 50 mg twice daily up to 33 cycles (1 cycle = 28 days)	Part 1: pre-dose and 0.5, 1, 1.5, 2, 4, 6, and 9 h post-dose on Days 1 and 15 of Cycle 1; pre-dose on Day 1 of Cycles 2 and 3.
				Part 2: 10 and 25 mg twice daily; 25, 50, and 100 mg once daily up to 33 cycles	Part 2: pre-dose and 0.5, 1, 1.5, 2, 4, 6, and 9 h post-dose on day 15 of cycle 1; pre-dose on Day 1 of cycles 2 and 3.
				Part 3: 10, 15, and 25 mg twice daily; 50, 100, and 200 mg once daily up to 33 cycles.	Part 3: pre-dose and 2 h after administration of morning dose on day 15 of cycle 1 and day 1 of cycles 2 and 3.
2	Randomized, double-blind, placebo-controlled Phase 3	309 (155 on Ruxolitinib and 154 on placebo)	Same as study 1	Subjects with baseline platelet count >200,000/μL begin dosing at 20 mg (four 5 mg tablets) twice daily for 24 weeks. subjects with baseline platelet count of 100,000–200,000/μL (inclusive) begin dosing at 15 mg (three 5-mg tablets) twice daily. Standardized dosing paradigm used to determine dose adjustments for safety and efficacy for 24 weeks	Weeks 4 and 12: pre-dose, 1 ± 0.25 h, 2 ± 0.25 h, and between 4 and 12 h post-dose. Weeks 8, 16, 24, 36, 48, 60, and 72, then every 24 weeks after week 72: random samples with dose time and sample collection time.
3	Randomized, controlled, compared to best available therapy Phase 3	219 (146 on Ruxolitinib and 73 on BAT)	Same as Study 1	The same as INCB18424-351 except dosing duration for 48 weeks	Weeks 4 and 12: pre-dose, between 0 and 2 h, and between 2 and 4 h post-dose. Weeks 8, 16, 24, 36, 48, 60, and 72 then every 24 weeks after week 72: random samples with dose time and sample collection time

PMF, primary myelofibrosis; PPV-MF, post-polycythemia vera myelofibrosis; PET-MF, post-essential thrombocythemia myelofibrosis; BAT, best available therapy.

medication were 5% or more of the sample population, in order to mitigate the detrimental impact of small sample size.

Handling of Missing Data and BQL Value

If the actual time for a dose was missing, the subsequent plasma concentration records for the dose were excluded. If a plasma concentration for a PK sample was missing, the record was excluded from the analysis. If date or time for a PK sample was missing, the relevant concentration was deleted from the analysis dataset.

For missing baseline clinical laboratory values and vital signs, baseline values were imputed by the values at screening or at the visit closest to the baseline visit. All other missing continuous covariates were replaced with the population median value for that covariate. For all missing categorical covariates, the number 99 was assigned and the group was tried as a separate category in the covariate testing. If a significant number (>15%) of any covariate value was missing, that covariate was not used in the analysis.

The plasma concentrations with BQL values were assigned as 0 and not included in the analysis dataset.

Population PK Analysis Methods

One- and two-compartment disposition models with first-order absorption and linear elimination were tested for their ability to characterize the pharmacokinetics of ruxolitinib. The magnitude of inter-individual variability (IIV) was assessed for all PK parameters. The IIV in PK parameters

was assumed a log normal distribution and was estimated using the following model with the random effect $\eta_{i:}$

$$P_i = P_{pop}\exp(\eta_i)$$

where P_{pop} was the typical value of the pharmacokinetic parameter in the population, P_i is the individual value in the ith individual and η_i is a random variable with a mean of zero and variance which was estimated as part of the model estimation. Proportional error, additive error, and mixed additive plus proportional error models were each evaluated for their ability to describe the magnitude of residual variability (RV). The first-order conditional estimation (FOCE) method with the INTERACTION option was used.

After a base model was identified, the effects of covariates on ruxolitinib PK were explored. A generalized additive model (GAM) analysis was utilized as a screening tool to initially identify covariates to be formally tested for statistical significance in NONMEM. The candidate covariates were incorporated into the PK model as fixed effect parameters by making the typical values of the structural PK parameters a function of the covariate. The effects of concomitant medications on ruxolitinib were tested in the PK model as time dependent categorical parameters in NONMEM regression analysis. The following model was used to estimate parameter estimates:

$$TVP = P_{pop} + \theta * CONMED$$

where TVP is the typical value of a model parameter, P_{pop} is an estimated parameter describing the typical parameter value for individuals not taken concomitant medications at specific time points. CONMED = 0 for not taken concomitant medications and 1 for taken concomitant medications. NONMEM regression analysis was performed on the model with covariate parameters being added in a stepwise univariate fashion during the forward selection process and subtracted stepwise in the model reduction (backward elimination) process. The likelihood Ratio Test was used to evaluate the significance of incorporating parameters into or removing parameters from the population model. Covariates contributing at least a 3.84 reduction in the objective function ($\alpha = 0.05$, one degree of freedom (df)) were considered significant in forward selection process and a covariate was considered significant if it contributed at least a 7.88 increase in the objective function value ($\alpha = 0.005$, 1 df) when removed from the model in backward elimination process. The covariate contributing the most significant reduction in the objective function (smallest *P* value <0.05) was included in the base covariate model. The new base covariate model (structural model + 1 significant covariate) was then used to test the next round of covariates. Each remaining covariate was added, one at a time, to the new base covariate model and tested for statistical significance using NONMEM. After this series of univariate analyses was completed, the covariate contributing the most significant reduction in the minimum value of the objective function (MVOF) was added to the new base covariate model. This process was repeated until there were no further covariates that produced significant changes in the MVOF. The resultant model was considered the full multivariable model. Univariate backward elimination proceeded after all adjustments had been made to the IIV and RV error models, and the full multivariable model was defined. Each covariate was removed from its respective parameter equation separately. The most non-significant covariate (the highest *P* value greater than 0.005) was removed from the model, and the reduced model then served as the new base multivariable model. The backward elimination procedure was repeated until all remaining covariates were significant. After the stepwise regression step was completed, the model was also checked for possible simplifications of covariate equations, such as power functions that could be reduced to linear functions (power term approximately 1.0), or significant discrete group covariates that could be redefined to fewer groups.

Data preparation was performed using SAS Version 9.1 (SAS Institute Inc., Cary, North Carolina). Exploratory data analyses and presentations of data were performed using S-Plus 7.0 (TIBCO Software, Polo Alto, California), R 2.15.2 (The R Foundation for Statistical Computing) and SAS Version 9.1. The PK analyses used NONMEM Version 7.1.0 (ICON Development Solutions, Ellicott City, MD).

Model Validation

Two methods were employed to assess the predictive performance of the final model, visual predictive check method, and external validation.

A total of 500 replications of the analysis dataset were simulated for visual predictive check method. Statistics of interest (5th, 10th, 50th [median], 90th, and 95th percentiles) were calculated from the simulated concentration values at each simulated sampling time point. Graphical and tabular summarization of the model evaluation results were prepared, including an overlay of the original data on a prediction interval based on the simulated replicate datasets.

Plasma ruxolitinib concentrations from study 3 were used as an external validation dataset. To assess the overall predictive performance of the final model at the population and individual levels, the differences between the measured and population predicted data and the differences between the measured and individual predicted data were evaluated for bias and precision. The population prediction error percent (PE%), absolute population prediction error percent (|PE|%), individual prediction error percent (IPE%), and absolute individual prediction error percent (|IPE|%)were computed for each external observation, as shown in Equation I, Equation II, Equation III, and Equation IV:

$$\mathrm{PE}\% = \frac{\text{Observed data} - \text{Population predicted data}}{\text{Observed data}} \times 100\% \quad \text{(I)}$$

$$|\mathrm{PE}|\% = |\mathrm{PE}\%| \quad \text{(II)}$$

$$\mathrm{IPE}\% = \frac{\text{Observed data} - \text{Individual predicted data}}{\text{Observed data}} \times 100\% \quad \text{(III)}$$

$$|\mathrm{IPE}|\% = |\mathrm{IPE}\%| \quad \text{(IV)}$$

The distribution of PE% and IPE% were evaluated as a measure of bias in the population and individual predictions, respectively, and the distribution of |PE%| and |IPE%| were evaluated as a measure of precision for the population and individual predicted data, respectively. Summary statistics of these prediction errors were calculated to assess bias and precision in the population predicted and individual predicted data relative to the observed data.

Results

Data Description

Only the PK samples from subjects taken ruxolitinib were included. After data cleaning, a total of 942 samples from 125 subjects enrolled in Study 1 and 1,245 samples from 147 subjects enrolled in Study 2 were included in the pooled PK model development analysis dataset and a total of 1,067 samples from 142 subjects enrolled in Study 3 included in the external validation dataset, with a range of 2–18 observations per subject.

The demographic characteristics and laboratory values for subjects, stratified by study, are shown in Table 2. The analysis population was approximately 56% male, primarily Caucasian (92%), and ranged in age from 39 to 87 years. The median body weight (WT) and body mass index (BMI) were 72.9 and 24.7 kg/m^2, respectively. The median value of the calculated creatinine clearance (CrCl) was 68.5 mL/min, indicating that the population, on average, had a modest decrease in glomerular filtration rate. The median value of 30 U/L for AST was indicative of mild elevations in this liver enzyme within the analysis population. Serum albumin (ALB) levels were generally within the normal range (median = 42 g/L). All demographic covariates and laboratory values that were assessed were considered as stationary (time-invariant).

Base Structural Model

The base structural PK model was a two-compartment disposition model with first-order absorption and linear elimination. All fixed effect parameters were estimated with very good precision (percent relative standard error (%RSE) $\leq$22.2%). The first-order absorption rate constant (k_a) was estimated to be 3.43 hours^{-1}, with a lag time of 0.0524 hours. CL/F was estimated to be 20.2 L/h, with estimates for V_c/F and apparent volume of distribution for the tissue (peripheral) compartment (V_p/F) of 57.7 and 11.8 L, respectively. Inter-compartmental clearance (Q/F)

Table 2. Baseline Demographic Characteristics of Patients

Subject characteristic	Modeling data (N = 272), mean ± SD	Validation data (N = 142), mean ± SD
Age (y)	65 ± 9	65 ± 10
Weight (kg)	74.3 ± 17.0	70.7 ± 13.5
BMI (kg/m^2)	25.4 ± 4.8	24.2 ± 3.7
Gender (%)	56% Male, 44% Female	44% Male, 56% Female
Race (%)	92% White, 8% Other	80% White, 20% Other
Creatinine clearance (mL/min)	69 ± 21	82 ± 24
Albumin (g/L)	42 ± 4	44 ± 4
Total bilirubin (μmol/L)	15 ± 8	17 ± 9
Alkaline phosphatase (U/L)	142 ± 97	119 ± 79
Alanine amino-transferase (U/L)	22 ± 15	25 ± 10
Aspartate Amino-transferase (U/L)	33 ± 15	25 ± 10
Baseline platelet count (10^3/μL)	330 ± 208	293 ± 164
Tumor type, (%)		
PMF	48	51
PPV-MF	32	34
PET-MF	20	15
CYP3A4 inhibitor		
No	258	142
Yes	14	0
CYP3A4 inducer		
No	264	142
Yes	8	0

PMF, primary myelofibrosis; PPV-MF, post-polycythemia vera myelofibrosis; PET-MF, post-essential thrombocythemia myelofibrosis.

was 2.6 L/h. The magnitude of estimated inter-individual variability (IIV) ranged from 30.9%CV for V_c/F to 85.7% CV for V_p/F. The poorest precision was for the estimate of IIV in V_p/F (59.7%RSE). The estimate of residual variability was 35.8%CV.

Covariate Evaluation

Forward Selection of Subject Covariates. After the general additive model (GAM) screening in Splus and univariate analysis of effect of concomitant medications on ruxolitinib clearance in NONMEM, six covariates were chosen to add to the model during the forward selection process including (presented in order of inclusion): the effect of body weight on V_c/F, gender effect on CL/F, gender effect on V_c/F, the influence of AST levels on V_c/F, the influence of serum ALB levels on CL/F, and the effect of CrCl on CL/F. Inclusions of effects of concomitant medications (CYP3A4 inhibitors, cytoreductive therapies, warfarin, digoxin, prednisone) on ruxolitinib clearance reduced the objective function value (OFV) by 2.91, 0.4, 0.005, 0.019, and 0.016, respectively. The lack of a significant difference in OFV indicated that these concomitant medication effects on CL/F were insignificant and were not included in the base structural model for further consideration. There were only eight out of 272 patients taken CYP3A4 inducer which was less than 5% of whole population and hence was not considered here. The effect of body weight on V_c/F and the effect of gender on CL/F explained the greatest amount of variability relative to the remaining statistically significant covariate effects. Moreover, inclusion of body weight effect on V_c/F reduced OFV by 70.7 (P-value $<.000001$), comparing OFV from the base structural model. Another 27.3 decrease was observed when the effect of gender on CL/F was added to model including body weight effect on V_c/F. No covariates were found to be statistically significant predictors of variability in k_a or V_p/F. The addition of these six covariate effects reduced the IIV in CL/F from 37.9%CV to 34.8% CV and reduced the IIV in V_c/F from 30.9%CV to 21.7%CV, which represent approximate 8% and 30% reductions in total inter-individual variability of the CL/F and V_c/F parameters, respectively. Inclusion of these covariates reduced the estimate of residual variability (RV) by 0.3%CV. None of the concomitant medications tested were significant predictors for CL/F.

Evaluation of the Full Multivariable Model and Statistical Error Models. The full multivariable model had an MVOF of 22850 which was a reduction of 126 units from the base structural model. The IIV models were further evaluated, including exploration of addition of other IIV terms and assessment of correlations between random effects. Correlation plots suggested a strong correlation existed between CL/F and V_c/F. Therefore, the corresponding covariance between CL/F and Vc/F was introduced and estimated. This covariance term was estimated with reasonable precision (15.3% RSE), described a correlation of 0.670 between IIV's on CL/F and V_c/F, and resulted in further reduction of the MVOF by 61 units.

Backward Elimination of Subject Covariates

Four covariates were removed from the model during the backward elimination process since no significant change was observed by excluding them (P-value $>.005$), including the effects of serum ALB and CrCl on CL/F and the effects of gender and AST on V_c/F. All remaining covariates were statistically significant ($\alpha = 0.005$). The removal of the non-significant covariates from the model increased the IIV in CL/F and V_c/F by less than 0.6%CV and 1.8% CV units, respectively.

Model Refinement

The estimate of the power term describing the fixed effect of body weight on V_c/F approached a value of 1, with a mean population estimate (95% confidence interval) of 0.873 (0.683–1.06). The significance of this power term was re-evaluated and was shown to not be statistically significant from 1 (increase in MVOF of 1.801 units, $P = 0.180$). Replacement of the power term with 1 only increased IIV in V_c/F by 0.1% CV and did not considerably impact estimates of any other parameters. Therefore, the effect of body weight on V_c/F was described using proportionality constant; this equates to a linear relationship between V_c/F and body weight described by a single slope term (with intercept = 0). Goodness-of-fit plots did not reveal bias in the residual variability model. No adjustments were made to the IIV models or the residual error model. Therefore, this model was considered the final PK model and subjected to the model evaluation procedure.

Final Model. The final population PK model parameter estimates, along with corresponding precisions (%RSE), are presented in Table 3. The goodness-of-fit plots from this model are shown in Figure 1. All fixed and random effect parameters were estimated with good precision (% RSE $\leq$20.3%), with the exception of IIV in V_p/F and k_a, which were estimated with moderate precision (36.6 and 43.7%RSE, respectively). The magnitude of the inter-individual variability was moderate for CL/F (39.1%CV) and V_c/F (28.0%CV). The magnitude of the estimated inter-individual variability was large for V_p/F and k_a (102.0%CV and 75.0%CV, respectively). The sizeable random variability in these two PK parameters is expected: Inter-individual variability in k_a is usually larger, mainly due to the inherent variability in the absorption process and the limited data available to inform this parameter due to sparse sampling at the absorption phase. The varying monophasic and biphasic disposition patterns observed in the full-profile data, as well as the sparse sampling strategy employed in the Phase 3 study, may have contributed to

Table 3. Population PK Parameters for Final PK Model

	Final parameter estimate		Magnitude of inter-individual variability (%CV)	
Parameter	Population mean	%RSE	Final estimate	%RSE
k_a (h^{-1})	4.12	14.3	75.0	43.7
$ALAG_1$ (h)	0.0545	5.96	NE	NE
CL/F (L/h) for males	22.1	3.40	39.1	9.22
CL/F (L/h) for females	17.7	3.50		
V_c/F for subject with body weight of 72.9 kg (L)	58.6	2.80	28.0	12.7
V_p/F (L)	11.2	18.6	102	36.6
Q/F (L/h)	2.53	20.3	NE	NE
RV (%CV)	35.5	6.19	NA	NA

%CV, percent coefficient of variation; %RSE, percent relative standard error; k_a, first-order absorption rate constant; $ALAG_1$, absorption lag time; CL/F, apparent oral clearance; V_c/F, apparent volume of distribution for the central compartment, $V_c/F_j = 58.6 \times \left(\frac{WTKG_j}{72.9}\right)$, where j represents the jth subject, V_p/F, apparent volume of distribution for the tissue (peripheral) compartment; Q/F, apparent inter-compartmental clearance; RV, residual variability; NA, not applicable; NE, not estimated.

the high IIV estimate of V_p/F. Residual variability was moderate (35.5%CV) and comparable to the base PK model estimate. The effect of CYP3A4 inducer on CL/F was tested only in an exploratory manner in the final model since there were only eight subjects that took CYP3A4 inducers (<5% of whole population). Inclusion of CYP3A4 inducer reduced the OFV by 5.84 (P-value = 0.016) which is larger than the pre-specified P-value of .005 and was considered insignificant. Therefore, it was not included in the final model.

Model Evaluation and Validation

The visual predictive check on the final PK model (Figure 2) using modeling dataset showed that the majority of the observed data fell within the 90% prediction interval and the median of the prediction interval tracks the middle of the observed data, demonstrating excellent model performance.

The final ruxolitinib population PK model was evaluated for its ability to accurately and precisely predict PK data collected in Study 3. A small proportion (n = 77, 7.2%) of observed concentrations in Study 3 with highly negative PE% values (<−300%) were excluded from these summary statistics. Forty-seven (57%) of these excluded samples were collected at ≤1 hour post-dose, representing concentrations collected during the absorption phase. The summary statistics of the PE%, |PE%|, IPE %, and |IPE%| are shown in Table S1.

The mean and median values for the population prediction error percent (PE%) for the observed concentrations in the model validation dataset are −10.8% and 5.3%, respectively. The distribution shows that the population prediction error percent was within approximately ± 30% for the majority of samples. The mean and median values for the absolute prediction error (|PE%|) for the observed concentrations in the model validation dataset are 51.0% and 38.3%, respectively. This indicates that the population model (without inter-individual variability effects included) has some degree of imprecision in its estimation of ruxolitinib concentrations in the external dataset. Nonetheless, this imprecision is within acceptable limits and the current model is considered useful in predicting ruxolitinib concentrations.

The mean and median values for the individual prediction error percents (IPE%) for the observed concentrations in the model validation dataset are −9.2% and 3.3%, respectively. The distribution of IPE % exhibits a leftward skew, although the individual prediction error percents are within approximately ±30% for the majority of samples, and only a small number of samples (<5% of the total number of samples) contribute to the left tail. The mean and median values for the absolute individual prediction error percents (|IPE%|) for the observed concentrations in the model validation dataset were 30.0% and 18.8%, respectively. This indicates that the PK model (including inter-individual variability) has adequate precision in its estimation of individual ruxolitinib concentrations in the external validation population.

Relevance of Covariates

The statistically significant covariates (gender and body weight) were further assessed for relevance on ruxolitinib exposure following 15 and 20 mg BID dosing. The covariates were split into two groups and the difference in geometric mean ratios of the individual predicted AUCss normalized to 10 mg BID between groups was evaluated. Gender was evaluated as male versus female (reference group). Body weight was divided into 2 groups at the median value of 72.9 kg and patients with higher body weight were used as the reference group (>72.9 kg).

The geometric mean ratios for both gender and body weight were maintained within the bounds of 0.5–2 for AUCss at both dose levels. Therefore, neither the

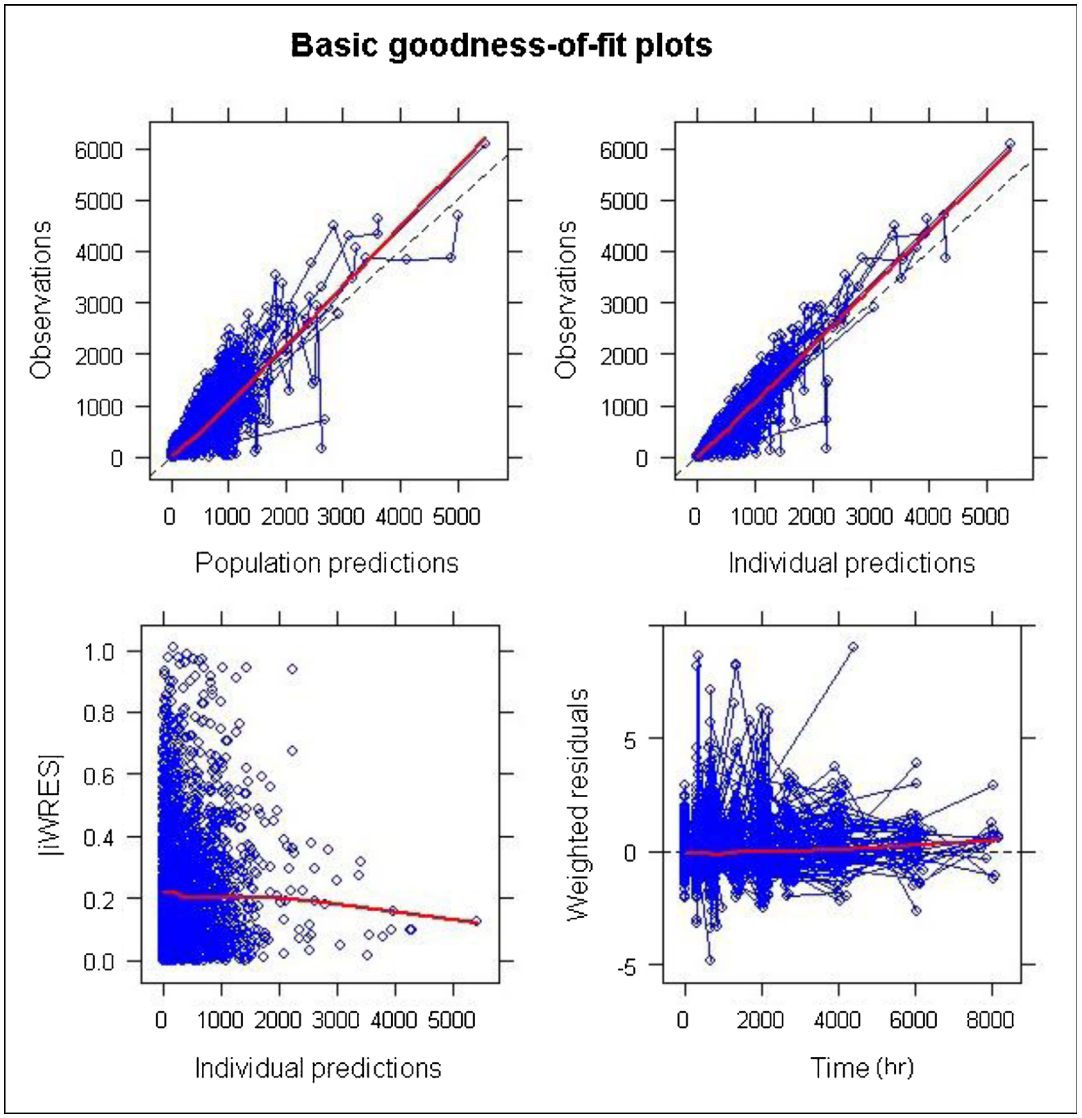

Figure 1. Goodness-of-fit plots for the final population PK model (IWRES, individual weighted residuals; ○, observed concentration; dash line, line of identity; solid line, regression line)

proportional relationship between Vc/F and body weight nor the estimated approximate 20% reduction in CL/F for females compared to males were deemed relevant with regard to steady-state ruxolitinib exposure. However, both covariate effects are retained in the final population model as significant predictors of PK parameters.

Discussion

A population PK model for ruxolitinib was developed using pooled data from studies 1 and 2 conducted in subjects diagnosed with PMF, PPV-MF, and PET-MF. The model was used to estimate ruxolitinib population PK parameters, to assess the influence of subject covariates (including demographic characteristics, laboratory indices, and concomitant medications) on ruxolitinib PK, and to obtain empirical Bayes PK parameter estimates to predict individual ruxolitinib exposures that will be utilized in related PK/PD analyses. This model was externally validated using data collected from a different Phase 3 study (Study 3) conducted in subjects who were also diagnosed with PMF, PPV-MF, or PET-MF.

The final PK model was parameterized in terms of k_a, $ALAG_1$, CL/F, V_c/F, Q/F, and V_p/F. The estimated mean population absorption half-life is approximately 0.168 hours (~10 minutes) following an estimated typical absorption lag time of less than 5 minutes (0.0545 hours) after administration of a dose. Despite minimal data available to inform the absorption parameters, both k_a and $ALAG_1$ were estimated with very good precision (14.3 and 5.96%RSE, respectively). The typical apparent terminal elimination (β) half-life estimated from

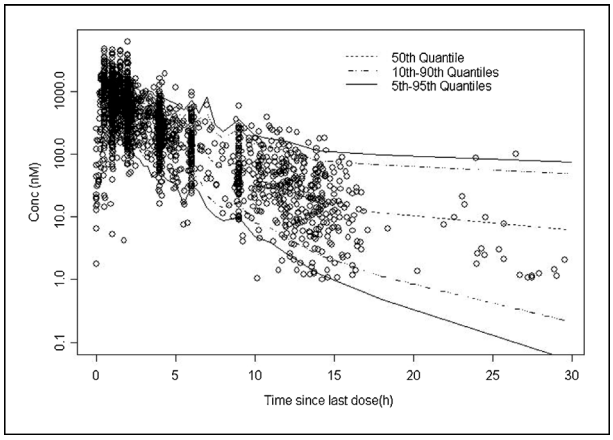

Figure 2. Visual Predictive Check of ruxolitinib concentration-time profile with 5th, 50th and 95th percentiles shown (o, observed concentration; 50th percentile (– – –); 5th–95th percentile (———); 10th–90th percentile (– · – · –)).

the final population PK model is approximately 3.76 hours for males and 4.07 hours for females, comparable to estimates derived from previous non-compartmental analyses (data not shown).

Inter-individual variability was estimated for k_a, CL/F, V_c/F, and V_p/F; the covariance for inter-individual random effects on CL/F and V_c/F was also estimated due to the strong correlation observed between $\eta_{CL/F}$ and $\eta_{Vc/F}$. The

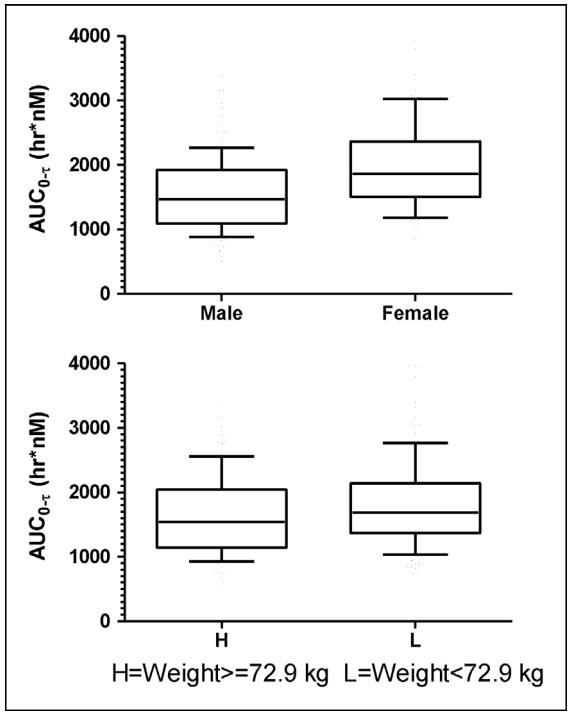

Figure 3. Sensitivity plots of comparing the effect of covariates on the steady state exposures normalized to 10 mg BID (Whiskers: 10–90 percentile)

estimated IIV in all 4 parameters (75.0%CV for k_a, 39.1% CV for CL/F, 28.0%CV for V_c/F, and 102.0%CV for V_p/F) following inclusion of significant covariate effects reflects a typical magnitude that is expected given the composition of PK data used in model development. The moderate IIV in k_a is anticipated, not only because of the typically high inter-individual variability inherent in the absorption phase, but also partly due to the lack of sufficient data to fully inform the absorption process because of the sparse sampling strategy. The large IIV in V_p/F is, in part, a reflection of the variable disposition patterns observed across the analysis population, with profiles exhibiting a mix of both mono-exponential and bi-exponential declines in plasma concentrations following peak (C_{max}).

Covariate analysis was performed to identify factors predictive of variability in ruxolitinib PK. Only gender was found to be a statistically significant predictor of CL/F, with males having a slightly higher apparent clearance compared to females (22.1 L/h versus 17.7 L/h, respectively). Body weight was the only statistically significant predictor of V_c/F. The influence of body weight on apparent central volume of distribution was described by a linear function, which predicts a V_c/F of 56.3 L for a typical 70 kg individual and an approximate 8 L increase in V_c/F for every 10 kg increase in body weight.

The gender difference in oral clearance may be explained by the difference of body weight between males and females. However, Figure S1 depicts a lack of correlation between body weight and individual clearance values as obtained by the post-hoc clearance values from the base population PK model. In addition, both female and male patients showed a lack of relationship between clearance and weight. Since body weight was not selected as significant covariate for CL/F by GAM, two additional post-hoc NONMEM analyses, base model plus weight as covariate on CL/F and base model plus gender and weight as covariates on CL/F, have been tried. The exercise confirms that addition of weight as covariate on clearance is not needed to adequately describe the ruxolitinib pharmacokinetics.

Neither the concomitant medications that were explored nor any of the laboratory indices of kidney and liver function were found to be significant descriptors of inter-individual variability in PK parameters.

Ruxolitinib is a CYP3A4 substrate. Co-administration with strong CYP inhibitors or inducers had effect on ruxolitinib clearance by two dedicated phase I studies.[6] However, only one patient received a strong CYP inhibitor and none of patients received a potent CYP3A4 inducer. Hence, it is unlikely to assess potent CYP inhibitors or inducers effect on ruxolitinib clearance. A pooled analysis of CYP inhibitors or CYP inducers did not show any statistical significance.

There were only two patients with severe renal impairment and the analysis showed that Modification of Diet in Renal Disease (MDRD) levels had no effect on ruxolitinib clearance, consistent with the dedicated phase I renal impairment study.[7] It is also worthwhile noting that changes in ruxolitinib metabolite burden were seen with worsening renal function[7], but that metabolites were not assessed in the pop PK model and that dose adjustment should be made for renal function as described in the label.

The changes with pharmacokinetic exposures seen in the dedicated phase I hepatic impairment study were not related to degree of hepatic function,[7] nor was there a direct relationship with laboratory markers of hepatic disease (AST, ALT, bilirubin, or albumin) so it was not surprising that this was also not evident in the pop PK model. The NCI Organ Dysfunction Working Group (ODWG) criteria for hepatic impairment were used to identify subjects with varying degrees of hepatic impairment. Subjects were classified as normal (bilirubin $\leq$1.0 ULN and AST $\leq$1.0 ULN (ULN, upper limit of normal)), mild impairment B1 (bilirubin $\leq$1.0 ULN and AST $>$1.0 ULN), mild impairment B2 (bilirubin $>$1.0 to $\leq$1.5 ULN), moderate impairment (bilirubin $>$1.5 to $\leq$3 ULN) and severe impairment (bilirubin $>$3 ULN). There were 191 patients in normal, 43 in mild impairment B1, 41 in mild impairment B2, 15 in moderate impairment and two unknown. None of patients were in severe hepatic impairment group. The analysis showed that hepatic function had no effect on ruxolitinib clearance.

Individual empirical Bayes estimates of k_a, CL/F, V_c/F, and V_p/F were obtained from the final PK model. The mean CL/F was similar between study 1 and study 2 (22.0 and 21.3 L/hr, respectively). Apparent central volume of distribution was fairly consistent across both studies, with mean V_c/F values of 59.1 and 61.0 L for studies 1 and 2, respectively. The mean predicted terminal elimination half-life for the overall population was 4.39 hours. Individual estimates of AUC_{ss} for each dosing regimen were also estimated using these Bayesian PK parameters. A comparison of predicted AUC_{ss} normalized to 10 mg between male and female subjects, as well as between subjects with high versus low body weight, generally showed a relatively wide distribution in exposures (Figure 3), but more importantly indicated that a nominal difference exists in ruxolitinib exposures at steady state between the two comparator populations, supporting lack of recommendation to dose adjustment based on these parameters.

In conclusion, although both gender and body weight were found to be statistically significant predictors of ruxolitinib PK, the geometric mean ratios in both cases fell within (0.5, 2) and hence are not clinically significant. The population PK model supports the dosing guidelines in the prescribing information.[8]

Declaration of Conflicting Interest

No conflict of interest.

References

1. Quintás-Cardama A, Vaddi K, Liu P, et al. Preclinical characterization of the selective JAK1/2 inhibitor RUXOLITINIB: therapeutic implications for the treatment of myeloproliferative neoplasms. *Blood* 2010;115:3109–3117.
2. Verstovsek S, Kantarjian H, Mesa RA, et al. Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. *N Engl J Med.* 2010;363:1117–1127.
3. Verstovsek S, Passamonti F, Rambaldi A, et al. Durable responses with the JAK1/JAK2 inhibitor, INCB018424, in patients with polycythemia vera (PV) and essential thrombocythemia (ET) refractory or intolerant to hydroxyurea (HU). Presentation on the American Society of Hematology (ASH) annual conference (http://investor.incyte.com/phoenix.zhtml?c=69764&p=irol-presentations). 2010.
4. Adam D. Shilling Frank M. Nedza Thomas EM, et al. Metabolism, excretion, and pharmacokinetics of [^{14}C] INCB018424, a selective janus tyrosine kinase 1/2 inhibitor, in humans. *Drug Metab Dispos.* 2010;38:2023–2031.
5. Shi JG, Chen X, McGee RF, et al. The pharmacokinetics, pharmacodynamics, and safety of orally dosed INCB018424 phosphate in healthy volunteers. *J Clin Pharmacol.* 2011;51, 1644–1654.
6. Shi JG, Chen X, Emm T, et al. The effect of CYP3A4 inhibition or induction on the pharmacokinetics and pharmacodynamics of orally administered ruxolitinib (INCB018424 phosphate) in healthy volunteers. *J Clin Pharmacol.* Online publication ahead of print, 20 May 2011 (DOI: 10.1177/0091270011405663)
7. Chen X, Shi JG, Emm T, et al. Pharmacokinetics and pharmacodynamics of orally-administered ruxolitinib (INCB 0184 24 phosphate) in renal and hepatic impairment patients (in press).
8. Full prescribing information for JAKAFI. Available at, http://www.incyte.com/products/uspi_jakafi.pdf (last access January 31, 2012).

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's website.

Figure S1. Lack of correlation between ruxolitinib clearance and weight using base model.

Table S1. Summary Statistics of Prediction Error Percent for the Final Population Pharmacokinetic Model Applied to Study 3