CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

202192Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

ruxolitinib (JAKAFI)

Date: October 27, 2011
To: File for NDA 202192
From: John K. Leighton, PhD, DABT
Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review of Dr. Chen and labeling and supervisory memorandum provided by Dr. Saber. I concur with Dr Saber's conclusion that JAKAFI may be approved for the proposed indication and that no additional nonclinical studies are needed.

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/s/

JOHN K LEIGHTON
10/27/2011

MEMORANDUM

Date: October 27, 2011
From: Haleh Saber, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
NDA: 202,192
Drug: JAKAFI (ruxolitinib)
Indication: For treatment of patients with myelofibrosis, including primary myelofibrosis, post-polycythemia vera myelofibrosis and post-essential thrombocythemia myelofibrosis

Ruxolitinib is a small molecule inhibitor of Janus Associated Kinases 1 and 2 (JAK 1/2). The inhibitory activity of ruxolitinib on JAKs was demonstrated *in vitro* and in animal models containing aberrant JAK/STAT signaling. Myelofibrosis, a type of myeloproliferative disorder, is known to be associated with dysregulation of JAK 1/2. This condition may present as a primary myelofibrosis or it may be secondary to polycythemia vera or essential thrombocythemia. The pharmacologic class assigned to ruxolitinib is "kinase inhibitor", as presented in the HIGHLIGHTS section of the label. The detailed information on the mechanism of action of the drug is under section 12.1 of the FULL PRESCRIBING INFORMATION.

Pharmacology, safety pharmacology, pharmacokinetic/ADME (absorption, distribution, metabolism and excretion), and toxicology studies were conducted in *in vitro* systems and/or in animal species. Ruxolitinib was administered orally to animals in toxicology studies, consistent with the intended route of administration in patients. Drug-related toxicities were similar after single- or repeat-dose administration, therefore only repeat-dose general toxicology studies were reviewed for this NDA. Toxicities were mostly related to pharmacology of the drug, with lymphoid depletion, and reduced size of thymus and spleen being the primary adverse effects. Single-dose administration of ruxolitinib to Beagle dogs in a safety pharmacology study resulted in reduced systolic, diastolic, and mean arterial pressure. Safety pharmacology studies also revealed CNS and respiratory effects in animals; e.g. lower body temperature, reduced activity, and increased tidal volume/decreased minute volume. While dizziness and balance disorder have been reported in patients treated with ruxolitinib, cardiovascular or respiratory adverse effects have not been observed.

When administered during the period of organogenesis, ruxolitinib was not teratogenic to rats or rabbits. Reduced fetal weight and/or increased post-implantation loss were seen in animals only at doses that resulted in maternal mortalities. In a designated fertility study, ruxolitinib did not impair male or female fertility but resulted in increased post-implantation loss. In a peri- and post-natal developmental study conducted in rats, there were no drug-related adverse findings in pups for fertility indices or for maternal or

embryofetal survival, growth, and development parameters at the doses evaluated. Reduced number of pups (F1) delivered compared to the control appears to be secondary to the post-implantation loss, as previously reported in the embryo-fetal developmental study.

Ruxolitinib was not genotoxic when tested *in vitro* or *in vivo* for mutagenic or clastogenic potential. When tested in a 6-motnh carcinogenicity study in Tg.rasH2 transgenic mouse, ruxolitinib was not carcinogenic. A 2-year carcinogenicity study in rat is ongoing. Considering the serious and life-threatening condition of the disease and lack of adequate therapy, the results of the 2-year carcinogenicity study may be submitted post-approval. Based on the QSAR (quantitative structure–activity relationship) models, six impurities were found to have structural alerts for genotoxicity. Further evaluation by Ames assay, showed these compounds to be negative for mutagenic potential. The Ames tests on impurities were non-GLP; however, upon further examination of the study criteria, conduct of the study, and concentrations used in the study, DHOT accepts results of the non-GLP studies for these impurity. Of note, one of the six impurities was present in the 6-month carcinogenicity study at (b) (4)

Considering the results of reproduction toxicology studies together with the negative results reported in the genetic toxicology studies, the Applicant's proposed Category C for pregnancy is acceptable.

The nonclinical studies were reviewed by Dr. Wei Chen. Results of the carcinogenicity study were reviewed by Dr. Miyun Tsai-Turton. The nonclinical findings are summarized in the "Executive Summary" of the NDA review and reflected in the product label.

Recommendation: I concur with Dr. Chen that from a nonclinical perspective, JAKAFI may be approved for the proposed indication.

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/s/

HALEH SABER
10/27/2011

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	202,192
Supporting document/s:	SDN 000
Applicant's letter date:	June 3, 2011
CDER stamp date:	June 3, 2011
Product:	Ruxolitinib phosphate
Indication:	Myelofibrosis
Applicant:	Incyte Corporation
Review Division:	Division of Hematology Oncology Toxicology
	(for Division of Hematology Products; DHP)
Reviewer:	Wei Chen, Ph.D.
Supervisor/Team Leader:	Haleh Saber, Ph.D.
Division Director:	John Leighton, Ph.D., D.A.B.T.
	(Ann Farrell, MD for DHP)
Project Manager:	Amy Baird

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1 Executive Summary

1.1 Introduction

Ruxolitinib phosphate, a new molecular entity, is a small molecule inhibitor of the Janus kinase family of protein tyrosine kinases (JAKs). JAKs play a role in the signal transduction following cytokine and growth factor binding to their receptors. After the receptor associates with its respective cytokine/ligand it goes through a conformational change, bringing the JAKs close enough to phosphorylate each other. Once phosphorylated/ activated, the JAKs transduce an intracellular signal by promoting and activating transcription factors called STATs. The activated STATs (signal transducer and activator of transcription) dissociate from the receptor and form dimers before translocating to the cell nucleus where they regulate transcription of selected genes [Kisseleva et al, Gene 285:1-24,(2002)]. The efficacy of ruxolitinib phosphate has been shown in *in vitro* studies and in *in vivo* nonclinical tumor models representing both hematological and solid tumors. Ruxolitinib is being developed as a monophosphate salt for the oral treatment of myelofibrosis, a type of myeloproliferative neoplasm (MPN). With this NDA, the Applicant is submitting nonclinical pharmacology, pharmacokinetic, and toxicology studies to support the approval of ruxolitinib phosphate for the treatment of myelofibrosis.

1.2 Brief Discussion of Nonclinical Findings

Ruxolitinib was rapidly absorbed in rats and dogs with a similarly short half-life after single and multiple oral dosing. The pivotal non-clinical general toxicity studies of ruxolitinib were conducted in rats and dogs, consistent with the clinical route of administration. Non-clinical studies also included safety pharmacology studies, reproductive toxicity studies, genotoxicity studies, and a carcinogenicity study. The pivotal toxicology studies were conducted in compliance with Good Laboratory Practice regulation. Nonclinical pharmacokinetic and toxicokinetic studies of ruxolitinib were also evaluated in rats and dogs.

Pharmacology

Ruxolitinib (also referred to as “INCB018424” or “INC424”) is a pyrrolo-pyrimidine derivative, and it is an inhibitor of JAKs, with relative selectivity for JAK1 and JAK2. Ruxolitinib inhibited JAK in enzyme-based and cell-based assays. Ruxolitinib showed its inhibitory activity on JAKs in animal models with aberrant JAK/STAT signaling. Ruxolitinib inhibited cell proliferation, decreased circulating inflammatory cytokines (eg, TNF-α, IL-6) and resulted in prolonged survival in the mice xenograft models of hematological malignancies.

Safety pharmacology

Safety pharmacology studies revealed adverse effects of ruxolitinib on the functions of central nervous, respiratory and cardiovascular systems. Administration of ruxolitinib resulted in various observations including lower body temperature, lower activity and observations of darkened skin and mucous membranes. Decreased respiratory frequency, increase in tidal volume and decrease in minute volume were noted in rats

following a single oral dose of INCB018424. Administration of ruxolitinib at a dose of 30 mg/kg (the highest dose tested) resulted in significantly lower pulse pressure, as well as lower systolic, diastolic, and calculated mean arterial pressure (up to 53%, 41%, 31%, and 33%, respectively) when compared to the control group.

General toxicology
The repeated dose toxicology studies in rats and dogs have shown that oral administration of ruxolitinib resulted in alterations in hematology parameters and microscopic observations of marked lymphoid depletion, which correlated with small thymus and spleen as well as low lymphocyte counts. No adverse effect of ruxolitinib on central nervous system, respiratory, or cardiovascular system was noted at the doses tested in the repeat-dose toxicology studies in rats and dogs. Clinical and anatomical pathology findings associated with the treatment of ruxolitinib were generally reversible after a recovery period. Toxicities observed are mostly direct effects based on the pharmacology of ruxolitinib.

Genetic toxicology
Ruxolitinib was not mutagenic or clastogenic in the *in vitro* and *in vivo* assays studied.

Reproductive toxicology
Ruxolitinib did not impair fertility when administered to either male or female rats prior to and during the mating time frame. However, embryo-fetal viability was reduced. Ruxolitinib was not teratogenic in either the rat or the rabbit when administered to animals during the period of organogenesis. It did, however, lead to increased postimplantation loss and/or reduced fetal weight at a maternally toxic/lethal dose. In a pre- and post-natal developmental study, ruxolitinib resulted in a slightly prolonged gestation period; the significance of this finding is unclear. Reduced number of pups (F1) delivered appears to be secondary to the increased post-implantation loss in F0 females..

Carcinogenicity studies
Treatment of Tg.rasH2 animals with ruxolitinib at daily oral doses up to 125 mg/kg for 26-weeks did not increase the incidence of neoplastic lesions.

1.3 Recommendations

1.3.1 Approvability

Recommending approval. The non-clinical studies adequately support the safety of ruxolitinib phosphate by oral route in myelofibrosis.

1.3.2 Additional Non Clinical Recommendations

Additional non-clinical studies are not needed at this time.

1.3.3 Labeling

Information needed for nonclinical sections of the label are provided in this review. Therefore, a separate labeling review is not deemed necessary.

2 Drug Information

2.1 Drug

CAS Registry Number: 941678-49-5

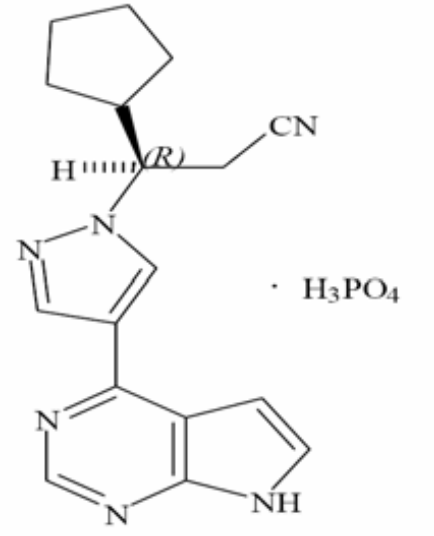
Generic Name: ruxolitinib phosphate

Code Name: INCB018424; INC424

Chemical Name: (R)-3-(4-(7H-pyrrolo [2,3-d] pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile phosphate

Molecular Formula/Molecular Weight: $C_{17}H_{21}N_6O_4P$ / 404.36

Structure or Biochemical Description



Pharmacologic Class: kinase inhibitor
Mechanism of action: inhibition of the Janus Associated Kinases 1 and 2 (JAKs 1 and 2)

2.2 Relevant INDs, NDAs, BLAs and DMFs:

IND 77456 (for myeloproliferative disorders)
IND 77101 (for psoriasis)
IND 77,455 (for rheumatoid arthritis)
DMF (b) (4)

2.3 Drug Formulation: 5mg, 10 mg, 15 mg, 20 mg, and 25 mg tablets

Composition of Ruxolitinib Phosphate Tablets, 5 mg

Component	Quality Standard	Amount (mg/tablet)	Function
Ruxolitinib Phosphate[a]	Incyte specification	(b) (4)	API
Microcrystalline Cellulose	NF/EP/JP		(b) (4)
Lactose Monohydrate	NF/EP/JP		
Colloidal Silicon Dioxide	NF/EP		
Hydroxypropyl Cellulose	NF/EP		
Povidone	USP/EP		
Sodium Starch Glycolate	NF/EP		
Magnesium Stearate	NF/EP/JP		
(b) (4)	USP/EP		
Total Tablet Weight (mg)			

(b) (4)

2.4 Comments on Novel Excipients: N/A

2.5 Comments on Impurities/Degradants of Concern: none

2.6 Proposed Clinical Population and Dosing Regimen

Myelofibrosis, including primary myelofibrosis (PMF), post-polycythemia vera Myelofibrosis (PPV-MF), and post-essential thrombocythemia-myelofibrosis (PET-MF)

The recommended starting dose is as follows:

Patients with platelet counts between 100,000 and 200,000/μL: 15 mg, oral, twice daily
Patients with platelet counts > 200,000//μL: 20 mg, twice daily

3 Studies Submitted

Studies Reviewed

ADME

	Title	Study no.	Folder/file
1	Pharmacokinetics of INCB018424 in Dogs	INCYTE-DMB-06.182.1	M4.2.2.2
2	*In vitro* and e*x vivo p*rotein binding of INCB018424 in rat, cynomolgus monkey, dog, minipig, and human serum and plasma	INCYTE-DMB-07.11.1	M4.2.2.3
3	Quantitatibe whole-body autoradioluminography of rats following oral administration of 14C-INCB018424	INCYTE-DMB-08.58.1	M4.2.2.3
4	Placental transfer and lacteal excretion of 14C-INCB018424 following administration of a single oral dose to Pregnant Sprague Dawley rats	INCYTE-DMB-10.50.1	M4.2.2.3
5	The in vitro metabolism of INCB018424 by rat liver microsomes and individual rat recombinant cytochrome P450(s)	INCYTE-DMB-10.51.2	M4.2.2.4
6	INCB018424: material balance and metabolism in male rats	INCYTE-DMB-08.61.1	M4.2.2.4
7	Identification of in vivo metabolites of INCB018424 in female rat following a single oral dose of ^{14}C-INCB018424	INCYTE-DMB-08.169.1	M4.2.2.4
8	Identification of in vivo metabolites of INCB018424 in beagle dogs	INCYTE-DMB-07.14.1	M4.2.2.4
9	Metabolism of [14C]INCB018424 in male and female beagle dogs after a single oral administration identification	INCYTE-DMB-08.149.1	M4.2.2.4
10	Excretion mass balance in female rats and pharmacokinetics of radioactivity in male and female rats following a single oral dose of [^{14}C]INCB018424	INCYTE-DMB-09.82.1	M4.2.2.5
11	Excretion/mass balance in male and female beagle dogs after a single oral administration of [^{14}C]INCB018424	INCYTE-DMB-08.62.2	M4.2.2.5

Drug interaction

	Title	Study no.	Folder/file name
1	Human CYP Isozymes that are Responsible for the In Vitro Metabolism of INCB018424	INCYTE-DMB-09.93.1	M5.3.2.2
2	In vitro evaluation o f INCB018424 as an inducer of cytochrome P450 expression in cultured human hepatocytes	INCYTE-DMB-11.06.1	M5.3.2.2

Toxicology studies

Repeat dose

	Title	Study no.	Folder/file name
1	A 6-month oral (gavage) toxicity study of INCB018424 in rats with a 6 week recovery period	T07-10-06	M4.2.3.2
2	A 6-month oral (gavage) toxicity study with INCB018424 in beagle dogs with 6-week recovery period	T07-10-07	M4.2.3.2
3	52-week oral gavage chronic toxicity and toxicokinetic study with INCB018424 in dogs with a 6-week recovery period	T08-07-03	M4.2.3.2

carcinogenicity

	Title	Study no.	Folder/file name
1	INCB018424: 26 week repeated dose oral carcinogenicity study in Tg.rasH2 mice	T09-02-03	M4.2.3.4

Reproductive toxicology

	Title	Study no.	Folder/file name
1	Oral gavage study of fertility and early embryonic development to implantation with INCB018424 in rats	8212204	M4.2.3.5
2	Oral administration of INCB018424 via gavage: definitive study for effects on embryo-fetal development in Sprague Dawley rats	T07-12-04	M4.2.3.5
3	Oral administration of INCB018424 via gavage: definitive study for effects on embryo-fetal development in New Zealand white rabbits	T07-12-05	M4.2.3.5
4	Oral gavage study for effects on pre- and post-natal development, Including maternal function with INCB018424 in rats	1001237	M4.2.3.5

Other studies

Genetic toxicology

	Title	Study no.	Folder/file name
1	(b) (4)	1012571	M4.2.3.7
2		1012575	M4.2.3.7
3		1012576	M4.2.3.7
4		1012577	M4.2.3.7
5		1012578	M4.2.3.7
6		1012579	M4.2.3.7

Studies submitted, but not reviewed

Pharmacology

	Title	Study no.	Folder/file name
1	In vitro activity of INCB018424 metabolites	INCYTE-IN VITRO-07.02.2	M4.2.1.1
2	In vitro activity of INCB018424 in cytokine-mediated cell-based assays	INCYTE-IN VITRO-09.08.1	M4.2.1.1
3	In vitro activity of INCB018424 metabolites	INCYTE-IN VITRO-069.11.1	M4.2.1.1
4	Pharmacological activity of metabolites of INCB018424 in rats and dogs	INCYTE-DMB-07.15.2	M4.2.1.1
5	Pharmacodynamic activity of INCB018424 following a single oral dose in gestating rabbits	INCYTE-IN VITRO-09.13.1	M4.2.1.1
6	Summary report: effect of INCB018424 in rodent models of cancer	INCYTE-PRECLIN -10.01.1	M4.2.1.1
7	Summary report: activity of INCB018424 in rodent pharmacodynamic models	INCYTE-PRECLIN -10.02.1	M4.2.1.1
8	Summary report: activity of INCB018424 in inflammatory disease models	INCYTE-PRECLIN -10.03.1	M4.2.1.1

Secondary Pharmacodynamics

	Title	Study no.	Folder/file name
1	*In Vitro* Pharmacology: ExpresSProfile- Study of INCB018424"	T-06-01-02	M4.2.1.2
2	*In Vitro* Pharmacology: Kinase Assays- Study of INCB018424	T-06-11-09	M4.2.1.2
3	Binding of INCB018424 to human adenosine A1, A2a and A2b receptors	INCYTE-IN VITRO-07.01.1	M4.2.1.2

ADME

	Title	Study no.	Folder/file name
1	*In vitro* permeation of INCB018424 in topical formulations across mouse skin	INCYTE-DMB-06.163.1	M4.2.2.2
2	Effect of Elevated Gastric pH on the Oral Absorption of INCB018424 in Cynomolgus Monkeys	INCYTE-DMB-09.60.1	M4.2.2.2
3	Pharmacokinetics of INCB018424 in CD-1 Mice After a Single Dose Oral Dose	INCYTE-DMB-08.170.1	M4.2.2.2
4	The Pharmacokinetics of INCB018424 in Rats	INCYTE-DMB-06.169.1	M4.2.2.2
5	Pharmacokinetics of INCB018424 in Cynomolgus Monkeys	INCYTE-DMB-07.08.1	M4.2.2.2
6	*Ex vivo* and *in vitro* protein binding of INCB018424 in CD-1 mouse plasma	INCYTE-DMB-08.191.1	M4.2.2.3
7	*Ex Vivo* and *In Vitro* Protein Binding of INCB018424 in CByB6F1 Hybrid (Wild-type TgRasH2) Mouse Plasma	INCYTE-DMB-08.158.1	M4.2.2.3
8	*In Vitro* and *Ex Vivo* Protein Binding of INCB018424 in Rabbit Plasma	INCYTE-DMB-09.61.1	M4.2.2.3
9	Brain and cerebrospinal fluid concentrations of INCB018424 in rats	INCYTE-DMB-07.12.1	M4.2.2.3
10	Quantitatibe whole-body autoradioluminography tissue distribution of 14C-INCB018424 in Sprague Dawley rats following a single oral dose	INCYTE-DMB-07.109.1	M4.2.2.3
11	Identification of in vivo metabolites of INCB018424 in CD-1 mice after a single oral dose	INCYTE-DMB-08.171.1	M4.2.2.4
12	Metabolism of [^{14}C]INCB018424 in male and female CByB6F1-Tg(HRAS)2Jic mice after a single oral administration	INCYTE-DMB-08.154.1	M4.2.2.4
13	Identification of *in vivo* metabolites of INCB018424 in female rat following a single oral dose of ^{14}C-INCB018424	INCYTE-DMB-09.84.1	M4.2.2.4
14	Identification of *in vivo* metabolites of INCB018424 in rabbit	INCYTE-DMB-09.95.1	M4.2.2.4
15	Identification of in vivo milk and plasma metabolites of INCB018424 in female lactating rats following a single oral dose of ^{14}C-INCB018424	INCYTE-DMB-10.51.2	M4.2.2.4

Drug interaction

	Title	Study no.	Folder/file name
1	Reaction phenotyping: identification of human CYP enzymes involved in the metabolism of [^{14}C]-VNP401101M	NCL-156	M5.3.2.2
2	Metabolic Stability of VNP40101M and VNP4090CE in Human Liver Microsomes and Cryopreserved Human Hepatocytes	NCL-147	M5.3.2.2
3	*In vitro* metabolism of [^{14}C]VNP40101M in cryopreserved hepatocytes from the rat, dog, and human	NCL-137	M5.3.2.2

Studies reviewed previously and referenced in this review

Safety pharmacology

	Title	Study no.	Folder/file name
1	Plethysmography-restrained respiratory assessment of orally administered INCB018424 to Sprague Dawley rats	T06-10-04	M4.2.1.3
2	Toxicokinetics of INCB018424 in rat acute CNS pharmacological profile study	T06-10-03	M4.2.1.3
3	Effects of INCB018424 on cloned HERG channels expressed in mammalian cells	DMB-06.187.1	M4.2.1.3
4	Cardiovascular assessment of orally administered INCB018424 to conscious radiotelemetry-instrumented beagle dogs	T06-10-01	M4.2.1.3

ADME

	Title	Study no.	Folder/file name
4	The Pharmacokinetics of INCB018424 in Rats	INCYTE-DMB-06.169.1	M4.2.2.2

Genetic toxicology

	Title	Study no.	Folder/file name
1	INCB018424: *Salmonella* plate incorporation mutagenicity assay Key findings: INCB01824 (free base) was not mutagenic in the *Salmonella* plate incorporation mutagenicity assay, under the conditions of this experiment	AB22ZU.501.BTL	M4.2.3.3
2	*Salmonella-Escherichia coli*/mammalian-microsome reverse mutation assay with a confirmatory assay	T06-08-01	M4.2.3.3
3	INCB018424: Chromosomal aberrations in cultured human peripheral blood lymphocytes	T06-08-02	M4.2.3.3
3	INCB018424: In vivo rat bone marrow micronucleus assay	T06-10-02	M4.2.3.3

4 Pharmacology

4.1 Primary Pharmacology

Brief summary

Ruxolitinib is an inhibitor of the Janus Associated kinases (JAKs) with selectivity for JAK1 and JAK2. Ruxolitinib was tested in cell-based assays and *in vivo* models relevant to the pathogenesis of myeloproliferative neoplasms (MPNs). Activity was observed in *in vitro* studies and in nonclinical tumor models representing both hematological and solid tumors. Ruxolitinib was shown to inhibit IL-2 stimulated phosphorylation of JAKs and STATs and IL-2 induced proliferation of T cells as well as the phosphorylation of STAT proteins and the production of pro-inflammatory factors (eg, IL-17, IL-22, MCP-1) induced by other cytokines, such as IL-23 and IL-6. Ruxolitinib inhibited splenomegaly in mice resulting from intravenous inoculation of cells expressing the clinically relevant JAK2V617F mutation. The *in vitro* pharmacological activity of eight ruxolitinib metabolites observed in human plasma was evaluated using enzyme and cell-based assays. The two major human metabolites, M18 and M27 (25% and 11% of parent based on AUC, respectively), as well as the six other metabolites tested were shown to be pharmacologically active. These metabolites had 2-5-fold weaker potency compared to that of the parent compound in a human whole blood assay measuring inhibition of IL-6 induced STAT3 phosphorylation.

In safety pharmacology assessments of ruxolitinib including respiratory and central nervous system studies in rats, and *in vitro* and *in vivo* (ECG study in conscious dogs) cardiovascular studies, treatment-related findings were lower body temperature, lower activity, observations of darkened skin and mucous membranes, decreases in minute volume in high dose female rats in the respiratory study and decreases in arterial blood pressure along with increases in heart rate at the highest dose evaluated in dogs in the cardiovascular study.

Primary pharmacodynamics

Mechanism of action:

Ruxolitinib is a small molecular inhibitor of JAKs with selectivity for JAK1 and JAK2 (IC50 value at 3.3 nM and 2.8 nM respectively). The *in vitro* studies showed that ruxolitinib inhibited JAK/STAT signaling and growth of a cell line expressing the constitutively active JAK2 mutant (JAK2V617F) that has been implicated in the pathogenesis of the majority of Philadelphia chromosome negative myeloproliferative neoplasms (MPNs). Ruxolitinib treatment resulted in a suppression of phosphorylated STAT3 (pSTAT3) and tumor growth in mice xenograft model with cells expressing JAK2V617F. These results suggest that the alteration of JAK/STAT signaling pathway could contribute to the therapeutic or toxic effect of ruxolitinib.

Drug activity related to proposed indication:

1- *Alteration of JAK/STAT pathway is associated with myeloproliferative disorders*

The Applicant cited publication
Lalentino and Pierre, Biochem Pharmacol (2006) 71: 715

Title: JAK/STAT signal transduction: Regulators and implication in hematological malignancies

This reference article is a commentary review paper; the following summary is copied from above review article.

Summary: Signal transducers and activators of transcription (STATs) comprise a family of several transcription factors that are activated by a variety of cytokines, hormones and growth factors. STATs are activated through tyrosine phosphorylation, mainly by JAK kinases, which lead to their dimerization, nuclear translocation and regulation of target genes expression. Stringent mechanisms of signal attenuation are essential for insuring appropriate, controlled cellular responses. Among them phosphotyrosine phosphatases (SHPs, CD45, PTP1B/TC-PTP), protein inhibitors of activated STATs (PIAS) and suppressors of cytokine signaling (SOCS) inhibit specific and distinct aspects of cytokine signal transduction.

Somatic point mutation in JH2 domain of JAK2 (JAK2V617F), leading also to constitutive tyrosine phosphorylation of JAK2 and its downstream effectors was reported in myeloproliferative disorders.

2- Effects of INCB018424 on kinase activities of JAKs and other enzymes
The Applicant cited publication
Quintas-Cardama et al. Blood (2010) 115: 3109

Title: Preclinical characterization of the selective JAK1/2 inhibitor INCB018424: therapeutic implications for the treatment of myeloproliferative neoplasms

Summary: INCB018424 inhibited JAK1 and JAK2 with IC50 values of 3.3 nM and 2.8 nM, respectively. The inhibitory effect of INCB018424 on JAK1 and JAK2 was 6 times more effective comparing to Tyk2 and 130 times comparing to JAK3.

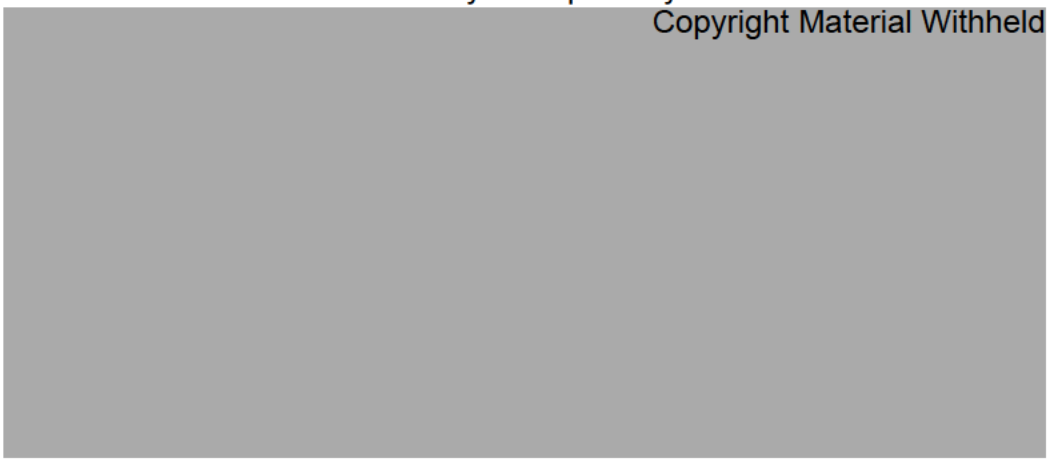
Methods: The kinase domains of human JAK1 (837-1142), JAK2 (828-1132), JAK3 (781-1124), and Tyk2 (873-1187) were cloned by PCR with N-terminal epitope tags. Recombinant proteins were expressed using Sf21 cells and baculovirus vectors and purified with affinity chromatography. JAK kinase assays used a homogeneous time-resolved fluorescence assay with the peptide substrate (-EQEDEPEGDYFEWLE). Each enzyme reaction was carried out with test compound or control, JAK enzyme, 500nM peptide, adenosine triphosphate (ATP; 1mM), and 2.0% dimethyl sulfoxide

(DMSO) for 1 hour. The 50% inhibitory concentration (IC50) was calculated as the compound concentration required for inhibition of 50% of the fluorescent signal.

Results:
1) INCB018424 inhibited kinase activities of JAK1 and JAK2 with IC50 values of 3.3nM and 2.8nM, respectively;
2) INCB018424 demonstrated inhibitory activity against Tyk2 with an IC50 value of 19 nM, and inhibitory activity against JAK3 with an IC50 value of 428 nM.

The following table is excerpted from this publication.

Enzymatic potency of INCB18424



3- The reduction of cell viability by INCB018424 was associated with inhibition of JAK/STAT signaling pathway

3-a. INCB018424 inhibited protein phosphorylation associated with JAK1 and JAK2

The Applicant cited publication
Quintas-Cardama et al. Blood (2010) 115: 3109

Title: Preclinical characterization of the selective JAK1/2 inhibitor INCB018424: therapeutic implications for the treatment of myeloproliferative neoplasms

Summary: treatment with INCB018424 reduced phosphorylation of JAK2, STAT5 and ERK1/2 in Ba/F3-EpoR-JAK2V617F cells; reduced phosphorylation of STAT3 and STAT5 in HEL cells; and reduced phosphorylation of JAK1 in primary MF patient samples.

Method: Ba/F3-EpoR-JAK2V617F or HEL cells, and primary MF patient samples were treated with increasing concentrations of INCB018424 for 2.5 hours, and extracts from these cells were subjected to immunoblot analysis for phosphorylation (p) or total forms of proteins associated with the JAK/STAT signaling pathway.

Results: There was a dose-dependent reduction in the phosphorylated form of JAK1, JAK2, STAT3 (pSTAT3), STAT5 (pSTAT5), and ERK1/2 with maximal effect at approximately 300 nM in the tested cell lines. However there was no change in total levels of the respective proteins.

The following figures are excerpted from this publication.

INCB018424 inhibited JAK2V617F-mediated signaling in Ba/F3-EpoR-JAK2V617F (A), HEL (B) cells, or primary MF patient peripheral blood mononuclear cells (C).

3-b. INCB018424 reduced cell viability

The Applicant cited publication
Quintas-Cardama et al. Blood (2010) 115: 3109

Title: Preclinical characterization of the selective JAK1/2 inhibitor INCB018424: therapeutic implications for the treatment of myeloproliferative neoplasms

Summary: A dose-dependent reduction in viability was observed in INCB018424 treated Ba/F3-EpoR-JAK2V617F cells and HEL cells.

Method: Hel cells and Ba/F3-EpoR-JAK2V617F cells (engineered cell system: Cytokine-dependent Ba/F3 cells were transformed to growth factor independence by ectopic expression of JAK2V617F and a requisite type I cytokine receptor EpoR) were treated with INCB018424 and a viable cell number was assessed after 48 hours. Viability was measured by cellular ATP determination using the Cell-Titer Glo (Promega) luciferase reagent or viable cell counting. Values were transformed to percent inhibition relative to vehicle control, and IC50 curves were fitted according to nonlinear regression analysis of the data using PRISM GraphPad.

Results: 1) A dose-dependent reduction in viability was observed with an IC50 of 126nM in Ba/F3-EpoR-JAK2V617F cells;
2) The growth of HEL cells was also affected by INCB018424 with a 50% effective concentration (EC50) of 186 nM.
The following figure is excerpted from this publication.

3-c. INCB018424 induced apoptosis

The Applicant cited publication
Quintas-Cardama et al. Blood (2010) 115: 3109

Title: Preclinical characterization of the selective JAK1/2 inhibitor INCB018424: therapeutic implications for the treatment of myeloproliferative neoplasms

Summary: Cells expressing JAK2V617F underwent apoptosis when treated with INCB018424.

Method: Ba/F3-EpoR-JAK2V617F cells were treated with various concentrations of INCB018424 for approximately 24 hours and analyzed for hallmarks of apoptosis by annexin V/propidium iodide staining or mitochondrial membrane depolarization. Cells treated with INCB018424 were stained with a combination of fluorescein isothiocyanate-annexin V and propidium iodide and analyzed using flow cytometry to determine the percentages of viable, early apoptotic, and late apoptotic or dead cells. The effects of JAK inhibition on mitochondrial membrane potential were determined by flow cytometry using JC-1 as a molecular probe.

Results: Treatment with INCB018424 increased apoptosis compared with DMSO, with a 4.3, 7.2-, and 13.2-fold increase at concentrations of 150, 400, and 1000 nM, respectively.

3-d. INCB018424 inhibited hematopoietic progenitor cell proliferation in primary MPN patient samples

The Applicant cited publication
Quintas-Cardama et al. Blood (2010) 115: 3109

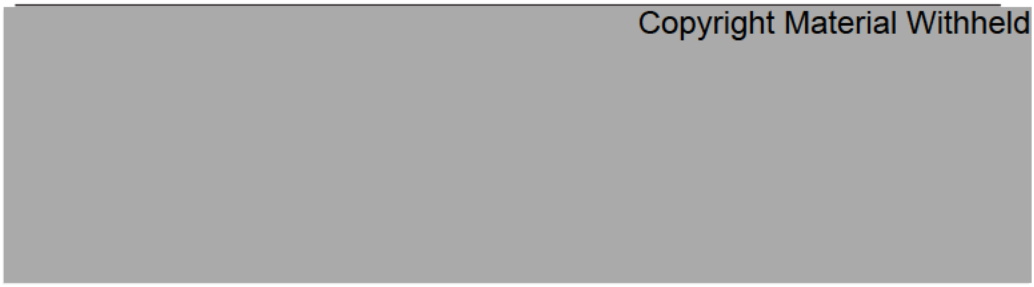
Title: Preclinical characterization of the selective JAK1/2 inhibitor INCB018424: therapeutic implications for the treatment of myeloproliferative neoplasms

Summary: INCB018424 inhibited hematopoietic progenitor cell colony formation. Cells derived from patients with polycythemia vera (PV) with mutated JAK2 are more sensitive to JAK1/2 inhibition than normal donors, particularly in the absence of hematopoietic growth factor support.

Method: Mononuclear cells from 3 healthy controls and 3 patients with PV expressing the JAK2V617F mutant allele at frequencies more than 90% were obtained. Growth of clonogenic progenitors of erythroid (BFU-E) and myeloid origin (CFU-M) was assessed in colony-forming assays in the presence of increasing concentrations of INCB018424

Results: 1) Inhibition of the growth of erythroid and myeloid progenitors was observed with INCB018424
2) INCB018424 demonstrated potency against erythroid colony formation in JAK2V617F^{+} human MPNs

The follow table is excerpted from this publication



3-e. The effect of INCB018424 on an in vivo model of JAK2V617F-driven malignancy

The Applicant cited publication
Quintas-Cardama et al. Blood (2010) 115: 3109

Title: Preclinical characterization of the selective JAK1/2 inhibitor INCB018424: therapeutic implications for the treatment of myeloproliferative neoplasms

Summary: Oral administration of INCB018424 inhibited JAK-STAT signaling, prevented splenomegaly, decreased JAK2V617F mutant cells in the spleen, decreased circulating inflammatory cytokines (eg, TNF-α, IL6) and resulted in prolonged survival in the mice at doses that did not cause changes of peripheral blood cell counts.

Method: 1 x 10^5 Ba/F3-EpoR-JAK2V617F cells were injected into the tail vein of Balb/c mice. The mice were monitored for splenomegaly and survival over a 3-week period to evaluate the impact of INCB018424 on the course of disease.

Results: 1) Vehicle treated animals had splenomegaly with a mean weight of 471 mg (~5 times normal), whereas spleen weights of mice treated with INCB018424 were 110 mg;
2) Beginning on day 15, mice from the vehicle group died of disease. By day 22, greater than 90% of the vehicle group had died, whereas, in contrast, greater than 90% of mice treated with INCB018424 survived;
3) Genomic PCR analysis of spleen samples showed that JAK2V617F cells were significantly decreased by treatment with INCB018424 (33%, $P< .01$);
4) INCB018424 normalized uncontrolled JAK/STAT signaling in spleens from mice inoculated with Ba/F3-EpoRJAK2V617F cells;
5) Treatment with INCB018424 resulted in significant suppression of elevated IL-6 levels and reduction of TNF-levels to normal;

The following figures and table are excerpted from this publication

4.2 Secondary Pharmacology

Both studies (T-06-01-02 and T-06-11-09) were non-GLP studies and are not reviewed here. The following summary is excerpted from the applicant's submission.

Ruxolitinib at 0.1 and 1 μM did not demonstrate significant (> 50%) cross reactivity with any of the 50 in vitro binding assays and enzyme assays (T-06-01-02). However, 26% inhibition at 1 μM was noted for the human adenosine 1 receptor (hA1). This observation prompted determination of an IC_{50} value for ruxolitinib -mediated inhibition of binding to this adenosine receptor, and the other adenosine family of receptors. Using membranes prepared from CHO-K1 cells expressing the human A1 receptor, the IC50 for ruxolitinib–mediated inhibition of radioligand binding was determined to be 2.1 μM. IC_{50} values for hA2a and hA2b receptors were > 30 μM and 11 μM respectively [INCYTE-IN VITRO-07.01.1].

Ruxolitinib was evaluated in a Cerep Kinase Assay screen (T06-11-09) at 0.2 μM to investigate the effect of ruxolitinib in 30 in vitro kinase assays. Ruxolitinib did not demonstrate significant cross reactivity (defined as > 50% inhibition) against any of the kinases tested in this panel except those of the JAK family: complete inhibition of JAK2 activity was confirmed and JAK3 activity was inhibited by 95%.

Note from reviewer: It would have been preferable if a higher concentration of INCB018424 (i.e., 10 μM) had been evaluated in the Cerep ExpresSProfile screen. However, based on the data available for INCB018424, it appears that there is little risk of unintended pharmacological activity due to binding to non-specific receptors/enzymes.

4.3 Safety Pharmacology

Safety pharmacology summary:

The safety pharmacology study reports were reviewed by Dr. Barbara Hill for the treatment of rheumatoid arthritis (IND 77,455). Dr. Hill summarized the safety pharmacology study results as follows.

- The NOAEL for central nervous systemic pharmacological effects noted in rats (lower body temperature and reduced total and ambulatory activity counts) following a single oral dose of INCB018424 was identified as 15 mg/kg for males and 50 mg/kg for females, under the conditions of this study;
- The NOAEL for respiratory effects noted in rats (decreased respiratory frequency, increase in tidal volume and increase in minute volume) following a single oral dose of INCB018424 was identified as 15 mg/kg for males and 50 mg/kg for females, under the conditions of this study;
- The IC_{50} for INCB018424 HERG potassium current was 131.6 μM, under the conditions of this study. The NOAEL for cardiovascular effects noted in male dogs (significant increase in heart rate, significant shortening of PR interval, slight increase in lengthening of the QRS complex, significant shortening of the RR interval and a slight prolongation of the heart-rate corrected QT interval) following a single oral dose of INCB018424 was identified as 10 mg/kg, under the conditions of this study.

Neurological effects:

From Dr. Hill's review

The neuropharmacologic effects of INCB018424 were evaluated in an oral rat GLP study using a Functional Observation Battery (FOB) and locomotor activity measurements. Single oral (gavage) doses of 0, 15, 50 and 150 mg/kg INCB018424 were administered to Sprague-Dawley rats (10/sex/group). Separate toxicokinetic groups for low-, mid- and high-dose groups were included in this study. Toxicology parameters included assessment of mortality. FOB (sensorimotor, neuromuscular and physiological observations) and locomotor activity (measured for a 60 minute interval; home cage, handling and open field observations) were recorded for all animals prior to

dose administration and 30 minutes post dose (T_{max}). Blood samples for toxicokinetic analysis were collected from 6 animals/sex/group at 0, 0.5, 1, 2, 4 and 8 hours post-dose.

No treatment related mortality was noted in this study. No treatment related effects on FOB or locomotor activity were noted in low-dose animals and mid dose females. The only treatment related effect on FOB was noted in high-dose females. A significant lower body temperature was noted in high-dose females (35.1°C) compared to control females (37.1°C) at 30 minutes post-dose. Total and ambulatory activity counts during the first 15 minutes of the 1-hour session were significantly reduced in mid dose males and high-dose animals. A summary of the mean pharmacokinetic parameters for INCB018424 measured in this study is provided in the following table.

Dose (mg/kg/day)	T_{max} (hr)		C_{max} (µM/ml)		$AUC_{0-8\ hr}$ (µM·hr)	
	Males	Females	Males	Females	Males	Females
15	0.5	0.5	0.15	1.02	0.14	1.29
50	0.5	0.5	0.68	2.85	1.15	9.62
150	0.5	0.5	1.94	13.6	4.0	36.1

A dose-related increase in systemic exposure was noted in this study. Females exhibited a greater systemic exposure compared to males. The NOAEL for central nervous systemic pharmacological effects noted in rats following a single oral dose of INCB018424 was identified as 15 mg/kg for males and 50 mg/kg for females, under the conditions of this study.

Respiratory effects:

From Dr. Hill's review

The respiratory effects of INCB018424 were evaluated in an oral rat GLP study using head-out neck-sealed plethysmography chambers. Single oral (gavage) doses of 0, 15, 50 and 150 mg/kg INCB018424 were administered to Sprague-Dawley rats (8/sex/group). Respiratory parameters included respiratory rate, tidal volume and derived minute volume. Data was captured from 60 minutes pre-dose and continuously for 4 hours post dose.

No treatment-related effects on mortality or clinical signs were noted in this study. A significant decrease in respiratory frequency was noted in high-dose animals. This effect lasted for up to 3 hours post dose with a maximum decrease of 23% in high dose males and 21% in high dose females. A significant increase in tidal volume was noted in mid-dose males and high-dose animals. This effect lasted for up to 4 hours post dose with a maximum increase of 21% in mid-dose males, 34% in high-dose males and 18% in high dose females. The higher tidal volume noted in high-dose animals may reflect a compensatory response to the lower respiratory frequency. A significant decrease in minute volume was noted in high-dose females (up to 17% noted at three timepoints from 0.75 to 1.75 hours post-dose). The NOAEL for respiratory effects noted in rats following a single oral dose of INCB018424 was identified as 15 mg/kg for males and 50 mg/kg for females, under the conditions of this study.

Cardiovascular effects:

From Dr. Hill's review

The effects of INCB018424 on the cardiovascular system were evaluated in a GLP *in vitro* HERG assay and in a GLP *in vivo* cardiovascular safety pharmacology study conducted in Beagle dogs.

INCB018424 (10, 100 and 300 µM) was tested for HERG-channel inhibition in embryonic kidney cells (HEK293). INCB018424 inhibited HERG current by (mean ± SEM): 3.8 ± 0.2% at 10 µM, 40.3 ± 1.6% at 100 µM and by 74.1 ± 0.2% at 300 µM versus 0.6 ± 0.5% for the vehicle control. Significant inhibition of HERG current was noted at 100 and 300 µM INCB018424 compared to vehicle control. The IC50 for INCB018424 HERG potassium current was 131.6 µM, under the conditions of this study. The positive control (60 nM terfenadine) inhibited HERG potassium current by 83.1% in this assay.

Four adult naïve male conscious radiotelemetry-implanted Beagle dogs were used in the in vivo cardiovascular safety pharmacology study. The sponsor states that only male dogs were used in this study because toxicokinetic parameters were similar in male and female dogs. Single oral doses of 0, 3, 10 and 30 mg/kg INCB018424 (dose volume = 10 ml/kg; vehicle: 0.5% methylcellulose) were administered in a Latin-square design. A 3-4 day washout period was incorporated between each dose. Heart rate, arterial blood pressure (systolic, diastolic), body temperature and ECG were collected for a 30-second period every 10 minutes for 24 hours post dose.

No treatment-related effects on mortality were noted in this study. Emesis was noted after dose administration in high-dose animals. A significantly lower pulse pressure as well as systolic, diastolic and calculated mean pressure (up to 53%, 41%, 31% and 33%, respectively) was noted in high-dose animals compared to control animals. These changes peaked approximately 2-3 hours post dose after which mean arterial blood pressure values began to recover. However, lower values for arterial blood pressure were still noted 24 hours post dose in high-dose animals. No treatment related effects on systolic, diastolic or mean pulse pressure were noted in low and mid-dose animals.

A significant increase in heart rate (increase up to 117% during hour 2) that lasted up to 10 hours post dose was noted in high-dose animals compared to vehicle control. The observed increase in heart rate may reflect a compensatory response to the decreases in arterial blood pressure noted in high-dose animals. No treatment-related effects on heart rate were noted in low- and mid-dose animals. A slight decrease in body temperature was noted at 3 and 4 hours (0.24^{o}C and 0.22^{o}C lower, respectively) in high-dose animals. No treatment-related effects on body temperature were noted in low- and mid-dose animals. A significant shortening of PR interval for up to 6 hours post dose (up to 21% shorter) was noted in high dose animals compared to vehicle control animals. The study report states that these changes in the PR interval were considered to be a consequence of the increases in heart rate. No treatment- related effects on PR interval were noted in low- and mid-dose animals. A slight increase in lengthening of the

QRS complex for up to 18 hours post-dose (up to 9% or 3.2 msec during hour 3) was noted in high-dose animals compared to vehicle control animals. No treatment related effects on QRS complex were noted in low and mid dose animals. A significant shortening of the RR interval for up to 6 hours post dose (up to 54% shorter during hour 2) was noted in high dose animals compared to vehicle control animals. The study report states that these changes in the RR interval were considered to be a consequence of the increases in heart rate. No treatment related effects on RR interval were noted in low and mid dose animals. A slight prolongation of the heart rate corrected QT interval (QTcV) was noted between hours 11 – 14 (5%) and 19 – 24 (3%) in high- dose animals compared to vehicle control animals. No treatment-related effects on QTcV were noted in low- and mid-dose animals.

Acute cardiovascular effects of INCB018424 after oral administration to high-dose dogs included decreased pulse pressure, systolic, diastolic and mean arterial pressure and increased heart rate. Addition treatment-related effects noted in high-dose dogs included shortening of the RR and PR interval, which may have been related to increased heart rate. A slight lengthening of the QRS complex and corrected QT interval was noted in high dose dogs. The NOAEL for cardiovascular effects noted in male dogs following a single oral dose of INCB018424 was identified as 10 mg/kg, under the conditions of this study.

Renal effects: no study conducted

Gastrointestinal effects: no study conducted

Abuse liability: no study conducted

Reviewer comment: The maximum daily dose proposed for BID dosing of ruxolitinib phosphate would be 50 mg/day, equivalent to 29.4 mg/m^2/day, based on a 1.7 m^2 surface area individual. The NOAEL identified in the conducted safety pharmacology studies (10-50 mg/kg or 60-300 mg/m^2 in rats, 10 mg/kg or 200 mg/m^2 in dogs) is 2-10 folds of the maximum daily dose proposed. Based on single dose toxicokinetics (rat: INCYTE-DMB-06.174.1; dog: T-07-10-07], the projected Cmax level in female rats at the NOAEL dose of 50 mg/kg is 2.85 μM (0.51 μM unbound), and the projected Cmax level in dogs at the NOAEL of 10 mg/kg is approximately 7.99 μM (0.77 μM unbound), which are 10.4-fold and 15.7-fold, respectively, of the Cmax at the highest proposed therapeutic dose in humans (25 mg bid) associated with a Cmax value of 1.48 μM (0.049 μM unbound, INCYTE-DMB-10.56.3). In addition, the adverse effects observed in the safety pharmacology studies were not noted in the repeated dose toxicology studies. Clinical studies have not recapitulated these findings at clinically relevant doses. Therefore, the potential for ruxolitinib to cause adverse alterations in respiratory, neurologic, and cardiovascular parameters in humans is considered to be low.

PHARMACOLOGY TABULATED SUMMARY

In Vitro Potency of Ruxolitinib in Cell Lines Relevant to MPNs

Cell Type	Stimulator	Measured Parameter	IC_{50} for ruxolitinib (mean ± standard deviation)[a]
INA-6	IL-6	STAT3 Phosphorylation	< 125 nM
	IL-6	Cell Proliferation	141 ± 43 nM
	BMSC	Cell Proliferation	185 nM
TF-1	GM-CSF	Cell Proliferation	80 ± 30 nM
Bcr-Abl TF-1	-	Cell Proliferation	> 25000 nM[b]
Ba/F3-JAK2V617F	-	JAK2/STAT5/ERK Phosphorylation	128-320 nM[b]
Ba/F3-JAK2V617F	-	Cell Proliferation	127 ± 17 nM[b]
HEL	-	STAT5 Phosphorylation	< 300 nM[b]
HEL	-	STAT3 Phosphorylation	< 300 nM[b]

INCB018424 had anti-tumor effect on the following tested cell lines *in vitro*- or *in vivo*

IL-6 dependent INA-6 cell line (plasma cell leukemia cells)
TF-1 (leukemia cells)
Ba/F3-JAK2V617F cell line (murine leukemia Ba/F3 cells expressing JAK2V617F with the erythropoietin (EPO) receptor)
HEL (human erythroleukemia cell line)
Progenitor colonies derived from mononuclear cells from patients with PV
INA-6 multiple myeloma mouse xenograft model
HH human T-cell cutaneous lymphoma xenograft model, mouse
Mouse xenograft model with cells expressing Ba/F3-JAK2V617F mutation
22Rv1 Tumor Model, hormone refractory prostate cancer xenograft tumor model, mouse
MM1.S Model, myeloma mouse xenograft model

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Note: PK studies conducted in minipigs are not reviewed.

Brief summary

Pharmacokinetic studies were performed in various species after either oral or intravenous administration of ruxolitinib. The PK of ruxolitinib is characterized by a rapid absorption with T_{max} value of 0.4-2 h, and a generally short terminal elimination half-life, ranging from 1.5 h to 2 h in rats and dogs. There was no accumulation with 1-month repeated daily dosing, however there was a small accumulation with 6-month

repeated daily dosing. The absolute oral bioavailability was variable across the nonclinical species, 29% in male rats and 105% in female rats, around 50% in dogs. There were generally no gender differences in exposure of ruxolitinib and its metabolites in dogs. In rats, the C_{max} and AUC values were several-fold higher in females compared to males.

In rats, after a single oral dose of 14C-ruxolitinib, drug-derived radioactivity was widely distributed with highest concentration in the GI system, and no detectable levels in the CNS. Ruxolitinib was eliminated from most tissues rapidly and completely. In pregnant rats administered a single oral dose of 14C-ruxolitinib, the fetal:maternal plasma and fetal tissue:maternal plasma concentration ratios were less than one for all fetal tissues indicating that fetal exposure was limited. The plasma protein binding of ruxolitinib was 3.3% in human.

Oxidation was the predominant metabolic pathways in rats and dogs. In vitro studies suggest that CYP3A4 is the predominant CYP isozyme responsible for the metabolism of ruxolitinib. Ruxolitinib is metabolized by isozymes including CPY1A2, CYP2C6 and CYP2D1 found in both male rats and female rats. In rat liver microsomes, ruxolitinib is metabolized to a greater extent in males compared to females, consistent with ruxolitinib being metabolized by the male-rat-specific isozymes CYP2C11, CYP2C13 and CYP3A2.

In rat, dog and human ^{14}C mass balance studies, elimination of drug-derived radioactivity after oral and IV dosing was similarly rapid and complete with excretion via urine, bile and feces. In rats, ruxolitinib-derived radioactivity preferentially partitions into milk with the qualitative metabolite profile being similar to plasma. Milk concentrations were less than 1% of C_{max} by 24 h. Ruxolitinib and M18 are not potent inhibitors of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 in human liver microsomes. In vitro data suggest that ruxolitinib is not an inducer of CYP1A2, CYP2B6 or CYP3A activity at therapeutically relevant concentrations.

Methods of Analysis

[See under individual study reviews]

Absorption

The pharmacokinetics of INCB018424 in rats

This study (study#INCYTE-DMB-06.169.1) was reviewed by Dr. Barbara Hill. The following summary is excerpted from Dr. Hill's review.

The pharmacokinetics of INCB018424 was determined in male and female rats given a single 10 mg/kg iv dose (2/sex) or a single 50 mg/kg oral dose (2/sex) of INCB018424 phosphate. The mean systemic plasma clearance of INCB018424 for males (9.4 L/hr/kg) and females (4.8 L/h/kg) were very high and approximated about 3- and 1.5- fold the hepatic blood flow in rats, respectively. The average clearance in males was about 2-fold greater than in females. The mean Cmax and AUC values after oral administration of 50 mg/kg INCB018424 were 2.24 μM and 5.40 μM.hr, respectively, in males and 30.4 μM and 39.3 μM.hr, respectively, in females. This

indicates an approximately 8-fold difference in oral clearance between male and female rats. The mean AUC values after iv administration of 10 mg/kg INCB018424 were 3.75 μM.hr and 7.49 μM.hr in males and females, respectively. The mean volumes of.distribution in male and female rats were 3.8 and 1.6 L/kg, respectively, indicating that.INCB018424 was distributed beyond the total volume of body water (0.7 L/kg) in rats. The apparent terminal disposition half-life of INCB018424 following oral dosing (1.5 hr) was longer than following iv dosing (0.4 hr), but did not show much difference between male and female rats. The average oral bioavailability of INCB018424 was 29% in male rats and 105% in female rats.

Study title: A 6-month oral (gavage) toxicity study of INCB018424 in rats with a 6 week recovery period

Key Study Findings:

- Plasma C_{max} and AUC_{0-t} values generally increased in a dose-proportional manner at doses ≥ 15 mg/kg, but increased not in a dose-proportional manner at doses < 15 mg/kg;
- C_{max} and AUC_{0-t} values were similar on study days 0 and 27; on study day 181, C_{max} and AUC_{0-t} values were higher than values on study days 0 and 27;
- Exposures were several fold higher in females than in males;
- The T_{max} was 0.5 h for all groups, while half-life values were short and not dose dependent, ranging from 0.358 to 1.98 h

Study no.: (b) (4)-519048
Volume #, and page #: electronic submission, Module 4,
Conducting laboratory and location: (b) (4)

Date of study initiation: 1 October 2007
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: INCB018424
Lot number: SS-IN3-139
Purity: 99.9%

Methods
Species/strain: Rat/Crl:CD(SD)
#/sex/group: 5/sex for control, 11/sex/group for treatment group
Schedule: once daily for 182 days
Doses in administered units: 0, 5, 10, 20/15 mg/kg, at the dose volume of 10 mL/kg
Route: oral gavage
Blood samples collection: prior to dosing and at 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after dose administration on study days 0, 27 and 181

Results: The table below is excerpted from the sponsor's application
Summary of INCBO18424 Toxicokinetics in Male and Female Rats Given Daily Oral Doses of 5, 15, 30 and 60 mg/kg of INCB018424

Gender	Day	Parameter	5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg
Male	0[a]	C_{max} (μM)	0.0116	0.0918	0.209	0.501
		T_{max} (h)	0.500	0.500	0.500	0.500
		AUC_{0-t}	0.00823	0.0902	0.298	1.07
		$t_{1/2}$ (h)	NC	0.358	1.07	0.787
	27	C_{max} (μM)	0.0139	0.0707	0.188	0.400
		T_{max} (h)	0.500	0.500	0.500	0.500
		AUC_{0-t}	0.00919	0.0924	0.237	0.720
		$t_{1/2}$ (h)	NC	0.887	1.94	0.899
	181	C_{max} (μM)	0.0492	0.165	0.445	0.707
		T_{max} (h)	0.500	0.500	0.500	0.500
		AUC_{0-t}	0.0533	0.296	0.662	1.32
		$t_{1/2}$ (h)	1.04	1.33	1.11	1.77
Female	0[a]	C_{max} (μM)	0.0968	0.660	2.83	3.03
		T_{max} (h)	0.500	0.500	0.500	0.500
		AUC_{0-t}	0.113	0.832	3.33	14.1
		$t_{1/2}$ (h)	0.931	1.98	1.22	1.07
	27	C_{max} (μM)	0.116	0.821	1.11	2.65
		T_{max} (h)	0.500	0.500	0.500	0.500
		AUC_{0-t}	0.130	1.03	2.41	10.1
		$t_{1/2}$ (h)	0.829	1.10	1.26	1.15
	181	C_{max} (μM)	0.209	1.72	4.98	6.11
		T_{max} (h)	0.500	0.500	0.500	0.500
		AUC_{0-t}	0.361	2.33	7.40	25.8
		$t_{1/2}$ (h)	1.79	1.66	1.47	1.40

[a] = Day 0 corresponds with the first day of dosing
NC = not calculated due to insufficient time points to determine the terminal phase

Study Title: Pharmacokinetics of INCB018424 in Dogs

Key Study Finding:

- The time profile following oral dosing was similar to that with IV dosing;
- The disposition half-life of INCB018424 was about same in both the PO and IV groups;
- The systemic plasma clearance was 0.48 L/hr/kg
- The bioavailability was 57% if plasma exposure was considered to increase in proportional manner with the dose escalation (see reviewer's comment below).

Study no: INCYTE-DMB-06.182.1
Volume #, and page #: electronic submission, page 1-9
Conducting laboratory and location: (b) (4)

Date of study initiation: July 10, 2006
GLP compliance: no, (no information provided)
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: INCB018424
Lot No.: SS-IN2-175
Purity: not provided

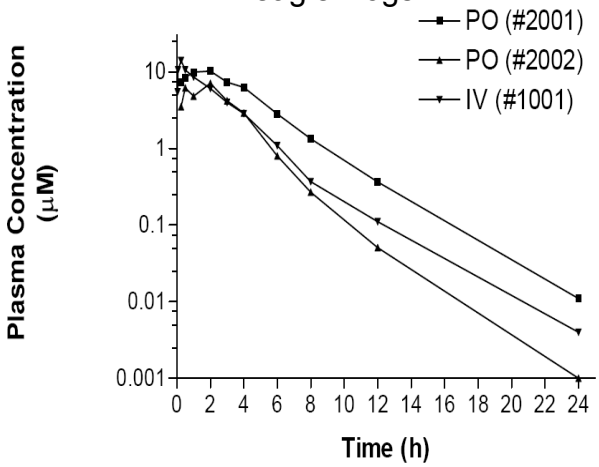
Formulation/vehicle:
IV study-1 mg/mL of INCB018424 in normal saline,
PO study-2 mg/mL of INCB018424 in 0.5% methylcellulose aqueous solution

Methods: Two male beagle dogs were given a single PO dose of 10 mg/kg and one male beagle dog was given a single IV dose of 5 mg/kg of INCB018424 (free base

equivalent). Blood samples were collected at predose, 15 min, 30 min, 1, 2, 3, 4, 6, 8, 12 and 24 h post-dose in the PO group, and predose, 2.5, 5, 15, 30 min, 1, 2, 3, 4, 6, 8, 12 and 24 h post-dose in the IV group. Analyses for ruxolitinib concentrations in plasma were conducted by liquid chromatographic tandem mass spectrometry (LC-MS/MS). The plasma concentration-time data was used to determine the pharmacokinetic parameters by standard non-compartmental methods using WinNonlin® version 5.0.1

Results: The following figures were copied from the sponsor's submission

Plasma Concentration-Time Profile Following IV (5 mg/kg) or PO (10 mg/kg) Dosing in Beagle Dogs



PK parameters

Plasma Concentrations (μM) and Pharmacokinetics of INCB018424 in Beagle dogs Given a 10 mg/kg PO Dose or 5 mg/kg IV Dose of INCB018424

PO				IV	
Time (h)	#2001	#2002	Mean	Time (h)	#1001
0	BQL	BQL	BQL	0	BQL
0.25	7.41	3.51	5.46	0.042	5.52
0.5	8.47	6.21	7.34	0.083	10.7
1.0	9.98	4.83	7.41	0.25	14.2
2.0	10.3	7.11	8.71	0.5	10.7
3.0	7.37	4.21	5.79	1.0	8.62
4.0	6.31	2.92	4.62	2.0	6.12
6.0	2.84	0.802	1.82	3.0	4.00
8.0	1.34	0.273	0.807	4.0	2.86
12	0.367	0.051	0.209	6.0	1.09
24	0.011	0.001	0.006	8.0	0.370
				12	0.111
				24	0.004
C_{max} (μM)	10.3	7.11	8.71	AUC (μM*h)	34.1
T_{max} (h)	2.0	2.0	2.0	CL (L/h/kg)	0.48
AUC* (μM*h)	52.3	25.4	38.9	V_{ss} (L/kg)	1.1
$T_{1/2}$ (h)	2.3	2.1	2.2	$T_{1/2}$ (h)	2.5
F (%)	77	37	57	% dose in urine#	<1

BQL, below quantifiable limit of 0.001 μM; # As unchanged parent

Reviewer comment:

It appears that the sponsor compared the ½ of the AUC from 10 mg/kg oral dosing to the AUC from 5 mg/kg IV dosing to determine bioavailability (57%). Considering the sample size and the PK profile from other conducted studies showing that AUC was not increased in a proportional manner with dose escalation, therefore the bioavailability claimed by the sponsor (57%) may not accurate by using above calculating method.

Study title: A 6-month oral (gavage) toxicity study with INCB018424 in beagle dogs with 6-week recovery period

Key Study Findings:

- Plasma C_{max} and AUC generally increased in a dose proportional manner at doses ≤ 5 mg/kg, increased greater than dose proportional escalation at dose > 5 mg/kg in both males and females;
- C_{max} and AUC values were generally similar on study days 0 and 40, but higher on study day 175;
- The mean Tmax and half-life values were generally similar for all dose groups ranging from 0.500 to 1.17 hours and 0.640 to 2.09 hours, respectively;
- TK parameters were generally comparable between males and females

Study no.: (b) (4)-519049
Volume #, and page #: electronic submission, Module 4,
Conducting laboratory and location: (b) (4)

Date of study initiation: 26 September 2007
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: INCB018424
Lot number: SS-IN3-139
Purity: 99.9%

Methods
Species/strain: Beagle dogs
#/sex/group: 7/sex/group
Schedule: daily for a total 6 months
Doses in administered units: 0.5, 2.5, 5.0 and 10 mg/kg/day
Route: oral gavage
Blood samples collection: prior to dosing and at 0.5, 1, 2, 4, 8, 12 and 24 hours after dose administration on study days 0, 40 and 175

Results: The following summary table was excerpted from the Sponsor's application

Summary of INCB018424Toxicokinetics in Male and Female Beagle Dogs Given Daily Oral Doses of 0.5, 2.5, 5 or 10 mg/kg of INCB018424

			Mean ± SD (N=7)			
Gender	Day[a]	Parameter	0.5 mg/kg	2.5 mg/kg	5 mg/kg	10 mg/kg[c]
Male	0	C_{max} (μM)	0.515 ± 0.32	2.24 ± 0.72	3.90 ± 1.5	7.99 ± 3.6
		T_{max} (h)	0.500 ± 0.0	0.500 ± 0.0	0.571 ± 0.19	0.714 ± 0.27
		AUC_{0-t} (μM*h)	0.752 ± 0.67	2.79 ± 0.96	7.38 ± 2.4	18.3 ± 6.4
		$t_{1/2}$ (h)	NC	0.640 ± 0.14	0.818 ± 0.13	1.21 ± 0.16
	40	C_{max} (μM)	0.471 ± 0.14	1.94 ± 0.36	3.51 ± 0.98	9.70 ± 3.3
		T_{max} (h)	0.500 ± 0.0	0.500 ± 0.0	0.643 ± 0.24	0.714 ± 0.27
		AUC_{0-t} (μM*h)	0.690 ± 0.52	2.75 ± 0.71	8.29 ± 2.4	27.5 ± 9.6
		$t_{1/2}$ (h)	NC	0.705 ± 0.17	1.04 ± 0.20	1.31 ± 0.36
	175	C_{max} (μM)	0.627 ± 0.23	3.01 ± 0.63	6.19 ± 0.79	15.0 ± 7.0
		T_{max} (h)	0.500 ± 0.0	0.643 ± 0.24	0.857 ± 0.24	1.17 ± 0.41
		AUC_{0-t} (μM*h)	1.16 ± 0.49	7.54 ± 2.0	18.3 ± 5.8	72.8 ± 55
		$t_{1/2}$ (h)	0.923 ± 0.26	1.16 ± 0.21	1.22 ± 0.25	2.09 ± 0.82
Female	0	C_{max} (μM)	0.491 ± 0.26	2.35 ± 1.1	4.24 ± 0.55	7.67 ± 2.4
		T_{max} (h)	0.500 ± 0.0	0.500 ± 0.0	0.500 ± 0.0	0.571 ± 0.19
		AUC_{0-t} (μM*h)	0.621 ± 0.48	4.16 ± 2.4	6.35 ± 2.0	21.0 ± 11
		$t_{1/2}$ (h)	NC	0.936 ± 0.32	0.686 ± 0.17	1.19 ± 0.41
	40	C_{max} (μM)	0.448 ± 0.25	2.14 ± 0.82	3.87 ± 0.99	9.53 ± 3.8
		T_{max} (h)	0.571 ± 0.19	0.500 ± 0.0	0.500 ± 0.0	0.786 ± 0.27
		AUC_{0-t} (μM*h)	0.663 ± 0.49	3.63 ± 2.0	6.19 ± 1.5	26.3 ± 17
		$t_{1/2}$ (h)	NC	0.846 ± 0.24	0.752 ± 0.16	1.23 ± 0.44
	175	C_{max} (μM)	0.588 ± 0.19	3.48 ± 1.1	4.46 ± 0.89	11.5 ± 7.3
		T_{max} (h)	0.571 ± 0.19	0.643 ± 0.24	0.786 ± 0.27	0.917 ± 0.20
		AUC_{0-t} (μM*h)	1.07 ± 0.72	8.10 ± 3.9	11.0 ± 2.2	38.8 ± 22
		$t_{1/2}$ (h)	0.965 ± 0.22[b]	1.08 ± 0.24	1.09 ± 0.21	1.48 ± 0.28

NC: Not calculated due to insufficient time points in the terminal phase in > 4 dogs in this group

a: Day 0 was the first day of dosing

b: N=5; two dogs had insufficient time points in the terminal phase to calculate $t_{1/2}$

c: N=6 for Day 175

Study title: 52-week oral gavage chronic toxicity and toxicokinetic study with INCB018424 in dogs with a 6-week recovery period

Key Study Findings:

- The mean plasma Cmax and AUC values generally increased dose proportionally or slightly greater between 0.75 and 6 mg/kg;
- The Cmax values on Day 1 and after multiple dosing were similar, while AUC values after multiple dosing were slightly higher at all doses in male dogs, but only at 6 mg/kg in females;
- The terminal half-life values were not dose-dependent and were slightly increase with multiple dosings;
- The mean T_{max} were generally similar for all dose groups ranging from 0.500 to 1.14 hours;
- TK parameters were generally comparable between males and females

Note: The AUC values in this study were consistent with corresponding values from the 6 month study

Study no.: 7456-271
Volume #, and page #: electronic submission, Module 4,
Conducting laboratory and location: (b) (4)

Date of study initiation: 02 July 2008
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: INCB018424
Lot number: BPR-07-101-B2-21
Purity: 97.89%

Methods
Species/strain: Beagle dogs
#/sex/group: 7/sex/group
Schedule: daily for a total 52 weeks
Doses in administered units: 0.75, 1.5, 3, and 6 mg/kg/day
Route: oral gavage
Toxicokinetic analysis: Day 1 and during Weeks 4, 13, 26, and 52 of the dosing phase. All animals will be bled predose (within 1 hour of dosing) and approximately 0.5, 1, 2, 4, 8, 12, and 24 hour postdose.

Results: The following summary table was excerpted from the Sponsor's application

Summary of INCB018424 Toxicokinetics in Male Beagle Dogs Given Daily Oral Doses of 0.75, 1.5, 3 or 6 mg/kg of INCB018424

			Mean ± SD (N=7)			
Gender	Day	Parameter	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg
Male	1	C_{max} (μM)	0.310 ± 0.13	0.486 ± 0.39	1.06 ± 0.62	3.97 ± 2.3
		T_{max} (h)	0.500 ± 0.0	0.500 ± 0.0	0.500 ± 0.0	0.571 ± 0.19
		AUC_{0-t} (μM*h)	0.463 ± 0.20	0.833 ± 0.75	2.31 ± 1.9	9.38 ± 8.1
		AUC_{0-24} (μM*h)	0.541 ± 0.21	0.897 ± 0.80	2.39 ± 1.9	9.61 ± 8.1
		$t_{1/2}$ (h)	1.16 ± 0.54	0.985 ± 0.40[a]	1.29 ± 0.67	1.02 ± 0.40
	28	C_{max} (μM)	0.262 ± 0.044	0.399 ± 0.24	0.958 ± 0.29	2.29 ± 1.5
		T_{max} (h)	0.571 ± 0.19	0.643 ± 0.24	0.643 ± 0.24	1.14 ± 0.63
		AUC_{0-t} (μM*h)	0.605 ± 0.19	1.15 ± 0.82	3.10 ± 1.4	11.0 ± 10
		AUC_{0-24} (μM*h)	0.673 ± 0.21	1.20 ± 0.82	3.21 ± 1.5	11.4 ± 10
		$t_{1/2}$ (h)	1.58 ± 0.41	1.33 ± 0.20	1.73 ± 0.37	1.95 ± 0.87
	87	C_{max} (μM)	0.294 ± 0.19	0.663 ± 0.31	1.74 ± 0.82	3.11 ± 1.7
		T_{max} (h)	0.643 ± 0.24	0.643 ± 0.24	0.500 ± 0.0	1.14 ± 0.63
		AUC_{0-t} (μM*h)	0.745 ± 0.46	1.86 ± 1.4	6.46 ± 2.8	15.9 ± 12
		AUC_{0-24} (μM*h)	0.784 ± 0.48	2.00 ± 1.4	6.59 ± 2.8	16.7 ± 13
		$t_{1/2}$ (h)	1.45 ± 0.49[a]	1.46 ± 0.43	1.63 ± 0.21	2.02 ± 0.60
	176[b]	C_{max} (μM)	0.326 ± 0.096	0.569 ± 0.25	1.49 ± 0.91	4.03 ± 1.6
		T_{max} (h)	0.571 ± 0.19	0.571 ± 0.19	0.643 ± 0.24	1.10 ± 0.55
		AUC_{0-t} (μM*h)	0.953 ± 0.21	2.13 ± 1.5	5.84 ± 3.3	18.5 ± 9.7
		AUC_{0-24} (μM*h)	1.02 ± 0.23	2.23 ± 1.7	6.08 ± 3.4	19.4 ± 10
		$t_{1/2}$ (h)	1.92 ± 0.73	2.07 ± 0.60	2.07 ± 0.68	2.01 ± 0.68
	357[c]	C_{max} (μM)	0.398 ± 0.12	0.630 ± 0.26	1.32 ± 0.57	4.91 ± 1.6
		T_{max} (h)	0.786 ± 0.27	0.929 ± 0.53	1.00 ± 0.50	0.875 ± 0.75
		AUC_{0-t} (μM*h)	1.12 ± 0.36	2.27 ± 1.2	5.90 ± 3.1	16.5 ± 0.84
		AUC_{0-24} (μM*h)	1.21 ± 0.37	2.36 ± 1.2	6.23 ± 3.2	16.8 ± 0.74
		$t_{1/2}$ (h)	1.68 ± 0.73	1.89 ± 0.19	2.48 ± 1.0	1.57 ± 0.37

a: N=6; one dog had insufficient time points in the terminal phase to calculate $t_{1/2}$
b: For 6 mg/kg group, N=5
c: Day 196 for 6 mg/kg (N=4)

Summary of INCB018424 Toxicokinetics in Female Beagle Dogs Given Daily Oral Doses of 0.75, 1.5, 3 or 6 mg/kg of INCB018424

			Mean ± SD (N=7)			
Gender	Day	Parameter	0.75 mg/kg	1.5 mg/kg	3 mg/kg	6 mg/kg
Male	1	C_{max} (μM)	0.511 ± 0.29	0.798 ± 0.62	1.68 ± 0.77	3.47 ± 1.1
		T_{max} (h)	0.571 ± 0.19	0.786 ± 0.57	0.714 ± 0.57	0.643 ± 0.24
		AUC_{0-t} (μM*h)	0.983 ± 0.58	1.78 ± 1.7	5.28 ± 4.3	8.58 ± 5.3
		AUC_{0-24} (μM*h)	1.05 ± 0.59	1.87 ± 1.7	5.38 ± 4.3	8.94 ± 5.8
		$t_{1/2}$ (h)	1.04 ± 0.57	1.20 ± 0.22	1.14 ± 0.22	1.42 ± 0.87
	28	C_{max} (μM)	0.280 ± 0.10	0.518 ± 0.37	1.10 ± 0.59	2.15 ± 0.73
		T_{max} (h)	0.714 ± 0.27	0.571 ± 0.19	1.00 ± 0.71	0.714 ± 0.27
		AUC_{0-t} (μM*h)	0.785 ± 0.34	1.38 ± 0.91	4.84 ± 2.7	8.74 ± 3.5
		AUC_{0-24} (μM*h)	0.855 ± 0.38	1.47 ± 0.89	5.08 ± 2.7	9.06 ± 3.8
		$t_{1/2}$ (h)	1.59 ± 0.48	1.83 ± 0.64	2.31 ± 1.1	1.84 ± 0.44
	87	C_{max} (μM)	0.328 ± 0.13	0.552 ± 0.25	1.76 ± 0.50	3.31 ± 1.0
		T_{max} (h)	0.643 ± 0.24	0.571 ± 0.19	0.929 ± 0.53	0.786 ± 0.57
		AUC_{0-t} (μM*h)	0.828 ± 0.45	1.82 ± 1.2	7.15 ± 3.8	13.1 ± 4.5
		AUC_{0-24} (μM*h)	0.878 ± 0.46	1.91 ± 1.2	7.46 ± 4.0	13.5 ± 4.8
		$t_{1/2}$ (h)	1.46 ± 0.46	1.73 ± 0.31	1.94 ± 0.49	1.72 ± 0.57
	176	C_{max} (μM)	0.304 ± 0.15	0.578 ± 0.19	1.41 ± 0.96	3.57 ± 2.4
		T_{max} (h)	0.857 ± 0.24	0.643 ± 0.24	0.714 ± 0.27	0.857 ± 0.24
		AUC_{0-t} (μM*h)	1.06 ± 0.56	2.33 ± 1.1	6.59 ± 5.5	17.6 ± 8.8
		AUC_{0-24} (μM*h)	1.14 ± 0.57	2.48 ± 1.1	6.83 ± 5.7	19.1 ± 9.4
		$t_{1/2}$ (h)	2.17 ± 0.88	2.41 ± 0.54	1.84 ± 0.53	3.21 ± 3.1
	357[b]	C_{max} (μM)	0.304 ± 0.26	0.844 ± 0.33	1.29 ± 0.51	3.90 ± 1.4
		T_{max} (h)	1.00 ± 0.55[a]	0.786 ± 0.27	0.786 ± 0.57	0.833 ± 0.26
		AUC_{0-t} (μM*h)	0.515 ± 0.69	2.22 ± 1.6	4.30 ± 2.7	16.4 ± 7.3
		AUC_{0-24} (μM*h)	0.688 ± 0.83	2.57 ± 1.6	4.83 ± 2.9	17.1 ± 7.7
		$t_{1/2}$ (h)	1.44 ± 0.15[c]	1.50 ± 0.41	1.90 ± 0.86	2.00 ± 0.50

a: N=6; could not be calculated for one dog due to insufficient time points
b: For 6 mg/kg group, N=6
c: N=4; could not be calculated for three dogs due to insufficient time points

Distribution

Quantitative whole-body autoradiography of rats following oral administration of ^{14}C-INCB018424

Key Study Findings:

- Radioactivity was mainly in the liver, bile, large intestine, small intestine, and uveal tract in both male and female rats; relative high exposure of radiation was also noted in the adrenal gland, renal cortex and renal medulla in female rats;
- Highest concentration of radiation in tissues was observed at 2 hours postdose;
- Radioactivity concentrations in central nervous system tissues (cerebellum, cerebrum, medulla, olfactory lobe, and spinal cord) were not measurable at any sampling time;
- Fecal, hepatobiliary, and renal excretion were indicated as elimination pathways;
- In general, elimination of administered radioactivity was rapid in most tissues, most tissue concentrations were below the limit of quantitation at 24 hours postdose and no 14C-INCB018424-derived radioactivity was detected by 336 hours postdose;
- Low concentrations of 14C-INCB018424-derived radioactivity were measured in the testis and accessory sex organs of male rats and the ovary and uterus of female rats, but concentrations were below the limit of quantitation by 8 hours postdose;
- Peak tissue concentrations of radioactivity tended to be higher in female rats than males, consistent with higher peak blood and plasma levels;
- The maximum blood and plasma concentrations of 14C-INCB018424-derived radioactivity were measured at 2 hours postdose, and declined steadily throughout the experimental timeframe. Blood radioactivity was not partitioned preferentially in plasma;
- Percentage of dosed radioactivity in blood and tissues was 80% in males and 53% in females at 8 hours postdose

Study no: (b) (4) 7456-241
Volume #, and page #: electronic submission, page 1-103
Conducting laboratory and location: (b) (4)

Date of study initiation: September 13, 2007
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: ^{14}C-INCB018424
Lot No.: 3562192
Purity: 96.8% (radiochemical purity)
INCB018424
Lot No.: SS-IN2-175
Purity: not provided
Formulation/vehicle: 0.5% methylcellulose in reverse osmosis water

Methods:

Group Designations and Dose Levels

Group	Number of Animals Male	Number of Animals Female	Dose Route	Target Dose Level (mg/kg)	Target Dose Volume (mL/kg)	Samples Collected
1	5 LE	5 LE	Oral	25	10	Carcass for WBA

LE Long Evans, pigmented rats
WBA Whole-body autoradiography.
Note: The dose was approximately 100 µCi/kg.

One animal/sex/time point was sacrificed at 2, 8, 24, 168, and 336 hours postdose. Blood was collected and plasma was harvested at specified time points and carcasses were prepared for quantitative whole-body autoradiography (QWBA). Blood and plasma were analyzed for concentrations of radioactivity using liquid scintillation counting (LSC). Exposed screens were scanned using a Storm scanner for QWBA.

Dosing:

Species/strain: Long Evans, pigmented rats (HsdBlu:LE)
#/sex/group or time point: 5/sex
Weight: 179-199 g
Doses in administered units: 25 mg/kg, 100 µCi
Route, form, volume, and infusion rate: oral gavage at dose volume of 10 mL/kg

Results: The following tables are excerpted from Applicant's submission

Concentrations of radioactivity in blood and tissues determined by whole-body autoradiography at specified times after a single oral administration of ^{14}C-INCB018424 to male rats (Group 1, 25 mg/kg)

	ng Equivalents ^{14}C-INCB018424/g				
	Animal Number (Sacrifice Time)				
Matrix	B05630 (2 Hours)	B05631 (8 Hours)	B05632 (24 Hours)	B05633 (168 Hours)	B05634 (336 Hours)
Adrenal gland	2120	ND	ND	ND	ND
Bile	30200	ND	ND	ND	ND
Blood	ND	ND	ND	ND	ND
Bone	BLQ	ND	ND	ND	ND
Bone marrow	950	ND	ND	ND	ND
Cecum	5710	121000	ND	ND	ND
Cecum contents	91500	498000	1550	ND	ND
Cerebellum	BLQ	ND	ND	ND	ND
Cerebrum	BLQ	ND	ND	ND	ND
Diaphragm	1420	ND	ND	ND	ND
Epididymis	1080	ND	ND	ND	ND
Esophageal contents	1400	ND	ND	ND	ND
Esophagus	5010	ND	ND	ND	ND
Exorbital lacrimal gland	754	ND	ND	ND	ND
Eye	2170	852	BLQ	BLQ	ND
Eye (lens)	BLQ	BLQ	BLQ	ND	ND
Fat (abdominal)	BLQ	ND	ND	ND	ND
Fat (brown)	844	ND	ND	ND	ND
Harderian gland	1010	ND	ND	ND	ND
Intra-orbital lacrimal gland	1030	ND	ND	ND	ND
Kidney	5420	673	ND	ND	ND
Large intestinal contents	1060	634000	4060	ND	ND
Large intestine	10500	31400	ND	ND	ND
Liver	9710	2140	BLQ	ND	ND
Lung	1060	ND	ND	ND	ND
Lymph nodes	ND	ND	ND	ND	ND
Medulla	BLQ	ND	ND	ND	ND
Muscle	1460	ND	ND	ND	ND
Myocardium	ND	ND	ND	ND	ND
Nasal turbinates	613	ND	ND	ND	ND
Olfactory lobe	BLQ	ND	ND	ND	ND
Pancreas	1550	BLQ	ND	ND	ND
Pituitary gland	835	ND	ND	ND	ND
Preputial gland	1410	590	ND	ND	ND

BLQ Below the limit of quantitation (<398 ng equivalents ^{14}C-INCB018424/g).
ND Not detectable (sample not discernible from background or surrounding tissue)

Note: ng Equivalent/g values are reported to three significant figures with a maximum of three decimal places.

Continued from last page

Matrix	ng Equivalents ^{14}C-INCB018424/g Animal Number (Sacrifice Time) B05630 (2 Hours)	B05631 (8 Hours)	B05632 (24 Hours)	B05633 (168 Hours)	B05634 (336 Hours)
Prostate	1240	ND	ND	ND	ND
Renal cortex	4910	ND	ND	ND	ND
Renal medulla	5950	ND	ND	ND	ND
Salivary gland	1250	ND	ND	ND	ND
Seminal vesicle	1080	ND	ND	ND	ND
Skin (nonpigmented)	1300	ND	ND	ND	ND
Skin (pigmented)	5860	3310	1400	ND	ND
Small intestinal contents	3980000[a]	26700	ND	ND	ND
Small intestine	104000	1370	ND	ND	ND
Spinal cord	BLQ	ND	ND	ND	ND
Spleen	1190	ND	ND	ND	ND
Stomach	1910	ND	ND	ND	ND
Stomach contents	65000	ND	BLQ	ND	ND
Testis	753	ND	ND	ND	ND
Thymus	1120	ND	ND	ND	ND
Thyroid	ND	ND	ND	ND	ND
Urinary bladder	ND	ND	ND	ND	ND
Urine	46600	ND	ND	ND	ND
Uveal tract	14400	6240	3320	1090	ND

BLQ Below the limit of quantitation (<398 ng equivalents ^{14}C-INCB018424/g).
ND Not detectable (sample not discernible from background or surrounding tissue)

Note: ng Equivalent/g values are reported to three significant figures with a maximum of three decimal places.
a One or more samples were above the upper limit of quantitation (>2230000 ng equivalents ^{14}C-INCB018424/g).

Concentrations of radioactivity in blood and tissues determined by whole-body autoradiography at specified times after a single oral administration of ^{14}C-INCB018424 to female rats (Group 1, 25 mg/kg)

	ng Equivalents ^{14}C-INCB018424/g				
	Animal Number (Sacrifice Time)				
Matrix	B05635 (2 Hours)	B05636 (8 Hours)	B05637 (24 Hours)	B05638 (168 Hours)	B05639 (336 Hours)
Adrenal gland	12200	409	ND	ND	ND
Bile	111000	ND	ND	ND	ND
Blood	3200	ND	ND	ND	ND
Bone	492	ND	ND	ND	ND
Bone marrow	3060	ND	ND	ND	ND
Cecum	15700	58900	ND	ND	ND
Cecum contents	73400	329000	7220	ND	ND
Cerebellum	BLQ	ND	ND	ND	ND
Cerebrum	BLQ	ND	ND	ND	ND
Diaphragm	5120	ND	ND	ND	ND
Esophageal contents	35100	ND	ND	ND	ND
Esophagus	5500	ND	ND	ND	ND
Exorbital lacrimal gland	5010	ND	ND	ND	ND
Eye	2910	1010	477	BLQ	ND
Eye (lens)	BLQ	BLQ	BLQ	ND	ND
Fat (abdominal)	515	ND	ND	ND	ND
Fat (brown)	5120	ND	ND	ND	ND
Harderian gland	5320	ND	ND	ND	ND
Intra-orbital lacrimal gland	4630	ND	ND	ND	ND
Kidney	18700	1020	ND	ND	ND
Large intestinal contents	1330	415000	9480	ND	ND
Large intestine	14600	27300	ND	ND	ND
Liver	25300	3070	449	ND	ND
Lung	3520	ND	ND	ND	ND
Lymph nodes	4050	ND	ND	ND	ND
Medulla	BLQ	ND	ND	ND	ND
Muscle	4160	ND	ND	ND	ND
Myocardium	5980	ND	ND	ND	ND
Nasal turbinates	2440	ND	ND	ND	ND
Olfactory lobe	BLQ	ND	ND	ND	ND
Ovary	1850	ND	ND	ND	ND
Pancreas	5920	ND	ND	ND	ND
Pituitary gland	4450	ND	ND	ND	ND
Preputial gland	3510	ND	ND	ND	ND

BLQ Below the limit of quantitation (<398 ng equivalents ^{14}C-INCB018424/g).
ND Not detectable (sample not discernible from background or surrounding tissue).

Note: ng Equivalent/g values are reported to three significant figures with a maximum of three decimal places.

Continued from last page

	ng Equivalents ^{14}C-INCB018424/g				
	Animal Number (Sacrifice Time)				
Matrix	B05635 (2 Hours)	B05636 (8 Hours)	B05637 (24 Hours)	B05638 (168 Hours)	B05639 (336 Hours)
Renal cortex	19500	1120	ND	ND	ND
Renal medulla	15900	739	ND	ND	ND
Salivary gland	5520	ND	ND	ND	ND
Skin (nonpigmented)	4680	ND	ND	ND	ND
Skin (pigmented)	6120	ND	ND	ND	ND
Small intestinal contents	2890000[a]	16400	ND	ND	ND
Small intestine	98500	ND	ND	ND	ND
Spinal cord	BLQ	ND	ND	ND	ND
Spleen	4400	ND	ND	ND	ND
Stomach	7660	ND	ND	ND	ND
Stomach contents	363000	1010	ND	ND	ND
Thymus	4070	ND	ND	ND	ND
Thyroid	4140	ND	ND	ND	ND
Urinary bladder	ND	ND	ND	ND	ND
Urine	138000	7150	ND	ND	ND
Uterus	3690	ND	ND	ND	ND
Uveal tract	19000	7360	3630	2350	ND

BLQ Below the limit of quantitation (<398 ng equivalents ^{14}C-INCB018424/g).
ND Not detectable (sample not discernible from background or surrounding tissue).

Note: ng Equivalent/g values are reported to three significant figures with a maximum of three decimal places.
a One or more samples were above the upper limit of quantitation (>2230000 ng equivalents ^{14}C-INCB018424/g).

Percent of radioactive dose in blood and tissues determined by whole-body autoradiography at specified times after a single oral administration of ^{14}C-INCB018424 to male rats (Group 1, 25 mg/kg)

	Percent of Radioactive Dose				
	Animal Number (Sacrifice Time)				
Matrix	B05630 (2 Hours)	B05631 (8 Hours)	B05632 (24 Hours)	B05633 (168 Hours)	B05634 (336 Hours)
Adrenal gland	0.00	NA	NA	NA	NA
Bile	NA	NA	NA	NA	NA
Blood	NA	NA	NA	NA	NA
Bone	NA	NA	NA	NA	NA
Bone marrow	0.01	NA	NA	NA	NA
Cecum	0.08	1.72	NA	NA	NA
Cecum contents	8.08	44.41	0.14	NA	NA
Cerebellum	NA	NA	NA	NA	NA
Cerebrum	NA	NA	NA	NA	NA
Diaphragm	NA	NA	NA	NA	NA
Epididymis	0.01	NA	NA	NA	NA
Esophageal contents	NA	NA	NA	NA	NA
Esophagus	NA	NA	NA	NA	NA
Exorbital lacrimal gland	NA	NA	NA	NA	NA
Eye	0.01	0.00	NA	NA	NA
Eye (lens)	NA	NA	NA	NA	NA
Fat (abdominal)	NA	NA	NA	NA	NA
Fat (brown)	0.00	NA	NA	NA	NA
Harderian gland	0.00	NA	NA	NA	NA
Intra-orbital lacrimal gland	NA	NA	NA	NA	NA
Kidney	0.18	0.02	NA	NA	NA
Large intestinal contents	0.05	27.53	0.18	NA	NA
Large intestine	0.28	0.85	NA	NA	NA
Liver	1.90	0.42	NA	NA	NA
Lung	0.02	NA	NA	NA	NA
Lymph nodes	NA	NA	NA	NA	NA
Medulla	NA	NA	NA	NA	NA
Muscle	2.57	NA	NA	NA	NA
Myocardium	NA	NA	NA	NA	NA
Nasal turbinates	NA	NA	NA	NA	NA
Olfactory lobe	NA	NA	NA	NA	NA
Pancreas	0.02	NA	NA	NA	NA
Pituitary gland	0.00	NA	NA	NA	NA
Preputial gland	0.00	0.00	NA	NA	NA

NA Not applicable.
SD Standard deviation

Continued from last page

	Percent of Radioactive Dose				
	Animal Number (Sacrifice Time)				
Matrix	B05630 (2 Hours)	B05631 (8 Hours)	B05632 (24 Hours)	B05633 (168 Hours)	B05634 (336 Hours)
Prostate	0.00	NA	NA	NA	NA
Renal cortex	0.16	NA	NA	NA	NA
Renal medulla	0.20	NA	NA	NA	NA
Salivary gland	0.01	NA	NA	NA	NA
Seminal vesicle	0.01	NA	NA	NA	NA
Skin (nonpigmented)	1.19	NA	NA	NA	NA
Skin (pigmented)	5.36	3.06	1.32	NA	NA
Small intestinal contents	314.56[a]	2.13	NA	NA	NA
Small intestine	8.86	0.12	NA	NA	NA
Spinal cord	NA	NA	NA	NA	NA
Spleen	0.01	NA	NA	NA	NA
Stomach	0.04	NA	NA	NA	NA
Stomach contents	6.67	NA	NA	NA	NA
Testis	0.03	NA	NA	NA	NA
Thymus	0.01	NA	NA	NA	NA
Thyroid	NA	NA	NA	NA	NA
Urinary bladder	NA	NA	NA	NA	NA
Urine	NA	NA	NA	NA	NA
Uveal tract	0.05	0.02	0.01	0.00	NA
Total	350.38[a]	80.28	1.65	0.00	0.00

NA Not applicable.
SD Standard deviation.
a One or more samples were above the upper limit of quantitation (>2230000 ng equivalents ^{14}C-INCB018424/g).

Percent of radioactive dose in blood and tissues determined by whole-body autoradiography at specified times after a single oral administration of ^{14}C-INCB018424 to female rats (Group 1, 25 mg/kg)

	Percent of Radioactive Dose				
	Animal Number (Sacrifice Time)				
Matrix	B05635 (2 Hours)	B05636 (8 Hours)	B05637 (24 Hours)	B05638 (168 Hours)	B05639 (336 Hours)
Adrenal gland	0.01	0.00	NA	NA	NA
Bile	NA	NA	NA	NA	NA
Blood	0.88	NA	NA	NA	NA
Bone	0.10	NA	NA	NA	NA
Bone marrow	0.04	NA	NA	NA	NA
Cecum	0.23	0.86	NA	NA	NA
Cecum contents	6.62	29.63	0.65	NA	NA
Cerebellum	NA	NA	NA	NA	NA
Cerebrum	NA	NA	NA	NA	NA
Diaphragm	NA	NA	NA	NA	NA
Esophageal contents	NA	NA	NA	NA	NA
Esophagus	NA	NA	NA	NA	NA
Exorbital lacrimal gland	NA	NA	NA	NA	NA
Eye	0.02	0.01	0.00	NA	NA
Eye (lens)	NA	NA	NA	NA	NA
Fat (abdominal)	0.15	NA	NA	NA	NA
Fat (brown)	0.03	NA	NA	NA	NA
Harderian gland	0.02	NA	NA	NA	NA
Intra-orbital lacrimal gland	NA	NA	NA	NA	NA
Kidney	0.70	0.04	NA	NA	NA
Large intestinal contents	0.06	19.58	0.44	NA	NA
Large intestine	0.41	0.77	NA	NA	NA
Liver	4.70	0.57	0.08	NA	NA
Lung	0.07	NA	NA	NA	NA
Lymph nodes	NA	NA	NA	NA	NA
Medulla	NA	NA	NA	NA	NA
Muscle	7.45	NA	NA	NA	NA
Myocardium	0.08	NA	NA	NA	NA
Nasal turbinates	NA	NA	NA	NA	NA
Olfactory lobe	NA	NA	NA	NA	NA
Ovary	0.00	NA	NA	NA	NA
Pancreas	0.10	NA	NA	NA	NA
Pituitary gland	0.00	NA	NA	NA	NA
Preputial gland	0.01	NA	NA	NA	NA

NA Not applicable.
SD Standard deviation.

Continued from last page

Percent of radioactive dose in blood and tissues determined by whole-body autoradiography at specified times after a single oral administration of ^{14}C-INCB018424 to female rats (Group 1, 25 mg/kg)

	Percent of Radioactive Dose				
	Animal Number (Sacrifice Time)				
Matrix	B05635 (2 Hours)	B05636 (8 Hours)	B05637 (24 Hours)	B05638 (168 Hours)	B05639 (336 Hours)
Renal cortex	0.73	0.04	NA	NA	NA
Renal medulla	0.59	0.03	NA	NA	NA
Salivary gland	0.04	NA	NA	NA	NA
Skin (nonpigmented)	4.35	NA	NA	NA	NA
Skin (pigmented)	5.69	NA	NA	NA	NA
Small intestinal contents	254.86a	1.44	NA	NA	NA
Small intestine	10.04	NA	NA	NA	NA
Spinal cord	NA	NA	NA	NA	NA
Spleen	0.05	NA	NA	NA	NA
Stomach	0.16	NA	NA	NA	NA
Stomach contents	35.30	0.10	NA	NA	NA
Thymus	0.04	NA	NA	NA	NA
Thyroid	0.00	NA	NA	NA	NA
Urinary bladder	NA	NA	NA	NA	NA
Urine	NA	NA	NA	NA	NA
Uterus	0.03	NA	NA	NA	NA
Uveal tract	0.10	0.04	0.02	0.01	NA
Total	333.64[a]	53.11	1.20	0.01	0.00

NA Not applicable.
SD Standard deviation.
a One or more samples were above the upper limit of quantitation (>2230000 ng equivalents ^{14}C-INCB018424/g).

Concentrations of radioactivity in blood, plasma, and cellular fraction at specified times after a single oral administration of ^{14}C-INCB018424to male and female rats (Group 1, 25 mg/kg)

Collection Time Point	ng Equivalents ^{14}C-INCB018424/g									
	Animal Number									
	Males					Females				
(hours)	B05630	B05631	B05632	B05633	B05634	B05635	B05636	B05637	B05638	B05639
					Blood					
2	1400	-	-	-	-	4830	-	-	-	-
8	-	178	-	-	-	-	183	-	-	-
24	-	-	44.8	-	-	-	-	40.7	-	-
168	-	-	-	14.6	-	-	-	-	14.4	-
336	-	-	-	-	BLQ	-	-	-	-	BLQ
					Plasma					
2	1420	-	-	-	-	5130	-	-	-	-
8	-	170	-	-	-	-	163	-	-	-
24	-	-	33.3	-	-	-	-	36.3	-	-
168	-	-	-	BLQ	-	-	-	-	BLQ	-
336	-	-	-	-	BLQ	-	-	-	-	BLQ
					Cellular Fraction					
2	1410	-	-	-	-	4710	-	-	-	-
8	-	172	-	-	-	-	197	-	-	-
24	-	-	54.7	-	-	-	-	54.3	-	-
168	-	-	-	26.1	-	-	-	-	25.4	-
336	-	-	-	-	16.9	-	-	-	-	16.4

BLQ Below the limit of quantitation.
- Not scheduled for collection.

Percent of radioactive dose in blood and tissues determined by whole-body autoradiography at specified times after a single oral administration of ^{14}C-INCB018424 to male rats (Group 1, 25 mg/kg)

	Percent of Radioactive Dose				
	Animal Number (Sacrifice Time)				
Matrix	B05630 (2 Hours)	B05631 (8 Hours)	B05632 (24 Hours)	B05633 (168 Hours)	B05634 (336 Hours)
Adrenal gland	0.00	NA	NA	NA	NA
Bile	NA	NA	NA	NA	NA
Blood	NA	NA	NA	NA	NA
Bone	NA	NA	NA	NA	NA
Bone marrow	0.01	NA	NA	NA	NA
Cecum	0.08	1.72	NA	NA	NA
Cecum contents	8.08	44.41	0.14	NA	NA
Cerebellum	NA	NA	NA	NA	NA
Cerebrum	NA	NA	NA	NA	NA
Diaphragm	NA	NA	NA	NA	NA
Epididymis	0.01	NA	NA	NA	NA
Esophageal contents	NA	NA	NA	NA	NA
Esophagus	NA	NA	NA	NA	NA
Exorbital lacrimal gland	NA	NA	NA	NA	NA
Eye	0.01	0.00	NA	NA	NA
Eye (lens)	NA	NA	NA	NA	NA
Fat (abdominal)	NA	NA	NA	NA	NA
Fat (brown)	0.00	NA	NA	NA	NA
Harderian gland	0.00	NA	NA	NA	NA
Intra-orbital lacrimal gland	NA	NA	NA	NA	NA
Kidney	0.18	0.02	NA	NA	NA
Large intestinal contents	0.05	27.53	0.18	NA	NA
Large intestine	0.28	0.85	NA	NA	NA
Liver	1.90	0.42	NA	NA	NA
Lung	0.02	NA	NA	NA	NA
Lymph nodes	NA	NA	NA	NA	NA
Medulla	NA	NA	NA	NA	NA
Muscle	2.57	NA	NA	NA	NA
Myocardium	NA	NA	NA	NA	NA
Nasal turbinates	NA	NA	NA	NA	NA
Olfactory lobe	NA	NA	NA	NA	NA
Pancreas	0.02	NA	NA	NA	NA
Pituitary gland	0.00	NA	NA	NA	NA
Preputial gland	0.00	0.00	NA	NA	NA

NA Not applicable.
SD Standard deviation

Continued from last page

Percent of radioactive dose in blood and tissues determined by whole-body autoradiography at specified times after a single oral administration of ^{14}C-INCB018424 to male rats (Group 1, 25 mg/kg)

	Percent of Radioactive Dose				
	Animal Number (Sacrifice Time)				
Matrix	B05630 (2 Hours)	B05631 (8 Hours)	B05632 (24 Hours)	B05633 (168 Hours)	B05634 (336 Hours)
Prostate	0.00	NA	NA	NA	NA
Renal cortex	0.16	NA	NA	NA	NA
Renal medulla	0.20	NA	NA	NA	NA
Salivary gland	0.01	NA	NA	NA	NA
Seminal vesicle	0.01	NA	NA	NA	NA
Skin (nonpigmented)	1.19	NA	NA	NA	NA
Skin (pigmented)	5.36	3.06	1.32	NA	NA
Small intestinal contents	314.56[a]	2.13	NA	NA	NA
Small intestine	8.86	0.12	NA	NA	NA
Spinal cord	NA	NA	NA	NA	NA
Spleen	0.01	NA	NA	NA	NA
Stomach	0.04	NA	NA	NA	NA
Stomach contents	6.67	NA	NA	NA	NA
Testis	0.03	NA	NA	NA	NA
Thymus	0.01	NA	NA	NA	NA
Thyroid	NA	NA	NA	NA	NA
Urinary bladder	NA	NA	NA	NA	NA
Urine	NA	NA	NA	NA	NA
Uveal tract	0.05	0.02	0.01	0.00	NA
Total	350.38[a]	80.28	1.65	0.00	0.00

NA Not applicable.
SD Standard deviation.
a One or more samples were above the upper limit of quantitation (>2230000 ng equivalents ^{14}C-INCB018424/g).

Percent of radioactive dose in blood and tissues determined by whole-body autoradiography at specified times after a single oral administration of ^{14}C-INCB018424 to female rats (Group 1, 25 mg/kg)

	Percent of Radioactive Dose				
	Animal Number (Sacrifice Time)				
Matrix	B05635 (2 Hours)	B05636 (8 Hours)	B05637 (24 Hours)	B05638 (168 Hours)	B05639 (336 Hours)
Adrenal gland	0.01	0.00	NA	NA	NA
Bile	NA	NA	NA	NA	NA
Blood	0.88	NA	NA	NA	NA
Bone	0.10	NA	NA	NA	NA
Bone marrow	0.04	NA	NA	NA	NA
Cecum	0.23	0.86	NA	NA	NA
Cecum contents	6.62	29.63	0.65	NA	NA
Cerebellum	NA	NA	NA	NA	NA
Cerebrum	NA	NA	NA	NA	NA
Diaphragm	NA	NA	NA	NA	NA
Esophageal contents	NA	NA	NA	NA	NA
Esophagus	NA	NA	NA	NA	NA
Exorbital lacrimal gland	NA	NA	NA	NA	NA
Eye	0.02	0.01	0.00	NA	NA
Eye (lens)	NA	NA	NA	NA	NA
Fat (abdominal)	0.15	NA	NA	NA	NA
Fat (brown)	0.03	NA	NA	NA	NA
Harderian gland	0.02	NA	NA	NA	NA
Intra-orbital lacrimal gland	NA	NA	NA	NA	NA
Kidney	0.70	0.04	NA	NA	NA
Large intestinal contents	0.06	19.58	0.44	NA	NA
Large intestine	0.41	0.77	NA	NA	NA
Liver	4.70	0.57	0.08	NA	NA
Lung	0.07	NA	NA	NA	NA
Lymph nodes	NA	NA	NA	NA	NA
Medulla	NA	NA	NA	NA	NA
Muscle	7.45	NA	NA	NA	NA
Myocardium	0.08	NA	NA	NA	NA
Nasal turbinates	NA	NA	NA	NA	NA
Olfactory lobe	NA	NA	NA	NA	NA
Ovary	0.00	NA	NA	NA	NA
Pancreas	0.10	NA	NA	NA	NA
Pituitary gland	0.00	NA	NA	NA	NA
Preputial gland	0.01	NA	NA	NA	NA

NA Not applicable.
SD Standard deviation.

Continued from last page

Percent of radioactive dose in blood and tissues determined by whole-body autoradiography at specified times after a single oral administration of ^{14}C-INCB018424 to female rats (Group 1, 25 mg/kg)

	Percent of Radioactive Dose				
	Animal Number (Sacrifice Time)				
Matrix	B05635 (2 Hours)	B05636 (8 Hours)	B05637 (24 Hours)	B05638 (168 Hours)	B05639 (336 Hours)
Renal cortex	0.73	0.04	NA	NA	NA
Renal medulla	0.59	0.03	NA	NA	NA
Salivary gland	0.04	NA	NA	NA	NA
Skin (nonpigmented)	4.35	NA	NA	NA	NA
Skin (pigmented)	5.69	NA	NA	NA	NA
Small intestinal contents	254.86a	1.44	NA	NA	NA
Small intestine	10.04	NA	NA	NA	NA
Spinal cord	NA	NA	NA	NA	NA
Spleen	0.05	NA	NA	NA	NA
Stomach	0.16	NA	NA	NA	NA
Stomach contents	35.30	0.10	NA	NA	NA
Thymus	0.04	NA	NA	NA	NA
Thyroid	0.00	NA	NA	NA	NA
Urinary bladder	NA	NA	NA	NA	NA
Urine	NA	NA	NA	NA	NA
Uterus	0.03	NA	NA	NA	NA
Uveal tract	0.10	0.04	0.02	0.01	NA
Total	333.64[a]	53.11	1.20	0.01	0.00

NA Not applicable.
SD Standard deviation.
a One or more samples were above the upper limit of quantitation (>2230000 ng equivalents ^{14}C-INCB018424/g).

In Vitro and _Ex Vivo_ protein binding of INCB018424 in rat, cynomolgus monkey, dog, minipig, and human serum and plasma

Key Study Findings

- In serum: the *in vitro* unbound fractions of INCB018424 in human, rat, cynomolgus monkey, dog and minipig serum were 3.2, 15, 5.6, 9.5 and 28%, respectively;
- In plasma: the *in vitro* unbound fractions of INCB018424 in human, rat, dog and minipig plasma were 3.3,1 4, 12 and 26%, respectively;
- The *ex vivo* unbound fractions of INCB018424 in rat, dog and minipig plasma were 18, 9.7 and 33%, respectively.

Study no: INCYTE-DMB-07.11.1
Volume #, and page #: electronic submission, page 1-11
Conducting laboratory and location: no information provided
Date of study initiation: October 20, 2005
GLP compliance: no,
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: INCB018424
Lot No.: 2, 7, 8, 9, 10 and SS-IN2-175;
Purity: not provided

Formulation/vehicle: not provided

Methods: The *in vitro* protein binding of INCB018424 was determined using serum and plasma from rats, cynomolgus monkeys (serum only), dogs, minipigs, and humans. The *ex vivo* protein binding of INCB018424 was determined using pooled plasma from rats (single oral or IV dose in rat pharmacokinetic study, INCYTE-DMB-06.169.1), minipigs (pharmacokinetic study in Gottingen minipigs administered a single IV dose of 18424, INCYTE-DMB-07.10.1) and dogs (10-day multiple oral dose toxicokinetic study in dogs, INCYTE-DMB-06.185.1). The Multi-Equilibrium Dialyzer SystemTM and diachema membranes from Harvard Apparatus (Holliston, MA) were used for the experiment.

Results: the following tables are excerpted from the Applicant's submission

Summary of *In Vitro* and *Ex Vivo* Fraction Unbound (fu) of INCB018424 in Serum and Plasma from Human, Rat, Cynomolgus Monkey, Dog and minipig

Species	Matrix	Type	INCB018424 Concentration (μM)	Lot #	f_u (%)	Mean ± SD (%)	Overall Mean ± SD (%)
Human	serum	*in vitro*	3.0	2	4.1	3.6 ± 0.4	3.2
			3.0	2	3.3		
			3.0	8	3.5		
			10	9	2.4	2.6	
			10	9	2.8		
	plasma	*in vitro*	10	9	2.9	3.3	3.3
			10	9	3.7		
Rat	serum	*in vitro*	3.0	2	20	15 ± 4	15
			3.0	2	17		
			3.0	9	9.4		
			10	8	17	14 ± 5	
			10	9	7.9		
			10	9	16		
	plasma	*in vitro*	3.0	10	17	17	14
			1.0	10	12	12	
		ex vivo	4.3	7	18	18	18
Cyno	serum	*in vitro*	3.0	2	5.6	5.6	5.6
Dog	serum	*in vitro*	3.0	2	8.9	9.2	9.5 ± 0.7
			3.0	10	9.5		
			10	10	10	10	
	plasma	*in vitro*	1.0	8	11	11	12 ± 1
			10	8	13	13	
			10	8	12		
		ex vivo	0.39	SS-IN2-175	7.3	7.3	9.7
			4.8	SS-IN2-175	12	12	
Minipig	serum	*in vitro*	3.0	2	31	30	28
			3.0	10	29		
			10	8	25	25	
	plasma	*in vitro*	3.0	10	26	26	26
		ex vivo	1.1	SS-IN2-175	33	33	33

Placental transfer and lacteal excretion of ^{14}C-INCB018424 following administration of a single oral dose to pregnant Sprague Dawley rats

Key Study Findings

- Peak tissue concentrations occurred at 1 hour postdose in most maternal and fetal tissues and then declined with time;
- Low concentrations of drug-derived radioactivity crossed the placenta and resulted in limited fetal exposure;
- Radioactivity concentrations in maternal central nervous system tissues were very low and only quantifiable in the Day 18 dams at 1 hour postdose;
- With the exceptions of fetal brain, fetal spinal cord, and fetal eye, radioactivity concentrations in fetal tissues were lower than the equivalent maternal tissues and were at BLQ by 8 hours postdose;
- Maternal elimination was nearly complete by 24 hours postdose;
- Peak radioactivity concentrations occurred at 1 hour postdose in plasma and blood and at 2 hours postdose in milk. The milk:plasma concentration ratios ranged from 4.02 to 24.8 (13.4 based on $AUC_{0-\infty}$), indicating that ^{14}C-INCB018424-related radioactivity preferentially partitioned into milk of rats.

Study no: INCYTE-DMB-10.50.1
Volume #, and page #: electronic submission, page 1-95
Conducting laboratory and location: (b) (4)

Date of study initiation: March 23, 2010
GLP compliance: yes,
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity: 14C-INCB018424
Lot No.: 65359001
Purity: Chemical purity: 99.6%
Radiopurity: 99.18%
INCB018424
Lot No.: BPR-09-134-B1-12
Purity: 98.9%

Formulation/vehicle: 0.5% methylcellulose in reverse osmosis water

Methods: The tissue distribution and lacteal excretion of 14C-INCB018424-derived radioactivity were assessed following a single oral administration of 14C-INCB018424 to timed-pregnant and lactating female Sprague Dawley (SD) rats. One animal/group/time point was sacrificed at 1, 2, 4, 8, and 24 hours postdose to examine tissue distribution of radioactivity in maternal and fetal tissues on Day 13 (Group 1) and Day 18 (Group 2) of gestation by whole-body autoradiography (WBA). Milk, blood, and plasma were collected from 3 animals/time point (Group 3) at 10

days postpartum and analyzed for concentrations of radioactivity using liquid scintillation counting (LSC) to assess the lacteal excretion of 14C-INCB018424-derived radioactivity.

The following summery table is copied from the sponsor.

Group	Number of Female Animals[a]	Dose Route	Target Dose Level (mg/kg)	Target Dose Volume (mL/kg)	Samples Collected
1[b]	5	Oral	30	10	Blood, Carcass for WBA
2[c]	5	Oral	30	10	Blood, Carcass for WBA
3[d]	15	Oral	30	10	Blood, Plasma, and Milk
1R[b,e]	4	Oral	30	10	Blood, Carcass for WBA

WBA Whole-body autoradiography.
Notes: The dose was approximately 50 µCi/kg.
a Animals were timed-pregnant.
b Animals were dosed on Day 13 of gestation.
c Animals were dosed on Day 18 of gestation.
d Animals were dosed approximately 10 days postpartum (the actual number of days postpartum was recorded in the data).
e Animals in Group 1 sacrificed at 1 and 8 hours postdose were either not pregnant or had few fetuses; data from the Group 1, 8-hour animal was not reported and only the radioanalysis data from the Group 1, 1-hour animal was reported. The Group 1, 1- and 8-hour animals were repeated as Group 1R. Two animals in Group 1R were sacrificed at 1 and 8 hours postdose, respectively.

Dosing:

Species/strain: pregnant Sprague Dawley rats (Hsd:Sprague Dawley SD)
Age: 15-17 weeks
#/sex/group or time point: see the table in Method section
Weight: 234-352 g
Doses in administered units: 30 mg/kg, ~50 µCi
Route, form, volume, and infusion rate: oral gavage at dose volume of 10 mL/kg

Results: the following tables were excerpted from the sponsor's submission

Pharmacokinetic parameters for radioactivity in blood and tissues collected from timed-pregnant rats after a single oral administration of ^{14}C-INCB018424 on Gestation Day 13 (Groups 1 and 1R, 30 mg/kg)

Matrix or Tissue	C_{max} (ng-eq/g)	T_{max} (Hours)	Half-Life (Hours)	AUC_{0-t} (ng-eq·hour/g)	$AUC_{0-\infty}$ (ng-eq·hour/g)
Adrenal gland	11900	1.00	2.41	43770	48538
Amniotic fluid	NC	NC	NC	NC	NC
Amniotic sac	10100	4.00	NC	67460	NC
Aorta	7470	1.00	44.0	59855	150616
Bile	36200	4.00	NC	108850	NC
Blood	2860	1.00	NC	7045	NC
Bone	NC	NC	NC	NC	NC
Bone marrow	3410	1.00	NC	7420	NC
Brain cerebellum	NC	NC	NC	NC	NC
Brain cerebrum	NC	NC	NC	NC	NC
Brain choroid plexus	2090	4.00	NC	4370	NC
Brain medulla	NC	NC	NC	NC	NC
Brain olfactory lobe	NC	NC	NC	NC	NC
Cecum	7860	2.00	7.63	75786	86214
Contents, cecum	547000	8.00	NC	6524220	NC
Contents, esophageal	113000	4.00	NC	150220	NC
Contents, large intestinal	289000	8.00	NC	3289530	NC
Contents, small intestinal	806000	2.00	NC	3219800	NC
Contents, stomach	1440000	1.00	0.484	3399380	3400559
Diaphragm	4240	1.00	1.87	19869	21956
Esophagus	4050	1.00	NC	10420	NC
Exorbital lacrimal gland	4540	1.00	NC	10935	NC
Eye	NC	NC	NC	NC	NC
Eye lens	NC	NC	NC	NC	NC
Eye uveal tract	2730	1.00	NC	4768	NC
Fat (abdominal)	NC	NC	NC	NC	NC
Fat (brown)	2940	1.00	NC	8005	NC
Fetal blood	NC	NC	NC	NC	NC
Fetal brain	NC	NC	NC	NC	NC
Fetal eye	NC	NC	NC	NC	NC
Fetal gastrointestinal tract	NC	NC	NC	NC	NC
Fetal heart	NC	NC	NC	NC	NC
Fetal kidney	NC	NC	NC	NC	NC
Fetal liver	NC	NC	NC	NC	NC
Fetal lung	NC	NC	NC	NC	NC
Fetal muscle	NC	NC	NC	NC	NC
Fetal spinal cord	NC	NC	NC	NC	NC
Fetus	923	1.00	7.97	1634	9808
Harderian gland	5490	1.00	8.10	30806	40883
Intra-orbital lacrimal gland	4470	1.00	NC	10050	NC
Kidney	17500	1.00	1.63	74400	80130
Kidney cortex	17400	1.00	1.67	78150	84568
Kidney medulla	15400	1.00	1.42	66950	70604

NC Not calculated.

Continued from last page

Pharmacokinetic parameters for radioactivity in blood and tissues collected from timed-pregnant rats after a single oral administration of ^{14}C-INCB018424 on Gestation Day 13 (Groups 1 and 1R, 30 mg/kg)

Matrix or Tissue	C_{max} (ng-eq/g)	T_{max} (Hours)	Half-Life (Hours)	AUC_{0-t} (ng-eq·hour/g)	$AUC_{0-\infty}$ (ng-eq·hour/g)
Large intestine	3790	4.00	37.9	51540	135212
Liver	26200	4.00	17.6	187430	252590
Lung	3000	1.00	NC	8175	NC
Lymph nodes	2770	1.00	NC	7260	NC
Mammary gland	2360	1.00	9.35	11250	26495
Muscle	2930	1.00	NC	7870	NC
Myocardium	5220	1.00	NC	13190	NC
Nasal turbinates	1080	4.00	NC	1860	NC
Ovary	1520	1.00	NC	4260	NC
Pancreas	5680	1.00	NC	13630	NC
Pituitary gland	3720	1.00	NC	10555	NC
Placenta	2770	1.00	NC	5825	NC
Preputial gland	6300	1.00	4.57	26700	41746
Salivary gland	4300	1.00	NC	11285	NC
Skin	2480	1.00	11.6	33552	44728
Small intestine	11000	2.00	4.49	67700	103037
Spinal cord	NC	NC	NC	NC	NC
Spleen	3560	1.00	NC	9105	NC
Stomach	4220	1.00	7.73	13470	50392
Thymus	3300	1.00	NC	8125	NC
Thyroid	4280	4.00	NC	25425	NC
Urinary bladder	8350	1.00	NC	31315	NC
Urine	243000	1.00	2.79	794000	929711
Uterus	4360	1.00	NC	8660	NC

NC Not calculated.

Pharmacokinetic parameters for radioactivity in blood and tissues collected from timed-pregnant rats after a single oral administration of ^{14}C-INCB018424 on Gestation Day 18 (Group 2, 30 mg/kg)

Matrix or Tissue	C_{max} (ng-eq/g)	T_{max} (Hours)	Half-Life (Hours)	AUC_{0-t} (ng-eq·hour/g)	$AUC_{0-\infty}$ (ng-eq·hour/g)
Adrenal gland	14600	4.00	NC	67415	NC
Amniotic fluid	NC	NC	NC	NC	NC
Amniotic sac	5770	4.00	25.0	43842	72274
Aorta	9660	1.00	14.0	83755	110019
Bile	84200	1.00	2.09	324250	364363
Blood	3540	1.00	NC	9045	NC
Bone	NC	NC	NC	NC	NC
Bone marrow	4620	1.00	1.58	21022	22552
Brain cerebellum	652	1.00	NC	NC	NC
Brain cerebrum	624	1.00	NC	NC	NC
Brain choroid plexus	NC	NC	NC	NC	NC
Brain medulla	NC	NC	NC	NC	NC
Brain olfactory lobe	855	1.00	NC	NC	NC
Cecum	13900	4.00	NC	25950	NC
Contents, cecum	498000	8.00	NC	5962020	NC
Contents, esophageal	19100	4.00	NC	30956	NC
Contents, large intestinal	470000	8.00	NC	5097340	NC
Contents, small intestinal	642000	2.00	2.58	2811980	2817232
Contents, stomach	1180000	2.00	0.624	3617280	3619658
Diaphragm	5730	1.00	1.95	26230	29406
Esophagus	7140	1.00	1.75	25705	27842
Exorbital lacrimal gland	5850	4.00	1.53	29670	31761
Eye	904	1.00	NC	NC	NC
Eye lens	NC	NC	NC	NC	NC
Eye uveal tract	3690	1.00	NC	7145	NC
Fat (abdominal)	682	4.00	NC	1332	NC
Fat (brown)	4270	1.00	NC	9915	NC
Fetal blood	1770	1.00	NC	4473	NC
Fetal brain	1730	1.00	NC	4539	NC
Fetal eye	2530	4.00	NC	6780	NC
Fetal gastrointestinal tract	2390	1.00	1.26	3080	5593
Fetal heart	3250	4.00	NC	9030	NC
Fetal kidney	2660	1.00	1.25	3425	6191
Fetal liver	3380	4.00	1.65	16806	18298
Fetal lung	2470	4.00	NC	6660	NC
Fetal muscle	2530	1.00	NC	NC	NC
Fetal spinal cord	2020	1.00	NC	5104	NC
Fetus	2260	4.00	NC	6210	NC
Harderian gland	8060	4.00	30.2	60996	103606
Intra-orbital lacrimal gland	6510	1.00	NC	16820	NC
Kidney	19700	1.00	5.40	127086	134071
Kidney cortex	20900	1.00	1.74	91975	100488
Kidney medulla	17400	1.00	2.40	82875	98443

NC Not calculated.

Continued from last page

Pharmacokinetic parameters for radioactivity in blood and tissues collected from timed-pregnant rats after a single oral administration of ^{14}C-INCB018424 on Gestation Day 18 (Group 2, 30 mg/kg)

Matrix or Tissue	C_{max} (ng-eq/g)	T_{max} (Hours)	Half-Life (Hours)	AUC_{0-t} (ng-eq·hour/g)	$AUC_{0-\infty}$ (ng-eq·hour/g)
Large intestine	8970	4.00	4.43	46040	76747
Liver	26200	1.00	7.75	254950	292965
Lung	5270	1.00	NC	13480	NC
Lymph nodes	4480	1.00	1.49	21407	22789
Mammary gland	6840	4.00	4.87	39235	66415
Muscle	4250	1.00	1.67	22439	24346
Myocardium	7430	1.00	1.65	32900	35475
Nasal turbinates	1900	1.00	NC	4249	NC
Ovary	2420	1.00	NC	6635	NC
Pancreas	7060	1.00	1.42	32748	34504
Pituitary gland	7470	1.00	1.66	25762	27583
Placenta	4280	1.00	1.52	20470	21839
Preputial gland	5290	1.00	3.05	10739	13780
Salivary gland	6520	1.00	1.50	28650	30428
Skin	4310	4.00	NC	21705	NC
Small intestine	12700	4.00	4.46	69135	113012
Spinal cord	NC	NC	NC	NC	NC
Spleen	5640	1.00	1.47	24480	25912
Stomach	6770	1.00	2.49	30040	34280
Thymus	4410	1.00	NC	12285	NC
Thyroid	7070	4.00	22.2	76315	129886
Urinary bladder	7720	1.00	0.458	8570	9693
Urine	27100	1.00	2.53	37400	112516
Uterus	4150	1.00	2.04	16063	17948

NC Not calculated.

Concentrations of radioactivity in blood, plasma, and milk as determined by LSC at specified times after a single oral administration of ^{14}C-INCB018424 to lactating female rats (Group 3, 30 mg/kg)

		ng Equivalents ^{14}C-INCB018424/g				
Sample	Time Point	1	2	3	Mean	SD
Blood[a]	1 h	2210	2920	3590	2910	691
Blood[b]	2 h	1960	2080	1970	2000	69.0
Blood[c]	4 h	818	980	1190	996	187
Blood[d]	8 h	275	384	251	303	71.2
Blood[e]	24 h	BLQ	BLQ	BLQ	0.00[f]	0.00
Plasma[a]	1 h	2290	3010	3750	3020	730
Plasma[b]	2 h	2120	2090	2060	2090	28.1
Plasma[c]	4 h	812	1010	1240	1020	212
Plasma[d]	8 h	285	400	237	307	83.6
Plasma[e]	24 h	BLQ	BLQ	BLQ	0.00[f]	0.00
Milk[a]	1 h	10500	10500	15000	12000	2600
Milk[b]	2 h	12600	16700	12800	14000	2280
Milk[c]	4 h	10800	13100	14200	12700	1750
Milk[d]	8 h	7990	6840	6910	7250	648
Milk[e]	24 h	105	132	147	128	21.5

BLQ Below the limit of quantitation. (<49.8 ng equivalents ^{14}C-INCB018424/g, blood)
(<43.2 ng equivalents ^{14}C-INCB018424/g, plasma)
(<51.1 ng equivalents ^{14}C-INCB018424/g, milk)

h Hours.
SD Standard deviation.
a Samples collected from Animal Nos. B20396, B20397, and B20398.
b Samples collected from Animal Nos. B20399, B20400, and B20401.
c Samples collected from Animal Nos. B20402, B20403, and B20404.
d Samples collected from Animal Nos. B20405, B20406, and B20407.
e Samples collected from Animal Nos. B20408, B20409, and B20410.
f BLQ was assigned a value of 0.00 for calculations.

Milk:plasma concentration ratios at specified times after a single oral administration of ^{14}C-INCB018424 to lactating female rats (Group 3, 30 mg/kg)

Time Point	Milk:Plasma Concentration Ratio				
	1	2	3	Mean	SD
1 h[a]	4.59	3.48	4.00	4.02	0.559
2 h[b]	5.97	7.98	6.22	6.72	1.09
4 h[c]	13.3	13.0	11.5	12.6	0.946
8 h[d]	28.0	17.1	29.1	24.8	6.65
24 h[e]	N.A.	N.A.	N.A.	N.A.	N.A.

h Hours.
N.A. Not applicable.
SD Standard deviation.
a Samples collected from Animal Nos. B20396, B20397, and B20398.
b Samples collected from Animal Nos. B20399, B20400, and B20401.
c Samples collected from Animal Nos. B20402, B20403, and B20404.
d Samples collected from Animal Nos. B20405, B20406, and B20407.
e Samples collected from Animal Nos. B20408, B20409, and B20410.

Pharmacokinetic parameters for radioactivity in blood, plasma, and milk, as applicable, collected from timed-pregnant or lactating rats after a single oral administration of ^{14}C-INCB018424 (Groups 1, 1R, 2, and 3, 30 mg/kg)

Matrix	C_{max} (ng-eq/g)	T_{max} (Hours)	Half-Life (Hours)	AUC_{0-t} (ng-eq·hour/g)	$AUC_{0-\infty}$ (ng-eq·hour/g)
		Groups 1 and 1R[a]			
Blood	3140	1.00	2.66	14627	16377
Plasma	3180	1.00	3.73	19637	19890
		Group 2			
Blood	4640	1.00	3.55	27796	28085
Plasma	4570	1.00	3.37	27737	27963
		Group 3[b]			
Blood	2910	1.00	2.22	9504	10475
Plasma	3020	1.00	2.19	9829	10798
Milk	14000	2.00	2.93	144624	145166

a Data from the 1-hour replacement animal (Animal No. B22229, Group 1R) was used to calculate pharmacokinetic parameters.
b Pharmacokinetic parameters were calculated using the mean concentrations obtained from 3 animals/time point.

Metabolism

The in vitro metabolism of INCB018424 by rat liver microsomes and individual rat recombinant cytochrome P450(s)

Key Study Findings:

- Liver microsomes from male rats metabolized INCB018424 *in vitro* to a greater extent than females;
- INCB018424 was metabolized by the following isozymes in both female and male rats; CYP1A2, CYP2C6 and CYP2D1
- INCB018424 was metabolized extensively by the male specific rat CYP isozymes CYP2C11, CYP2C13 and CYP3A2.

Study number: INCYTE-DMB-07.01.2-153
Volume #, and page #: Electronic submission; page 108
M4/42-stud-rep/422-pk/4224-metab/ncl-153.pdf, page 1-43
Conducting laboratory and location: Incyte Corporation
Date of study initiation: August 7, 2006
GLP compliance: no,
QA report: yes (x) no (x)
Drug, lot #, radiolabel, and % purity: INCB018424
Lot SS-IN2-175
Purity: not provided
Formulation/vehicle: 50 mM potassium phosphate buffer (pH 7.4), 2mM NADPH,
Methods:

Metabolism by Rat Liver Microsomes

INCB018424 was incubated in triplicate with either female or male rat liver microsomes at 37°C. Each incubation contained 0.5 mg/mL of the either female (pool of 10 rats) or male (pool of 10 rats) rat liver microsome, 50 mM potassium phosphate buffer (pH 7.4), 2mM NADPH, and INCB018424 (1 µM). Aliquots were taken at 0, 10, 20 and 30 minutes, deproteinized with acetonitrile and stored at -20°C until analysis.

Metabolism by Individual Rat Recombinant CYP Isozymes

INCB018424 was incubated in triplicate with individual rat cytochrome P-450 (CYP) isozymes at 37°C. Each incubation contained 5 pmols of the individual isozyme, 50 mM potassium phosphate buffer (pH 7.4), 2mM NADPH, and INCB018424 (1 µM). Aliquots were taken at 0, 30 and 60 minutes, deproteinized with acetonitrile and stored at -20^{o}C until analysis.

Results: The Applicant only presented the summary table. The following summary tables are excerpted from the Applicant's submission.

Extrapolated *In Vivo* Parameters Based on the *In Vitro* Rate of Metabolism of INCB018424 by Rat Liver Microsomes

	Extraploted *In Vivo* Parameter	
Gender	CL (L/h/kg)	Heptic Extraction Ratio
Female	1.02	0.35
Male	2.54	0.88

The *In Vitro* Metabolism of INCB018424 by Individual Rat Recombinant Cytochrome P450s

		Percent of INCB018424 Remaining	
CYP Isozyme	Gender	0 minutes	60 minutes
CYP1A2	Male/Female	100	5 ± 1
CYP2B1	Male/Female	100	102 ± 3
CYP2C6	Male/Female	100	1 ± 0
CYP2C11	Male	100	2 ± 1
CYP2C12	Female	100	101 ± 4
CYP2C13	Male	100	78 ± 4
CYP2D1	Male/Female	100	42 ± 1
CYP3A2	Male	100	2 ± 1

INCB018424: Material Balance and Metabolism in Male Rats

Key Study Findings:

- The route and extent of elimination was similar between orally and intravenously dosed rats with urine being the most significant route (~50%);
- INCB018424 was also excreted via bile and feces;
- Elimination was rapid; most of the dose was eliminated within 12 hrs of dosing (92% and 87%, respectively);
- The metabolic profile was similar between dosing routes;
- Metabolite breakdown was extensive with very few single metabolites accounting for more than 5% of the dose.

Study number: (b) (4)-22603; INCYte-DMB-08.61.1
Volume #, and page #: Electronic submission; page 1-82
Conducting laboratory and location: (b) (4)

Date of study initiation: March 20, 2007
GLP compliance: no,
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: INCB018424
Lot SS-IN2-175
Purity: not provided
[^{14}C]INCB018424
Lot 3562192
Purity: 99% (radiochemical purity)
Formulation/vehicle: 50 mM potassium phosphate buffer (pH 7.4), 2mM NADPH,
Methods: INCB018424 was administered by the oral and intravenous routes to male Crl:CD(SD) rats. Dose solutions were prepared as follows:

Dose Route	Dose Level (mg/kg bw)	Dose Volume (mL/kg bw)	Radiochemical Dose (µCi/kg bw)	Specific Activity in Dose (µCi/mg)	Chemical Concentration (mg/mL)	Radiochemical Concentration (µCi/mL)
Oral	50	10	600	12	5	60
Intravenous	10	5	300	30	2	60

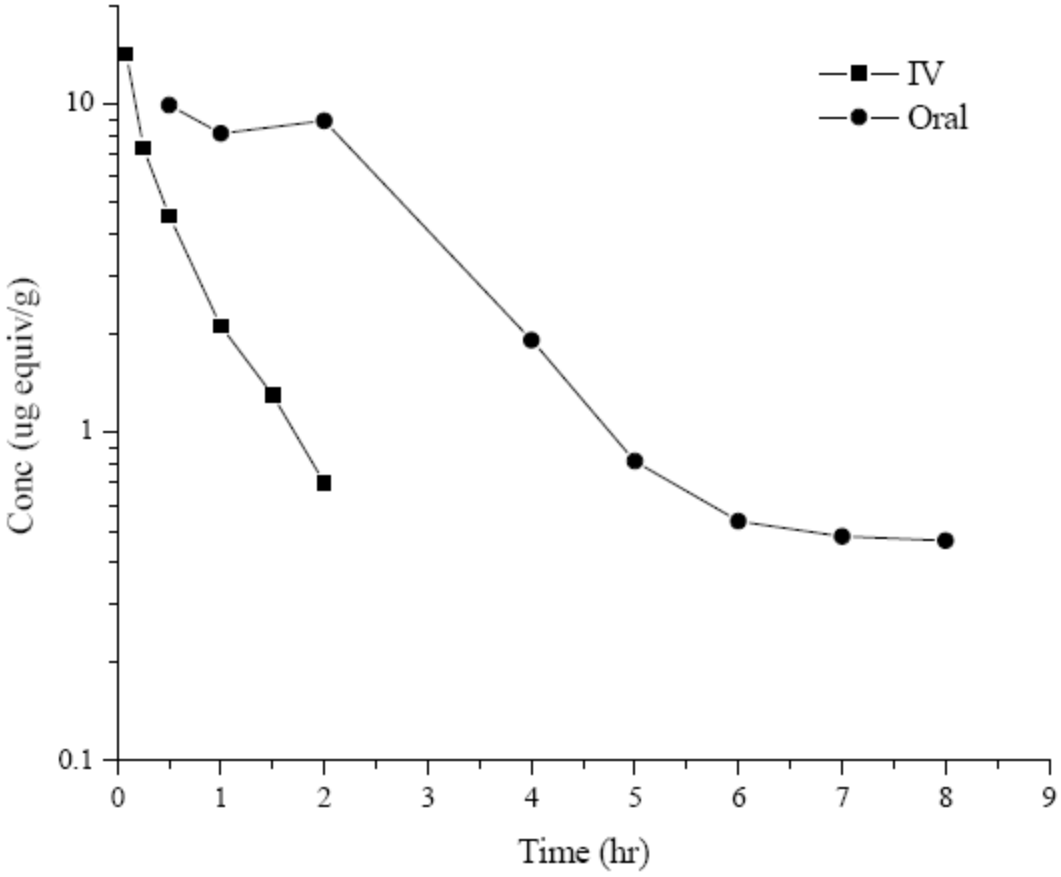
Study design is summarized in the following table:

Experiment	Dose Route	Dose Level (mg/kg bw)	Number of Animals	Time of Sacrifice (hours)	Samples
Mass Balance and Pharmacokinetics	Oral	50	4M	96	Urine, Feces, Bile, Carcass, Cage Wash, Metabolite profile in selected samples
	Intravenous	10	4M	96	
Metabolite Identification	Oral	50	2M	3	Metabolite profile in plasma

Following dosing, rats were housed individually in metabolism units suitable for the collection of urine and feces up to a maximum of 4 days. Blood samples were collected pre-dose, and at 5, 15, 30, 60, 90, and 120 minutes post-dose for the intravenous group, and 0.5, 1, 2, 4, 5, 6, 7, and 8 hours post-dose for the oral group. Urine, feces, bile and/or plasma samples were analyzed by HPLC and mass spectroscopy to determine the molecular weight of any radioactively labeled metabolites.

Results: the following figures and tables are excerpted from the Applicant's submission.

Total ^{14}C Concentration in Plasma Following Oral or Intravenous Dosing



Pharmacokinetic Parameters

Nominal Dose		Half-Life	Tmax	Cmax	Observed AUC
Oral (50 mg/kg)	Mean	1.338	1.25	11.342	30.325
	SD	0.257	0.866	2.913	9.03
IV (10 mg/kg)	Mean	0.579	0.083	58.557	13.002
	SD	0.072	0	88.633	7.45

Percent Recovery

Sample		Oral Mean	Oral SD	IV Mean	IV SD
urine	Predose	N.A.	N.A.	N.A.	N.A.
urine	4 h	28.813	7.697	35.172	5.712
urine	8 h	17.05	9.136	9.009	2.679
urine	12 h	4.089	2.39	1.601	1.034
urine	24 h	1.6	0.771	2.553	1.102
urine	48 h	0.387	0.261	0.303	0.157
urine	72 h	0.067	0.038	0.102	0.085
urine	96 h	0.025	0.014	0.021	0.008
	Subtotal[a]	52.03	4.285	48.76	5.548
feces	Predose	N.A.	N.A.	N.A.	N.A.
feces	4 h	N.A.	N.A.	N.A.	N.A.
feces	8 h	1.302	1.018	N.A.	N.A.
feces	12 h	4.788	4.51	5.079	0.81
feces	24 h	6.465	2.91	7.845	1.978
feces	48 h	0.583	0.405	1.18	1.085
feces	72 h	0.206	0.227	0.1	0.055
feces	96 h	0.11	0.09	0.163	0.265
	Subtotal[a]	11.931	0.916	11.826	0.766
bile	Predose	N.A.	N.A.	N.A.	N.A.
bile	4 h	29.321	5.674	34.691	1.155
bile	8 h	4.594	1.545	0.943	0.177
bile	12 h	1.723	2.262	0.325	0.015
bile	24 h	0.867	0.412	0.383	0.137
bile	48 h	0.121	0.035	0.122	0.03
bile	72 h	0.042	0.016	0.031	0.003
bile	96 h	0.019	0.006	0.024	0.014
	Subtotal[a]	36.687	5.778	36.518	1.122
cage wash	24 h	1.042	0.618	6.501	3.741
cage wash	48 h	0.382	0.312	0.535	0.566
cage wash	72 h	0.068	0.048	0.201	0.09
cage wash	96 h	0.08	0.024	0.366	0.219
	Subtotal[a]	1.571	0.885	7.368	4.072
	Total	102.22	1.312	104.47	4.709

a The subtotals are the average of the subtotals from each animal, not the sum of the values listed here. Please see appendix data for individual values.

Metabolites observed in pooled bile, urine, and feces after oral and intravenous dosing

Designation	RT (min)[a]	m/z	Metabolite description	Matrix observed[b]
M1	3.25	357	Tri-hydroxylation and reduction	U
M2	4.25	357	Tri-hydroxylation and reduction	U (IV)
M3	4.25	339	Di-hydroxylation of cyclopentyl	U (oral)
M4	4.75	339	Di-hydroxylation of cyclopentyl	U
M5	4.75	499	*O*-glucuronidation of 3-cyclopentyl	U (oral), B
M6	5.25	499	*O*-glucuronidation of 3-cyclopentyl	B
M7 (INCB025257)	5.42	323	3-hydroxylation of cyclopentyl	U, B, F
M9 (INCB025255)	5.75	321	3-ketone on cyclopentyl	U, B (oral), F (oral)
M10	5.75	499	*O*-glucuronidation of 3-cyclopentyl	B
M11 (INCB025256)	5.92	321	3-ketone on cyclopentyl	U, F
M12	5.92	325	2-hydroxylation of cyclopentyl and reduction	U (oral)
M13	5.92	339	Dihydroxylation	U
M14 (INCB027596)	5.92	323	2-hydroxylation of cyclopentyl	U (IV), F
M15	6.25	499	*O*-glucuronidation of 3-cyclopentyl	B (oral)
M16 (INCB025264)	6.75	323	3-hydroxylation of cyclopentyl	U, F (oral)
M17	6.75	499	*O*-glucuronidation of 3-cyclopentyl	U, B
M18 (INCB027598)	7.25	323	2-hydroxylation of cyclopentyl	U
M19	7.25	341	Di-hydroxylation of pyrazol or pyrolopyrimidine and reduction	U
M20	8.08	339	Hydroxylation on cyclopentyl and pyrazol or pyrolopyrimidine	U
M21	8.42	339	Hydroxylation on cyclopentyl and pyrazol or pyrolopyrimidine	U
M22	8.42	403	Sulfate of 3-hydroxylated cyclopentyl	U (IV)
18424	10.3	307	None	U, F

a approximate retention time based on radiochromatograms for urine under experimental conditions
b Unless noted in parentheses, metabolite observed after oral and IV dosing for the indicated matrix
U = urine
B = bile
F = feces

Percent of peaks and metabolite identification of pooled bile, urine, and feces after oral dosing

Bile			Urine			Feces		
Run Time (min)	Peak ID	% of Dose	Run Time (min)	Peak ID	% of Dose	Run Time (min)	Peak ID	% of Dose
			3.25	M1	4.88			
			4.25	M3	1.58			
4.917	M5	1.91	4.75	M4,M5	2.25			
5.25	M6, M7	1.99	5.417	M7	14.76	5.583	M7	4.03
5.75	M9,M10	2.49	5.917	M9,M11,M12,M13	14.05	5.917	M9	1.05
6.25	M15	3.12	6.75	M16,M17	1.98	6.25	M11,M14	2.60
6.75	M17	8.45	7.25	M18,M19	1.15	6.917	M16	0.77
			8.083	M20	1.82	10.583	18424	0.55
			8.417	M21	1.54			
			10.25	18424	0.15			

Percent of peaks and metabolite identification of pooled bile, urine, and feces after intravenous dosing

Bile			Urine			Feces		
Run Time (min)	Peak ID	% of Dose	Run Time (min)	Peak ID	% of Dose	Run Time (min)	Peak ID	% of Dose
			3.25	M1	2.40			
4.917	M5	2.78	4.25	M2	1.42	5.583	M7	4.87
5.25	M6,M7	2.33	4.75	M4	2.38	6.25	M11,M14	2.11
5.75	M10	2.72	5.417	M7	9.93	10.583	18424	0.34
6.75	M17	6.11	5.917	M9,M11,M13,M14	9.90			
			6.75	M16,M17	2.17			
			7.25	M18,M19	2.94			
			8.083	M20	1.23			
			8.417	M21,M22	1.31			
			10.25	18424	1.99			

Identification of rat plasma metabolites of INCB018424 after a single oral dose of ^{14}C-INCB018424

Key study findings:

- INCB018424 was present in plasma from the 1 and 4 hour timepoints, with 12 radiochromatographic peaks related to INCB018424 observed;
- A total of seven metabolites of INCB018424 were identified and confirmed using previously isolated or synthesized metabolites of INCB018424**;**
- The two most abundant circulating plasma metabolites were INCB025258 (M8), a 3-hydroxyl metabolite of the cyclopentyl ring and INCB025256 (M11), a 3-oxocyclopentyl metabolite.

Study no: INCYTE-DMB-08.169.1
Volume #, and page #: electronic submission, page 1-43
Conducting laboratory and location: INCYTE
Date of study initiation: September 13, 2008
GLP compliance: no,
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: INCB018424
Lot SS-IN2-175
Purity: not provided
^{14}C-INCB018424
Lot 3562192
Purity: not provided
Formulation/vehicle: not provided
Methods: Plasma samples from male rats orally dosed with 25 mg/200 µCi/kg ^{14}C-INCB018424 were obtained on day 1 of a single dose tissue distribution study conducted at (b) (4) study # 59N-070. Residual plasma samples collected 1, 4 and 24 h post-dose from each rat were pooled by time point. Samples were assayed using electrospray ionization LC-MS with a Thermo Finnigan LCQ Deca-XP Plus Ion-Trap Mass Spectrometer (Thermo-Fisher Scientific Waltham MA), operated in positive ionization mode.

Source of Study Samples for Biotransformation Experiments

Subject ID	Study #	Total Dose	^{14}C Dose	Time Point
1 and 2	59N-0701	25 mg/kg	200 µCi/kg	1 Hour
3 and 4	59N-0701	25 mg/kg	200 µCi/kg	4 Hour
5 and 6	59N-0701	25 mg/kg	200 µCi/kg	24 Hour

Results:

Summary Table of Metabolites of INCB018424 Found in Male Rat Plasma at 1 Hour Post-Dose

1 Hour Post-Dose Sample	RT	m/z	Average DPM	% of Parent	Plasma DPM/Gram	Plasma uCi/gram	Plasma ug/gram
Metabolite M41	7.4	339	1577	110	2896	0.00130	0.1819
Metabolite M43	10.7	339	639	43	1172	0.00053	0.0737
INCB025257 (M7)	13.8	323	2225	150	4084	0.00184	0.2566
INCB025258 (M8)	14.7	323	4201	280	7713	0.00347	0.4846
INCB025255 (M9)	17.9	321	3102	210	5696	0.00257	0.3579
INCB025264 (M16)	20.1	323	1501	100	2756	0.00124	0.1732
INCB025256 (M11)	21.0	321	4046	270	7428	0.00335	0.4667
INCB032568 (M48)	27.5	323	1067	72	1958	0.00088	0.1230
INCB027598 (M18)	32.8	323	387	26	711	0.00032	0.0446
Metabolite M52	45.5	312	385	26	707	0.00032	0.0444
Metabolite M53	51.2	305	599	40	1100	0.00050	0.0691
INCB018424	52.0	307	1492	100	2739	0.00123	0.1721
		Sum =	**22202**				

Sample Mass Extracted (g)	2.014
Extraction Efficiency (%)	90.14
Recon Volume (µL)	100
Injection Volume (µL)	30
Mass Eq. of Plasma on Column (g)	0.545

Specific Activity of Dose = 7.169 uCi/mg
0.00717 uCi/ug

Summary Table of Metabolites of INCB018424 Found in Male Rat Plasma at 4 Hours Post-Dose

4 Hour Post-Dose Sample	RT	m/z	Average DPM	% of Parent	Plasma DPM/Gram	Plasma uCi/gram	Plasma ug/gram
Metabolite M41	7.4	339	237	6800	435	0.00020	0.0273
Metabolite M43	10.7	339	61	1700	112	0.00005	0.0071
INCB025257 (M7)	13.8	323	142	4100	261	0.00012	0.0164
INCB025258 (M8)	14.7	323	538	15000	989	0.00045	0.0621
INCB025255 (M9)	17.9	321	297	8500	545	0.00025	0.0343
INCB025264 (M16)	20.1	323	171	4900	315	0.00014	0.0198
INCB025256 (M11)	21.0	321	535	15000	984	0.00044	0.0618
INCB032568 (M48)	27.5	323	174	5000	320	0.00014	0.0201
INCB027598 (M18)	32.8	323	70	2000	129	0.00006	0.0081
Metabolite M52	45.5	312	0	0	0	0.00000	0.0000
Metabolite M53	51.2	305	18	510	33	0.00001	0.0021
INCB018424	52.0	307	4	100	6	0.00000	0.0004
		Sum =	**2245**				

Sample Mass Extracted (g)	2.01
Extraction Efficiency (%)	90.14
Recon Volume (µL)	100
Injection Volume (µL)	30
Mass Eq. of Plasma on Column (g)	0.544

Specific Activity of Dose = 7.169 uCi/mg
0.00717 uCi/ug

Identification of in vivo metabolites of INCB018424 in female rat following a single oral dose of ^{14}C-INCB018424

Key study findings:

- INCB018424 was present in plasma from female rat at the 1, 2, and 4 hour timepoints, with a total of 18 radiochromatographic peaks related to INCB018424 observed;
- Concentrations of metabolites in plasma were low (less than 0.8 µg) equivalents/g in all cases;
- There were eight circulating plasma metabolites present at 1 or 2 hours post-dose at levels that were >10% of parent (based on radiochemical detection) in order of decreasing amount: INCB025258 (M8); INCB025256 (M11); INCB018424 (M7),
- Structures for INCB025257 (M7); INCB032568 (M48); INCB025264(M16); INCB027598 (M18); INCB025262 (M27); and M35 have not been elucidated;
- The overall metabolic profile is similar in males and females, with the three most abundant metabolites of INCB025258 (M8), INCB025256 (M11), and INCB025257 (M7), in decreasing amount, respectively.

Study no: INCYTE-DMB-08.169.1

Volume #, and page #: electronic submission, page 1-120
Conducting laboratory and location: INCYTE
Date of study initiation: September 22, 2009
GLP compliance: no,
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: INCB018424
Lot BPR-07-101-B2-21
Purity: not provided
^{14}C-INCB018424
Lot 65359001
Purity: not provided
Formulation/vehicle: not provided
Methods:

Source of Study Samples for Biotransformation Experiments

Species	Gender	Dose / Route	Study # / Group	Matrix
Rat (intact)	F, M	25 mg/kg PO	59N-0901 / 2, 3	Plasma
Rat (bile duct-cannulated)	F	25 mg/kg PO	59N-0901 / 1	Bile, Urine, Feces

Plasma, bile, urine, feces obtained from either male or female Sprague-Dawley rats were prepared by pooling each sample obtained from individual animals at the following collection time points:
Plasma: 1, 2, 4, 8, 24, hours for females; 1, and 4 hours for male rats
Urine, bile and feces: 0-8, 8-24, 24-48, 48-72, 72-96 hours for female rats

Samples were assayed using electrospray ionization LC-MS with a Thermo Finnigan
LCQ Deca-XP Plus Ion-Trap Mass Spectrometer (Thermo-Fisher Scientific Waltham MA), operated in positive ionization mode.

Results:

Quantitative Summary (as % of Parent Present and µg equivalent/g) of Metabolites of ^{14}C-INCB018424 Found in Female Rat Plasma Following a Single 25 mg/kg Oral Dose

Metabolite	RT	m/z	1 Hour		2 Hour		4 Hour	
			% of Parent	µg/gram	% of Parent	µg/gram	% of Parent	µg/gram
Unknown	7.3	Unk	0.6	0.009	1.31	0.02	104.56	0.04
Unknown	8.0	Unk	2.6	0.035	2.60	0.03	57.40	0.02
M1	8.1	357	N/F	N/F	N/F	N/F	N/F	N/F
M2	12.2	357	N/F	N/F	N/F	N/F	N/F	N/F
M54	12.3	367	3.3	0.044	4.26	0.06	79.92	0.03
Di-Hydroxyl (m/z 339)	13.5	339	3.5	0.047	2.53	0.03	48.76	0.02
M2-B	14.4	357	N/F	N/F	N/F	N/F	N/F	N/F
M10	15.4	499	N/F	N/F	N/F	N/F	N/F	N/F
Unknown	16.6	Unk	1.4	0.020	2.28	0.03	22.30	0.01
M15	17.2	499	N/F	N/F	N/F	N/F	N/F	N/F
M3	17.7	339	N/F	N/F	N/F	N/F	N/F	N/F
Quad-Hydroxyl (m/z 371)	18.0	371	4.6	0.062	5.80	0.08	84.36	0.04
INCB025257 (M7)	19.9	323	12.3	0.166	15.93	0.21	102.42	0.04
M4	20.9	339	N/F	N/F	N/F	N/F	N/F	N/F
M53	21.1	305	N/F	N/F	N/F	N/F	N/F	N/F
INCB025255 (M9)	21.9	321	6.6	0.089	8.49	0.11	63.28	0.03
INCB025258 M8)	24.0	323	58.6	0.795	46.00	0.62	374.20	0.16
M25	24.3	499	N/F	N/F	N/F	N/F	N/F	N/F
INCB025256 (M11)	26.0	321	32.8	0.445	39.79	0.53	416.45	0.18
M35	26.9	355	9.5	0.128	9.44	0.13	63.14	0.03
INCB025264 (M16)	27.9	323	11.1	0.151	7.22	0.10	27.51	0.01
M19	28.6	341	N/F	N/F	N/F	N/F	N/F	N/F
INCB025262 (M27)	29.6	323	9.8	0.133	9.44	0.13	168.91	0.07
M26	31.2	499	N/F	N/F	N/F	N/F	N/F	N/F
Oxo Metabolite (m/z 321)	31.6	321	0.4	0.005	N/F	N/F	0.98	0.00
M20	35.6	339	N/F	N/F	N/F	N/F	N/F	N/F
M28	36.9	499	N/F	N/F	N/F	N/F	N/F	N/F
M21	38.0	339	N/F	N/F	N/F	N/F	N/F	N/F
INCB032568 (M48)	38.8	323	11.9	0.161	14.46	0.19	103.34	0.04
Glycosylation of 323	42.5	485	3.4	0.047	2.52	0.03	77.82	0.03
INCB027598 (M18)	43.0	323	10.6	0.144	7.87	0.11	64.52	0.03
M50-B	57.1	323	6.6	0.090	5.17	0.07	187.02	0.08
INCB018424	64.7	307	100.0	1.356	100.00	1.34	100.00	0.04

* Quantitative data for the metabolism of INCB018424 in Male Rat Plasma for this study may be found in Table 10, and Appendix A, Tables 19 and 20.

** No drug related material was radiochemically detected in the 8 and 24 hour post-dose plasma samples.

Quantitative Summary (as % of Administered Radioactivity) of Metabolites of ^{14}C-INCB018424 Found in Female Rat Excreta Following a Single 25 mg/kg Oral Dose

Metabolite	RT	m/z	Urine	Bile	Feces	Total
Unknown	7.3	Unk	0.39	1.63	N/F	**2.02**
Unknown	8.0	Unk	0.10	N/F	1.29	**1.39**
M1	8.1	357	1.06	1.60	N/F	**2.65**
M2	12.2	357	0.43	0.91	N/F	**1.34**
M54	12.3	367	N/F	N/F	N/F	**N/F**
Di-Hydroxyl (m/z 339)	13.5	339	N/F	N/F	N/F	**N/F**
M2-B	14.4	357	1.02	0.52	N/F	**1.54**
M10	15.4	499	N/F	1.16	N/F	**1.16**
Unknown	16.6	Unk	N/F	0.19	N/F	**0.19**
M15	17.2	499	N/F	3.38	N/F	**3.38**
M3	17.7	339	2.38	N/F	N/F	**2.38**
Quad-Hydroxyl (m/z 371)	18.0	371	N/F	1.83	N/F	**1.83**
INCB025257 (M7)	19.9	323	2.44	1.26	2.63	**6.33**
M4	20.9	339	1.42	N/F	0.69	**2.11**
M53	21.1	305	N/F	1.34	N/F	**1.34**
INCB025255 (M9)	21.9	321	2.26	0.64	0.48	**3.38**
INCB025258 M8)	24.0	323	7.89	N/F	5.46	**13.35**
M25	24.3	499	N/F	5.07	N/F	**5.07**
INCB025256 (M11)	26.0	321	8.59	2.42	1.04	**12.05**
M35	26.9	355	N/F	N/F	N/F	**0.00**
INCB025264 (M16)	27.9	323	1.54	0.47	1.05	**3.06**
M19	28.6	341	4.88	1.73	0.90	**7.51**
INCB025262 (M27)	29.6	323	2.62	N/F	2.29	**4.92**
M26	31.2	499	N/F	1.44	N/F	**1.44**
Oxo Metabolite (m/z 321)	31.6	321	0.12	0.27	N/F	**0.39**
M20	35.6	339	1.15	N/F	N/F	**1.15**
M28	36.9	499	N/F	8.30	N/F	**8.30**
M21	38.0	339	1.37	N/F	N/F	**1.37**
INCB032568 (M48)	38.8	323	1.03	N/F	0.51	**1.54**
Glycosylation of 323	42.5	485	N/F	N/F	N/F	**0.00**
INCB027598 (M18)	43.0	323	0.27	N/F	0.18	**0.45**
M50-B	57.1	323	1.42	N/F	N/F	**1.42**
INCB018424	64.7	307	0.05	0.04	0.26	**0.35**

Quantitative Summary of Metabolites of ^{14}C-INCB018424 (as µg equivalents/g and % of Administered Dose) Found in Female Rat Urine Following a Single 25 mg/kg Oral Dose (0-24 Hour)

Metabolite	RT	m/z	(µg/g equivalents) 0-8 Hr	(µg/g equivalents) 8-24 Hr	% of Dose 0-24 Hrs
Unknown	7.3	Unk	4.570	0.220	0.4
Unknown	8.0	Unk	N/F	0.557	0.1
M1	8.1	357	13.578	N/F	1.1
M2	12.2	357	5.556	N/F	0.4
M54	12.3	367	N/F	N/F	N/F
Di-Hydroxyl (m/z 339)	13.5	339	N/F	N/F	N/F
M2-B	14.4	357	13.061	N/F	1.0
M10	15.4	499	N/F	N/F	N/F
Unknown	16.6	Unk	N/F	N/F	N/F
M15	17.2	499	N/F	N/F	N/F
M3	17.7	339	26.758	1.688	2.4
Quad-Hydroxyl (m/z 371)	18.0	371	N/F	N/F	N/F
INCB025257 (M7)	19.9	323	25.680	2.482	2.4
M4	20.9	339	18.213	N/F	1.4
M53	21.1	305	N/F	N/F	N/F
INCB025255 (M9)	21.9	321	26.059	1.298	2.3
INCB025258 M8)	24.0	323	91.592	4.249	7.9
M25	24.3	499	N/F	N/F	N/F
INCB025256 (M11)	26.0	321	98.392	5.235	8.6
M35	26.9	355	N/F	N/F	N/F
INCB025264 (M16)	27.9	323	17.513	0.968	1.5
M19 (DiHydrodiol)	28.6	341	62.120	0.196	4.9
INCB025262 (M27)	29.6	323	29.400	1.887	2.6
M26	31.2	499	N/F	N/F	N/F
Oxo Metabolite (m/z 321)	31.6	321	1.087	0.198	0.1
M20	35.6	339	13.015	0.757	1.1
M28	36.9	499	N/F	N/F	N/F
M21	38.0	339	15.000	1.146	1.4
INCB032568 (M48)	38.8	323	11.317	0.815	1.0
Glycosylation of m/z 323	42.5	485	N/F	N/F	N/F
INCB027598 (M18)	43.0	323	3.308	0.077	0.3
M50-B	57.1	323	14.882	1.457	1.4
INCB018424	64.7	307	0.662	N/F	0.1

Quantitative Summary of Metabolites of ^{14}C-INCB018424 (as µg equivalents/g and % of Administered Dose) Found in Female Rat Bile Following a Single 25 mg/kg Oral Dose (0-24 Hour)

Metabolite	RT	m/z	(µg/g equivalents) 0-8 Hr	8-24 Hr	% of Dose 0-24 Hrs
Unknown	7.3	Unk	13.360	N/F	1.6
Unknown	8.0	Unk	N/F	N/F	N/F
M1	8.1	357	13.117	N/F	1.6
M2	12.2	357	7.443	N/F	0.9
M54	12.3	367	N/F	N/F	N/F
Di-Hydroxyl (m/z 339)	13.5	339	N/F	N/F	N/F
M2-B	14.4	357	4.286	N/F	0.5
M10	15.4	499	9.574	N/F	1.2
Unknown	16.6	Unk	N/F	0.376	0.2
M15	17.2	499	27.812	N/F	3.4
M3	17.7	339	N/F	N/F	N/F
Quad-Hydroxyl (m/z 371)	18.0	371	15.029	N/F	1.8
INCB025257 (M7)	19.9	323	10.383	N/F	1.3
M4	20.9	339	N/F	N/F	N/F
M53	21.1	305	11.006	N/F	1.3
INCB025255 (M9)	21.9	321	5.238	N/F	0.6
INCB025258 M8)	24.0	323	N/F	N/F	N/F
M25	24.3	499	38.279	0.833	5.1
INCB025256 (M11)	26.0	321	19.917	N/F	2.4
M35	26.9	355	N/F	N/F	N/F
INCB025264 (M16)	27.9	323	3.891	N/F	0.5
M19	28.6	341	14.213	N/F	1.7
INCB025262 (M27)	29.6	323	N/F	N/F	N/F
M26	31.2	499	11.300	0.131	1.4
Oxo Metabolite (m/z 321)	31.6	321	N/F	0.547	0.3
M20	35.6	339	N/F	N/F	N/F
M28	36.9	499	66.573	0.403	8.3
M21	38.0	339	N/F	N/F	N/F
INCB032568 (M48)	38.8	323	N/F	N/F	N/F
Glycosylation of 323	42.5	485	N/F	N/F	N/F
INCB027598 (M18)	43.0	323	N/F	N/F	N/F
M50-B	57.1	323	N/F	N/F	N/F
INCB018424	64.7	307	0.349	N/F	0.0

Quantitative Summary of Metabolites of ^{14}C-INCB018424 (as µg equivalents/g and % of Administered Dose) Found in Female Rat Feces Following a Single 25 mg/kg Oral Dose (0-24 Hour)

Metabolite	RT	m/z	(µg/g equivalents) 0-24 Hr	% of Dose 0-24 Hrs
Unknown	7.3	Unk	N/F	N/F
Unknown	8.0	Unk	1.966	1.3
M1	8.1	357	N/F	N/F
M2	12.2	357	N/F	N/F
M54	12.3	367	N/F	N/F
Di-Hydroxyl (m/z 339)	13.5	339	N/F	N/F
M2-B	14.4	357	N/F	N/F
M10	15.4	499	N/F	N/F
Unknown	16.6	Unk	N/F	N/F
M15	17.2	499	N/F	N/F
M3	17.7	339	N/F	N/F
Quad-Hydroxyl (m/z 371)	18.0	371	N/F	N/F
INCB025257 (M7)	19.9	323	3.992	2.6
M4	20.9	339	1.045	0.7
M53	21.1	305	N/F	N/F
INCB025255 (M9)	21.9	321	0.734	0.5
INCB025258 M8)	24.0	323	8.299	5.5
M25	24.3	499	N/F	N/F
INCB025256 (M11)	26.0	321	1.576	1.0
M35	26.9	355	N/F	N/F
INCB025264 (M16)	27.9	323	1.599	1.1
M19	28.6	341	1.372	0.9
INCB025262 (M27)	29.6	323	3.483	2.3
M26	31.2	499	N/F	N/F
Oxo Metabolite (m/z 321)	31.6	321	N/F	N/F
M20	35.6	339	N/F	N/F
M28	36.9	499	N/F	N/F
M21	38.0	339	N/F	N/F
INCB032568 (M48)	38.8	323	0.779	0.5
Glycosylation of 323	42.5	485	N/F	N/F
INCB027598 (M18)	43.0	323	0.269	0.2
M50-B	57.1	323	N/F	N/F
INCB018424	64.7	307	0.389	0.3

Identification of in vivo metabolites of INCB018424 in beagle dogs

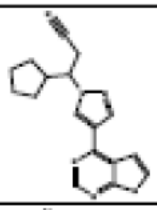
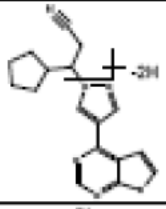
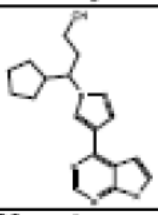
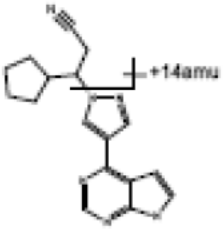
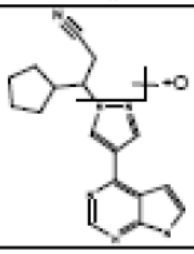
Key study findings:

- INCB018424 underwent extensive metabolism, and the major metabolites were derived from oxidation and subsequent glucuronidation.

Study no: INCYTE-DMB-07.14.1
Volume #, and page #: electronic submission, page 1-48
Conducting laboratory and location: INCYTE
Date of study initiation: January 7, 2007
GLP compliance: no,
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: INCB0018424
Lot # SS-IN2-175
Formulation/vehicle: not provided
Methods: The plasma and urine samples were obtained from the beagle dogs following a single intravenous administration of INCB018424 at 5 mg/kg (Report: INCYTE-DMB-06.182.1). The residual plasma samples from this pharmacokinetic study were pooled from individual dogs and time points to generate samples of ample volume to characterize the metabolic profile. Samples were analyzed by liquid chromatography tandem mass spectrometry (LC/MS/MS).

Results:

Metabolites Observed in Beagle Dog Plasma and Urine Samples

$[MH]^+$	Inference	Beagle dog Plasma t_R (min)	Beagle dog Urine t_R (min)
307 INCB018424 Parent drug		23.3	23.5
M1 *m/z* 305 (-2 amu)		22.6 22.8	22.6 22.8
M3 *m/z* 312 (+5 amu)		19.8 22.5	22.3
M4 *m/z* 314 (+7 amu)	No assignment	x	10.3 12.0 13.0 15.8 16.4 16.6
M5 *m/z* 321 +14 amu		12.7 14.3 15.2 17.2 18.8	11.2 13.1 14.8 15.8 16.3 17.8 19.6
M6a *m/z* 323 +16 amu		13.3 13.7 14.9 15.5 17.3 18.6 18.9	13.7 14.1 15.8 18.0 19.2 19.6

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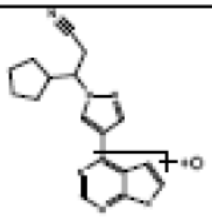
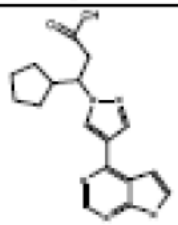
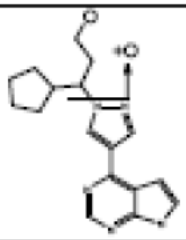
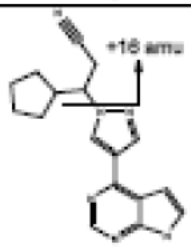
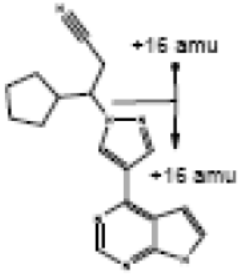
M6b *m/z* 323 +16 amu		22.8 23.9	21.5 23.1
M9 *m/z* 326 +19 amu		x	trace
M10 *m/z* 328 +21 amu		x	11.4 13.2 16.3 16.9
M12a *m/z* 339 [+32 amu]		9.4 10.4 10.6 11.2 11.4 11.8 14.2	7.4 9.7 10.3 10.7 10.9 12.6 14.8
M12b *m/z* 339 [+32 amu]		15.1 15.5 17.1 19.3 20.3 22.2	13.8 14.4 15.4 15.8 17.4 18.9 19.2 19.4 20.2 20.4

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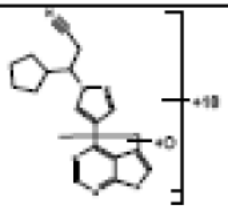
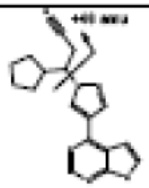
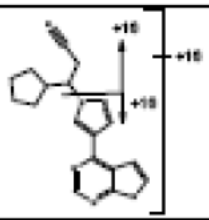
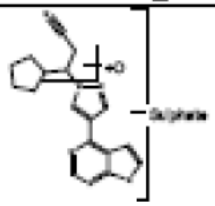
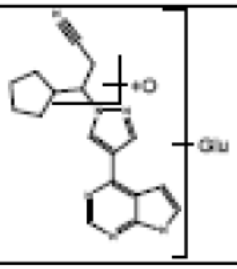
M13 m/z 341 +34 amu		18.3 18.8	18.5 18.8 19.0
M14 m/z 353 [+46 amu]		x	11.2 17.6 20.4
M15 m/z 355 [+48 amu]		x	11.2 12.2 12.4 12.9
M16 m/z 403 [+16 +80 amu]		13.2 21.3	10.8 11.9 12.7 14.1 14.7 17.0 21.5
M17 m/z 483 [+176 amu]	Glucuronide of Parent Drug	19.1 21.6	21.8
M21a m/z 499 [+16 +176 amu]		12.2 15.2 16.0 17.2	11.0 12.6 14.2 15.4 18.0

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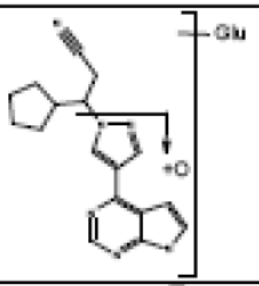
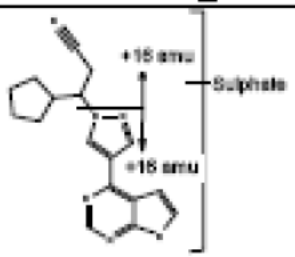
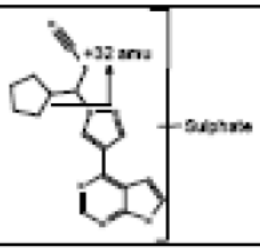
M21b *m/z* 499 [+16 +176 amu]		17.9 23.1	16.4 18.5
M25a *m/z* 419 [+112 amu]		x	9.2 10.3 12.4 13.3 15.5 16.1 16.4
M25b *m/z* 419 [+112 amu]		x	9.8 10.4 10.8

Metabolism of [14C]INCB018424 in Male and Female Beagle Dogs after a Single Oral Administration

Key study findings:

- Several peaks were observed in plasma, urine and feces;
- The number of components and the profile patterns in urine, feces and plasma were similar between male and female dogs;
- These results indicate that oxidation, primarily resulting in hydroxylated and ketone metabolites, is the major Phase I metabolic pathway for INCB018424 and that the hydroxylated metabolites can undergo conjugation (e.g. glucuronidation).

Study no: INCYTE-DMB-08.149.1, (b) (4) Study No. 63161
Volume #, and page #: electronic submission, page 1-76
Conducting laboratory and location: (b) (4)

Date of study initiation: September 28, 2007
GLP compliance: no,
QA report: yes ( ) no (x)

Drug, lot #, radiolabel, and % purity: INCB018424 Phosphate
Lot # SS-IN2-175
Purity: 99%

[14C]INCB018424
Lot#356219
Purity: 99%

Formulation/vehicle: not provided

Methods:

Group Designations and Sample Collection Times.

Study Type[a]	Number of Animals/ Sex/Animal ID	Dose Route and Level	Matrix	Sample Collection Times
MB[a]	2♂ (1M1 and 1M2) 2♀ (1F1 and 1F2)	PO[a] 3 mg//kg BW 10 µCi/kg BW	Whole Blood (Blood Cells and Plasma)	Predose, 1, 2, 4, 8, 24, 48, 72 and 96 hours post dose administration.
			Urine	Predose, 0-4, 4-12, 12-24, 24-48, 48-72 and 72-96 hour interval after dose administration.
			Feces	Predose, 0-24, 24-48, 48-72 and 72-96 hour interval after dose administration.
			Cage wash and Cage wipes	Approximately 24, 48, 72 and 96 hour after dose administration.

[a] MB = Mass Balance; PO = Oral

Selected plasma, urine and fecal samples were analyzed by HPLC for metabolite characterization.

Excretion

Excretion Mass Balance in Female Rats and Pharmacokinetics of Radioactivity in Male and Female Rats Following a Single Oral Dose of [14C]INCB018424

Key Study Findings:

- Elimination of INCB018424-derived radioactivity occurred via the urine, bile and feces, accounting for an average of 45%, 40% and 20% of the administered dose, respectively;
- Excretion of radioactivity in urine and bile was rapid, with 40% of the dose recovered in the 0-8 h urine specimen, 37% in the 0-8 h bile specimen and 19% in the 0-24 h feces specimen
- The pharmacokinetics of blood and plasma total radioactivity were similar;

- Maximum plasma and blood radioactivity concentrations were observed at 1.0 h after oral dosing of [^{14}C]INCB018424 in male and female rats, the first blood collection time.

Study no: INCYTE-DMB-09.82.1 (b) (4) Study Number: 59N-0901
Volume #, and page #: electronic submission, page 1-70
Conducting laboratory and location: (b) (4)

Date of study initiation: August 12, 2009
GLP compliance: no
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity:

INCB018424
Lot No.: BPR-07-101-B2-21
Purity: 97.8%
[Pyrimidine-2-^{14}C]INCB018424
Lot#6535900
Purity: 99.18% (radiochemical purity)

Formulation/vehicle: 30% ethyl alcohol, 70% polyethylene glycol 300, 6 mg/mL citric acid, and 5% dextrose in water (D5W)

Methods: Sprague Dawley rats were used in the study. Each animal was administered a single oral dose of [^{14}C]INCB018424 by gavage using a dosing volume of 10 mL/kg. A summary of groups and doses is presented in the following table (excerpted from the Applicant's submission):

Group & Gender	N	Dose Route	Target Dose Level (mg/kg)	Target Dose Volume (mL/kg)	Target Dose Conc. (mg/mL)	Target Radioactivity Level (µCi/kg)
1 (BDC) Female	4	PO	25	10	2.5	200
2 (Intact) Female	15	PO	25	10	2.5	200
3 (Intact) Male	6	PO	25	10	2.5	200

Bile, urine, feces, and plasma samples were collected at the time points shown in the table below:

Sample Collection Summary

Group	Bile	Urine	Feces	Cage Residue	Blood and Plasma*
1	Pre-dose, 0-8, 8-24, 24-48, 48-72, and 72-96 h	Pre-dose, 0-8, 8-24, 24-48, 48-72, and 72-96 h	Pre-dose, 0-24, 24-48, 48-72, and 72-96 h	Rinse at 24, 48, 72 h; wash & wipe at study termination	No Sample
2	No Sample	No Sample	No Sample	No Sample	1, 2, 4, 8, 24 h
3	No Sample	No Sample	No Sample	No Sample	1, 4 h

* Terminal sample collections from 3 animals per sample time

The radioactivity content was then quantified by LSC. All LSC analyses were performed using a Model 2800TR liquid scintillation analyzer (Perkin Elmer), counting for at least 5 min or 100,000 counts.

Dosing:

Species/strain: Sprague-Dawley rats
#/sex/group or time point: see the table above
Age: not provided
Weight: 242-256 g
Doses in administered units: 25 mg/kg; 200 μCi/rat
Route, volume: oral gavage at dose volume of 10 mL/kg

Results: The following figure was submitted by the Applicant

Recovery of Radioactivity for Group 1 Female Rats Following Oral Administration of [^{14}C]INCB018424 at a Target Dose of 25 mg/kg

Matrix	Collection Time	% of Dose Recovered						
		Rat #1	Rat #2	Rat #3A	Rat #4	Mean	SD	Cumulative
Feces	Pre-dose	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	0-24 h	20.374	17.562	19.240	17.076	18.563	1.522	18.563
	24-48 h	0.869	1.503	1.544	1.068	1.246	0.331	19.809
	48-72 h	0.146	0.242	0.215	0.139	0.186	0.051	19.995
	72-96 h	0.075	0.077	0.123	0.066	0.085	0.026	20.080
	Sub-total	**21.464**	**19.384**	**21.122**	**18.349**	**20.080**	**1.470**	
Urine	Pre-dose	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	0-8 h	43.969	40.865	30.249	45.464	40.137	6.864	40.137
	8-24 h	4.652	5.624	4.321	4.039	4.659	0.690	44.796
	24-48 h	0.485	0.376	0.356	0.316	0.383	0.072	45.179
	48-72 h	0.193	0.184	0.185	0.113	0.169	0.037	45.348
	72-96 h	0.100	0.068	0.063	0.073	0.076	0.017	45.424
	Sub-total	**49.399**	**47.117**	**35.174**	**50.005**	**45.424**	**6.945**	
Bile	Pre-dose	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	0-8 h	31.619	34.907	43.811	36.714	36.763	5.150	36.763
	8-24 h	2.339	3.101	1.753	2.114	2.327	0.570	39.090
	24-48 h	0.214	0.317	0.250	0.253	0.259	0.043	39.348
	48-72 h	0.115	0.097	0.103	0.131	0.112	0.015	39.460
	72-96 h	0.063	0.041	0.045	0.017	0.042	0.019	39.501
	Sub-total	**34.350**	**38.463**	**45.962**	**39.229**	**39.501**	**4.811**	
Cage Rinse	0-24 h	1.695	0.590	0.624	0.322	0.808	0.607	0.808
	24-48 h	0.152	0.096	0.077	0.088	0.103	0.033	0.911
	48-72 h	0.050	0.026	0.062	0.021	0.040	0.020	0.951
	Sub-total	**1.897**	**0.712**	**0.763**	**0.431**	**0.951**	**0.648**	
Cage Wash/	96 h	0.069	0.060	0.149	0.150	0.107	0.049	0.107
Wipe	96 h	0.142	0.067	0.119	0.049	0.094	0.044	0.094
	Total	**107.321**	**105.803**	**103.289**	**108.213**	**106.157**	**2.155**	

Plasma and Blood Concentrations of Total Radioactivity in Group 2 Female Rats Following Oral Administration of [14C]INCB018424 at a Target Dose of 25mg/kg

Time	Plasma µg Equivalents/mL				
(h)	Rat 5	Rat 6	Rat 7	Mean	SD
1	11.405	7.123	10.191	9.573	2.207
	Rat 8	Rat 9	Rat 10		
2	9.477	10.663	6.502	8.881	2.144
	Rat 11	Rat 12	Rat 13		
4	3.313	3.167	2.299	2.926	0.548
	Rat 14	Rat 15	Rat 16		
8	0.519	0.654	0.552	0.575	0.070
	Rat 17	Rat 18	Rat 19		
24	0.072	0.058	0.065	0.065	0.007

Time	Blood µg Equivalents/mL				
(h)	Rat 5	Rat 6	Rat 7	Mean	SD
1	9.438	4.943	7.390	7.257	2.250
	Rat 8	Rat 9	Rat 10		
2	7.941	8.401	5.234	7.192	1.711
	Rat 11	Rat 12	Rat 13		
4	2.516	2.247	1.663	2.142	0.436
	Rat 14	Rat 15	Rat 16		
8	0.376	0.453	0.394	0.408	0.040
	Rat 17	Rat 18	Rat 19		
24	BQL	BQL	BQL	BQL	ND

Plasma and Blood Concentrations (Mean and SD, µg equivalents/mL) of Total Radioactivity in Group 2 Female and Group 3 Male Rats Following Oral Administration of [^{14}C]INCB018424 at a Target Dose of 25 mg/kg

Time (h)	Group 2, Females			
	Plasma		Blood	
	Mean	SD	Mean	SD
1	9.573	2.207	7.257	2.250
2	8.881	2.144	7.192	1.711
4	2.926	0.548	2.142	0.436
8	0.575	0.070	0.408	0.040
24	0.065	0.007	BQL	ND
C_{max} (µg equiv/mL)	9.573		7.257	
T_{max} (h)	1.0		1.0	
AUC_{0-24} (µg equiv*h/mL)	37.94		28.55	
$t_{1/2}$ (h)	3.4		1.5	
	Group 3, Males			
	Plasma		Blood	
Time (h)	Mean	SD	Mean	SD
1	10.146	2.085	8.767	1.813
4	0.586	0.033	0.441	0.024

BQL: Below quantitation limit (<0.123 µg equivalents/mL) and assigned a value of 0 for calculations

Blood / Plasma Total Radioactivity Concentration Ratios Following Administration of [^{14}C]INCB018424 at a Target Dose of 25 mg/kg to Group 2 Female and Group 3 Male Rats

Group 2, Female

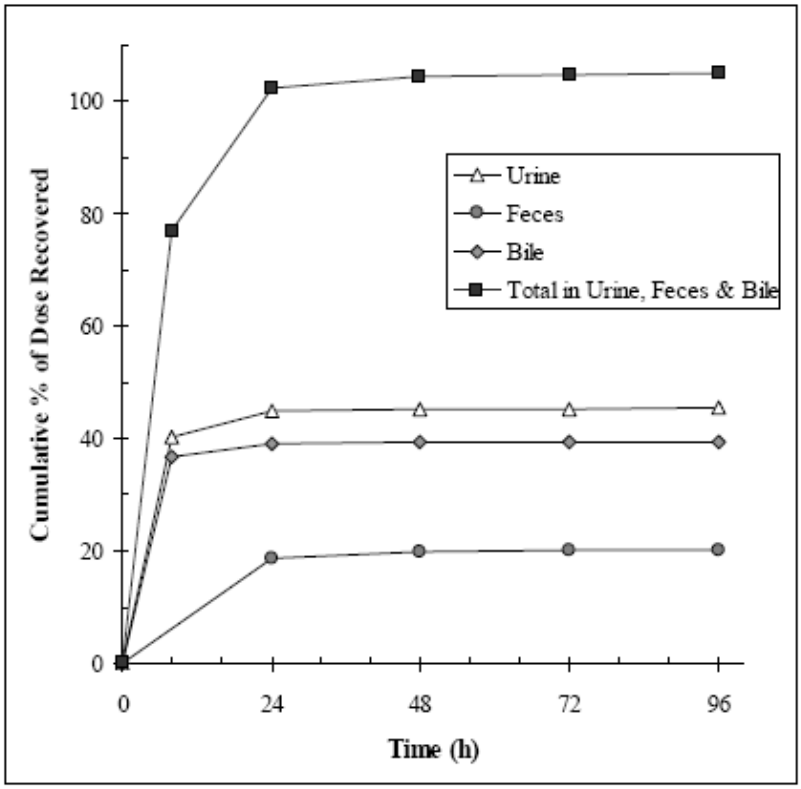
Time	Blood / Plasma Ratio				
(h)	Rat 5	Rat 6	Rat 7	Mean	SD
1	0.828	0.694	0.725	0.749	0.070
	Rat 8	Rat 9	Rat 10		
2	0.838	0.788	0.805	0.810	0.025
	Rat 11	Rat 12	Rat 13		
4	0.759	0.710	0.723	0.731	0.026
	Rat 14	Rat 15	Rat 16		
8	0.724	0.693	0.714	0.710	0.016
	Rat 17	Rat 18	Rat 19		
24	ND	ND	ND	ND	ND

ND Not Determined

Group 3, Male

Time	Blood / Plasma Ratio				
(h)	Rat 20	Rat 21	Rat 22	Mean	SD
1	0.861	0.867	0.864	0.864	0.003
	Rat 23	Rat 24	Rat 25		
4	0.755	0.752	0.752	0.753	0.002

Time Course of Excretion of Radioactivity by Group 1 (Bile Cannulated) Female Rats Following Oral Administration of [^{14}C]INCB018424 at a Target Dose of 25 mg/kg



Excretion/Mass Balance in Male and Female Beagle Dogs after a Single Oral Administration of [^{14}C]INCB018424

Key Study Findings:

- INCB018424 equivalent concentrations and elimination rate in blood cells were similar in male and female dogs; The INCB018424 concentration and elimination rate in plasma was similar to that of blood cells
- The excretion pattern of dosed INCB018424 radioactivity was consistent in both male and female Beagle dogs;
- The peak concentration was reached in blood cells at 1 hr post dose, the concentration decreased dramatically from 1 hr through 8 hrs post dose;
- Feces was the main route for excretion, which accounted for 54.8% and 57.9% of the dose in male and female, respectively. In urine, 34.4% and 36.3% of the dose was recovered for male and female, respectively.

Study no: INCYTE-DMB-08.62.2, (b) (4) Study No. 63161
Volume #, and page #: electronic submission, page 1-37
Conducting laboratory and location: (b) (4)

Date of study initiation: September 28, 2007
GLP compliance: no
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity:

INCB018424
Lot No.: BPR-07-101-B2-21
Purity: 97.8%
[Pyrimidine-2-14C]INCB018424
Lot#6535900
Purity: 99.18% (radiochemical purity)

Formulation/vehicle: 0.5% methylcellulose in H_2O
Methods: Two male and two female Beagle dogs received a single PO administration of [12+14C]INCB018424. The blood samples were collected from each animal before dose administration and at specified timepoints after dosing. The urine, feces, cage wash and cage wipes were collected from each animal for excretion and mass balance determination. See below the study design as provided by the Applicant. The radioactivity analysis was performed with collected samples by either direct liquid scintillation counting (LSC) of duplicate aliquots or oxidization (combustion) of duplicate aliquots followed by LSC.

Study Type[a]	Number of Animals/Sex	Dose Route/Level[b]	Matrix	Sample Collection Times
MB	2♂ 2♀	PO 3 mg/kg BW 10 µCi/kg BW	Whole Blood[c], (Blood Cells and Plasma)	Predose, 1, 2, 4, 8, 24, 48, 72 and 96 hrs post dose administration.
			Urine[d],	Predose, 0-4, 4-12, 12-24, 24-48, 48-72 and 72-96 hr interval after dose administration.
			Feces[e],	Predose, 0-24, 24-48, 48-72 and 72-96 hr interval after dose administration.
			Cage Wash[f], and Cage Wipes[g]	Approximately 24, 48, 72 and 96 hr after dose administration.

Dosing:

Species/strain: Beagle dog
#/sex/group or time point: see the table above
Age: 6 to 12 months
Weight: 7.9 to 9.8 kg
Doses in administered units: 3 mg/kg, 10 µCi/kg
Route, volume: oral gavage at dose volume of 10 mL/kg

Results (presented for total radioactivity only):

[^{14}C]INCB018424 Radioactivity in Blood Cells Collected from Male and Female Beagle Dogs after a Single PO Administration of INCB018424 at Approximately 3 mg/kg Body Weight

Blood Collection Time Interval	[^{14}C]INCB018424 Radioactivity Concentration in Blood Cells (dpm/g)							
	Male Beagle Dogs				Female Beagle Dogs			
(hr)	1M1	1M2	Average	%Difference	1F1	1F2	Average	%Difference
0	na	na	na	na	na	na	na	na
1	13775	14930	14353	4.0%	14414	17952	16183	10.9%
2	10647	10625	10636	0.1%	9734	13443	11588	16.0%
4	6322	5492	5907	7.0%	5214	8140	6677	21.9%
8	2232	1673	1952	14.3%	2337	3153	2745	14.9%
24	494	398	446	10.8%	519	714	617	15.9%
48	313	254	284	10.4%	277	422	349	20.8%
72	254	180	217	17.0%	227	318	273	16.6%
96	214	162	188	13.8%	189	243	216	12.5%

na = not applicable.

[^{14}C]INCB018424 Radioactivity in Plasma Collected from Male and Female Beagle Dogs after a Single PO Administration of INCB018424 at Approximately 3 mg/kg Body Weight

Blood Collection Time Interval	[^{14}C]INCB018424 Radioactivity Concentration in Plasma (dpm/g)							
	Male Beagle Dogs				Female Beagle Dogs			
(hr)	1M1	1M2	Average	%Difference	1F1	1F2	Average	%Difference
0	na	na	na	na	na	na	na	na
1	15436	12651	14044	9.9%	11225	14542	12884	12.9%
2	11369	9452	10410	9.2%	9024	11723	10374	13.0%
4	7302	5367	6335	15.3%	4214	7146	5680	25.8%
8	2099	1425	1762	19.1%	1928	2794	2361	18.3%
24	290	210	250	15.9%	389	439	414	6.0%
48	70	83	77	8.4%	56	220	138	59.4%
72	125	105	115	8.8%	63	139	101	37.2%
96	68	60	64	5.9%	87	92	90	3.0%

na = not applicable.

Total Recovery of Dosed Radioactivity in Male and Female Beagle Dogs after Administration of INCB018424 at Approximately 3 mg/kg Body Weight

Matrix	Time point (hr)	Total Dose % Recovery from Urine, Feces, Cage Wash and Cage Wipes					
		Male Beagle Dogs			Female Beagle Dogs		
		1M1	1M2	Average	1F1	1F2	Average
Urine	Predose	NA	NA	NA	NA	NA	NA
	0-4	7.1%	13.6%	10.3%	10.8%	0.0%	5.4%
	4-12	18.5%	12.6%	15.6%	12.3%	1.2%	6.7%
	12-24	4.8%	7.5%	6.2%	6.6%	34.8%	20.7%
	24-48	1.6%	2.1%	1.8%	1.6%	4.4%	3.0%
	48-72	0.3%	0.4%	0.4%	0.3%	0.6%	0.4%
	24-48	0.2%	0.1%	0.1%	0.1%	0.1%	0.1%
Urine Subtotal		32.5%	36.4%	34.4%	31.5%	41.1%	36.3%
Feces	Predose	NA	NA	NA	NA	NA	NA
	0-24	48.5%	45.2%	46.8%	51.9%	39.0%	45.5%
	24-48	6.5%	6.1%	6.3%	11.1%	1.7%	6.4%
	48-72	1.0%	1.8%	1.4%	1.2%	9.5%	5.4%
	72-96	0.2%	0.2%	0.2%	0.1%	1.1%	0.6%
Fecal Subtotal		56.2%	53.4%	54.8%	64.4%	51.3%	57.9%
Cage Wash	24	4.6%	2.3%	3.5%	1.7%	1.1%	1.4%
	48	0.9%	1.0%	1.0%	0.3%	0.2%	0.2%
	72	0.4%	0.2%	0.3%	0.1%	0.3%	0.2%
	96	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%
Cage Wash Subtotal		6.0%	3.6%	4.8%	2.1%	1.7%	1.9%
Cage Wipes Extract	96	0.04%	0.02%	0.03%	0.02%	0.01%	0.01%
Fecal Container Wipe [A]	0-96	0.00%	0.00%	0.00%	0.02%	0.01%	0.01%
Total Sum		94.70%	93.40%	94.06%	98.10%	94.10%	96.10%

Note:
NA = Not Applicable
A. For homogenization, fecal sample was removed from the original container and transferred to new container due to bigger volume of the resulted fecal homogenate. The original container was cleaned with wipe and radioactivity in the wipe was counted.

Pharmacokinetic drug interactions (only two studies are reviewed below; see the review by the clinical pharmacology team for review of other drug interaction studies)

Human CYP Isozymes that are Responsible for the In Vitro Metabolism of INCB018424

Key Study Finding:

- Study results suggested that CYP3A4 is the predominant CYP isozyme responsible for the metabolism of INCB018424.

Study no: INCYTE-DMB-09.93.1
Volume #, and page #: electronic submission, page 1-8
Conducting laboratory and location: Incyte Corporation

(b) (4)

(b) (4)

Date of study initiation: July 15, 2008
GLP compliance: no
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: INCB018424
Lot SS-IN2-175
Purity: not provided

Formulation/vehicle: not provided
Methods: INCB018424 (1 µM) was incubated (N=3) with human liver microsomes (2.0 mg/mL of protein), NADPH (2 mM), and 50 mM potassium phosphate buffer (pH 7.4) at 37°C. Parallel incubations using the same conditions included either furafylline (5 µM),
sulfaphenazole (10 µM), (S)-(+)-N-3 Benzylnirvanol (5 µM), quindine (5 µM), or ketoconazole (1 µM) to selectively inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6 and
CYP3A4, respectively. Aliquots were taken at 0, 10, 20 and 30 minutes and deproteinized with acetonitrile, centrifuged and the supernatants analyzed by LC/MS. Incubations without NADPH served as controls.

Results: the following table is excerpted from the Applicant's submission

INCB018424 Incubation Condition	Percent of Initial INCB018424 Remaining after 30 Minute Incubation (N=3)
No inhibitor	35±1
With Furafylline (5 µM, CYP1A2 inhibitor)	38±1
With Sulfaphenazole (10 µM, CYP2C9 inhibitor)	40±1
With Benzylnirvanol (5 µM, CYP2C19 inhibitor)	35±2
With Quinidine (5 µM, CYP2D6 inhibitor)	42±2
With Ketoconazole (1 µM, CYP3A4 inhibitor)	74±1
All Inhibitors Added	86±5

Summary: When ketoconazole, a selective inhibitor of CYP3A4, was co-incubated with INCB018424, 74% of the parent compound remained, whereas co-incubations with selective inhibitors of CYP1A2, CYP2C9, CYP2C19 and CYP2D6, had a minimal effect with 38±1%, 40±1%, 35±2% and 42±2%, respectively of the initial concentration of INCB018424 remaining.

In vitro evaluation o f INCB018424 as an inducer of cytochrome P450 expression in cultured human hepatocytes

Key Study Finding:

- INCB018424, at concentrations up to 10 µM did not induce CYP1A2 or CYP2B6 activity in cultured human hepatocytes

Study no: INCYTE-DMB-11.06.1
Volume #, and page #: electronic submission, page 1-31
Conducting laboratory and location: (b) (4)

Date of study initiation: February 11, 2011
GLP compliance: no
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: INCB018424
Lot # BPR-07-101-B2-21
Purity: 98%

Formulation/vehicle: DMSO, 0.1% v/v

Methods: Three preparations of cultured human hepatocytes (two cryopreserved and one fresh) from three separate livers were treated once daily for three consecutive days with dimethyl sulfoxide (DMSO, 0.1% v/v, vehicle control), one of three concentrations of INCB018424 (1, 3 or 10 μM) or one of two known human CYP inducers, namely, omeprazole (100 μM) and phenobarbital (750 μM). After treatment, the cells were treated *in situ* with the appropriate marker substrates for the analysis of phenacetin *O*-dealkylation (marker for CYP1A2) and bupropionhydroxylation (marker for CYP2B6) by LC/MS/MS.

Discussion and Conclusions

The absorption, distribution, metabolism and excretion of ruxolitinib has been studied in mice (CD-1, hairless and transgenic), rats (albino and pigmented), rabbits, dogs, monkeys and minipigs. Summary and conclusion below is mostly based on the study results from rat and dog studies.

Oral pharmacokinetics studies have shown that ruxolitinib was absorbed rapidly with Tmax values ranging from 0.45 to 2 h; the terminal elimination half-life of ruxolitinib was generally short after oral and IV dosing, ranging from 1.5 h (rat) to 2 h (dog). The absolute oral bioavailability was variable across the nonclinical species, 29% in male rats and 105% in female rats, and approximately 50% in dogs. No accumulation was observed after 1-month repeated daily dosing, while small accumulation was observed after 6-month daily administration of ruxolitinib (the plasma exposures at the end of 6-month treatment were about 2-fold of those observed on study day 1). There were generally no gender differences in the exposure of ruxolitinib and its metabolites in dogs. In rats, the Cmax and AUC values were several-fold higher in females compared to males, in part due to higher metabolic clearance in male rats. In addition, the human metabolites of ruxolitinib were identified and observed at similar or higher exposures compared to human during the course of nonclinical toxicology studies in mice, rat and dog and in the carcinogenicity study in Tg.rasH2 mouse. The terminal half-life, routes and rate of excretion for ruxolitinib-derived radioactivity and metabolites observed between male and female rats were similar. Following IV administration of ruxolitinib, the total systemic clearance ranged from moderate in dogs to very high in rats. Following IV administration of ruxolitinib, the total systemic clearance and volume of distribution was species-dependent and ranged from moderate in dogs (26% of hepatic blood flow in dogs) to very high in rats (greater than 100% of hepatic blood flow). The apparent steady-state volume of distribution (Vss) of ruxolitinib in male rats, female rats, and dogs was 3.8, 1.6, and 1.1 L/kg, respectively, indicating ruxolitinib is not distributed extensively beyond body water in dog (consistent with humans), and may be distributed to a greater extent in rat. Ruxolitinib is not distributed significantly beyond body water in dog and monkey and may be distributed to a greater extent in rats.

In rats, after a single oral dose of ^{14}C-ruxolitinib, drug-derived radioactivity was widely distributed with rapid and complete elimination from most tissues. High concentration of ruxolitinib was detected in the GI system, levels in the CNS were below LOQ. Ruxolitinib was eliminated from most tissues rapidly and completely. In pregnant rats administered a single oral dose of ^{14}C-ruxolitinib, fetal exposure was observed but

was less than maternal exposure with fetus:maternal plasma and fetal tissue:maternal plasma concentration ratios being less than one for all fetal tissues. The plasma protein binding of ruxolitinib is species-dependent 3.3% in human. In rat and dog mass balance studies, elimination of drug-derived radioactivity after oral and IV dosing is similarly rapid and complete with excretion via urine, bile and feces. In rats, ruxolitinib-derived radioactivity preferentially partitions into milk with the qualitative metabolite profile being similar to plasma. Milk concentrations were less than 1% of Cmax by 24 h. In milk, the qualitative metabolite profile was similar to plasma, with quantitative differences likely due to the higher exposure of ruxolitinib-related radioactivity in milk relative to plasma.

Oxidation was the predominant metabolic pathways in rats and dogs. In vitro studies suggest that CYP3A4 is the predominant CYP isozyme responsible for the metabolism of ruxolitinib. Ruxolitinib is metabolized by isozymes including CPY1A2, CYP2C6 and CYP2D1 found in both male rats and female rats. In rat liver microsomes, ruxolitinib is metabolized to a greater extent in males compared to females, consistent with ruxolitinib being metabolized by the male-rat-specific isozymes CYP2C11, CYP2C13 and CYP3A2. For the major human metabolites (M18 and M27) and most of the measured minor metabolites, they were studied in one or more animal species at the exposure levels exceeding or approaching corresponding human exposure at the highest proposed therapeutic dose (25 mg twice daily).

Tables and figures to include comparative TK summary

The following tables are from the Applicant. Some of the studies summarized in the table (e.g. mouse, minipig, monkey studies) are not reviewed.

2.6.5.3.a. Pharmacokinetics: Absorption After a Single Dose

					Test Article: ruxolitinib		
Study No.	**[INCYTE-DMB-08.170.1]**		**[INCYTE-DMB-06.169.1]**		**[INCYTE-DMB-06.182.1]**	**[INCYTE-DMB-07.08.1]**	**[INCYTE-DMB-08.143.1]**
Species	**CD-1 Mouse**		**Rat**		**Dog**	**Monkey**	**Human**
Gender (M/F)/Number of animals:	3M[a]	3F[a]	2M	2F	2M	3M	6M
Feeding condition:	Fasted		Fasted		Fasted	Fasted	Fasted
Vehicle/Formulation:	Suspension / 0.5% methylcellulose		Suspension / 0.5% methylcellulose		Solution / 0.5% methylcelluose	Solution/0.2% Tween-20 in 0.5% methylcellulose	Solution / Water
Method of Administration:	Gavage		Gavage		Gavage	Gavage	Oral
Dose (mg/kg):	15[b]		50		10	10	25 mg
Sample (eg, whole blood, plasma, serum):	Plasma		Plasma		Plasma	Plasma	Plasma
Analyte:	INCB018424		INCB018424		INCB018424	INCB018424	INCB018424
Assay:	HPLC/MS		HPLC/MS		HPLC/MS	HPLC/MS	HPLC/MS
PK parameters:							
T_{max} (hr)	0.5	0.5	0.5	0.5	2.0	1.7	0.63
C_{max} (µM)	2.09	1.76	2.24	30.4	8.71	1.52	1.09
AUC (µM*h) (Time for calculation – hr)	2.64 (0–24)	3.05 (0–24)	5.40 (0–∞)	39.3 (0–∞)	38.9 (0–∞)	8.05 (0–∞)	3.20 (0–∞)
T ½ (hr) (Time for calculation – hr)[c]	0.899 (2-24)	0.821 (2-24)	1.7	1.2	2.2	1.5	2.3 (2-36)

Additional Information:

A single oral dose was well absorbed in mice, rats, dogs, monkeys and humans. Similarly rapid clearance across species.

In rats, plasma C_{max} and AUC values were higher in females compared to males, but the terminal elimination half-lives were similar. No gender difference after a single oral dose in mice.

[a] per time point (21M/21F total)

[b] 100 mg/kg in male and female CD-1 also performed in same study, but not shown (C_{max} = 39.8 and 62.5 µM, respectively; AUC = 97.0 and 168 µM*h)

[c] where not indicated, at least three datapoints in the terminal phase were used to calculate half-life

2.6.5.3.b. Absorption After a Single Intravenous (iv) Dose

	Test Article: ruxolitinib					
Study No.	[INCYTE-DMB-06.169.1]		[INCYTE-DMB-06.182.1]	[INCYTE-DMB-07.10.1]		[INCYTE-DMB-07.08.1]
Species	**Rat**		**Dog**	**Minipig**		**Monkey**
Gender (M/F)/Number of animals:	2M	2F	1M	1M	1F	2M
Feeding condition:	Fasted		Fasted	Fasted		Fasted
Vehicle/Formulation:	Solution/2.0 mg/mL in 10% dimethyl acetamide, 30% SBE-beta-cyclodextrin (50% w/v) and 60% saline		Solution/1 mg/mL in saline	Solution/1 mg/mL in 10% dimethyl acetamide, 30% hydroxypropyl betacyclodextrin (45% w/v) and 60% normal saline		Solution/1 mg/mL in 10% dimethyl acetamide, 30% hydroxypropyl betacyclodextrin (45% w/v) and 60% normal saline
Method of Administration:	iv		iv	iv		iv
Dose (mg/kg):	10		5	5		5
Sample (eg, whole blood, plasma, serum):	Plasma		Plasma	Plasma		Plasma
Analyte:	INCB018424		INCB018424	INCB018424		INCB018424
Assay:	HPLC/MS		HPLC/MS	HPLC/MS		HPLC/MS
PK parameters:						
CL (L/h/kg)	9.4	4.8	0.48	9.0	7.7	0.91
V_{ss} (L/hr)	3.8	1.6	1.1	5.0	7.7	0.81
AUC (μM*h) (Time for calculation – hr)	3.75 (0–∞)	7.49 (0–∞)	34.1 (0–∞)	1.82 (0–∞)	2.10 (0–∞)	18.0 (0–∞)
T ½ (hr) (Time for calculation – hr)[a]	0.41	0.43	2.5	0.42	0.72	0.88
% dose in urine as unchanged parent	< 1	< 1	< 1	< 1	< 1	< 1

Additional Information:

Total systemic clearance and volume of distribution was species-dependent and ranged from moderate (26% of hepatic blood flow in dogs) to very high (450% of hepatic blood flow in minipigs).

Ruxolitinib not distributed extensively beyond body water in dogs and monkeys; may be distributed to a greater degree in rats and minipigs.

[a] where not indicated, at least three datapoints in the terminal phase were used to calculate half-life

2.6.5.6.a. In Vitro Plasma Protein Binding

			Test Article: ruxolitinib
Study system: In vitro			
Target entity, Test system and method: Plasma, Equilibrium dialysis			
Species	**Conc. Tested**	**% Unbound**	**Study No.**
CByB6F1-Tg(HRAS)2Jic (Tg.rasH2) mice	11 µM	5.2	[INCYTE-DMB-08.158.1]
CD-1 mouse	1 – 10 µM	2.7 - 3.4	[INCYTE-DMB-08.191.1]
Hairless mouse	0.43 – 4.3 µM	3.8 – 5.2	[INCYTE-DMB-09.62.1]
Rat	1.0 – 3.0 µM	12 - 17	[INCYTE-DMB-07.11.1]
Rabbit (gestating)	0.5 – 5 µM	10.0 – 13.7	[INCYTE-DMB-09.61.1]
Dog	1.0 – 10 µM	11 - 13	[INCYTE-DMB-07.11.1]
Minipig	3.0 µM	26	[INCYTE-DMB-07.11.1]
Human	10 µM	3.3	[INCYTE-DMB-07.11.1]
Additional Information: Non-gestating rabbits also determined (11.2% bound).			

2.6.5.6.b. In Vitro Serum Protein Binding

			Test Article: ruxolitinib
Study system: In vitro			
Target entity, Test system and method: Serum, Equilibrium dialysis			
Species	**Conc. Tested**	**% Unbound**	**Study No.**
Rat	3.0 – 10 µM	14 - 15	[INCYTE-DMB-07.11.1]
Dog	3.0 – 10 µM	9.2 - 10	[INCYTE-DMB-07.11.1]
Monkey	3.0 µM	5.6	[INCYTE-DMB-07.11.1]
Minipig	3.0 - 10 µM	25 - 30	[INCYTE-DMB-07.11.1]
Human	3.0 - 10 µM	2.6 – 3.6	[INCYTE-DMB-07.11.1]

2.6.5.6.c. Ex Vivo Plasma Protein Binding

			Test Article: ruxolitinib
Study system: Ex vivo			
Target entity, Test system and method: Plasma, Equilibrium dialysis			
Species	**Conc. Tested**	**% Unbound**	**Study No.**
CByB6F1-Tg(HRAS)2Jic (Tg.rasH2) mice	1.2 – 13.6 µM	4.9 – 5.0	[INCYTE-DMB-08.158.1]
CD-1 mouse	5.7 – 19.5 µM	2.4 - 3.0	[INCYTE-DMB-08.191.1]
Hairless mouse	1.3 – 14 µM	3.0	[INCYTE-DMB-09.62.1]
Rat	4.3 µM	18	[INCYTE-DMB-07.11.1]
Rabbit (gestating)	0.7 – 3.1 µM	11.8 – 13.1	[INCYTE-DMB-09.61.1]
Dog	0.39 – 4.8 µM	7.3 – 12	[INCYTE-DMB-07.11.1]
Minipig	1.1	33	[INCYTE-DMB-07.11.1]

2.6.5.7.b. Excretion into Milk

						Test Article: Ruxolitinib
Excretion into milk						**Study No. [INCYTE-DMB-10.50.1]**
Species: Rats						
Lactating date/Number of animals: Lactation Day 10/3 animals per time point						
Feeding condition: Not fasted						
Vehicle/Formulation: 0.5% methylcellulose						
Method of Administration: Oral gavage						
Dose (mg/kg): 30						
Analyte: Total radioactivity, ^{14}C						
Assay: LSC						
Time (hr)	1	2	4	8	24	$AUC_{0-\infty}$ [b]
Concentration/Amount (µg/g) [a]						
Milk:	12.0	14.0	12.7	7.25	0.128	145
Plasma:	3.02	2.09	1.02	0.307	BLQ	10.8
Milk/plasma:	4.02	6.72	12.6	24.8	NA	13.4
Neonates:						

Additional Information:

Milk:plasma ratio of radioactivity based on $AUC_{0-\infty}$ was 13.4, indicating ruxolitinib-derived radioactivity preferentially partitions into milk. Milk concentrations were < 1% of C_{max} by 24 h.

Metabolite profile in milk and plasma also examined, but not shown. The qualitative metabolite profile in milk was similar to plasma, with quantitative differences likely due to the higher exposure of INCB018424-related radioactivity in milk relative to plasma.

Blood also examined, but not shown (blood/plasma between 1 and 8 h ranged from 0.96 to 0.99)

[a] µg Equivalent/g values are reported to three significant figures with a maximum of three decimal places.
[b] $AUC_{0-\infty}$ reported as µg equivalent*h/g.
BLQ - Below the limit of quantitation (< 43.2 ng equivalents ^{14}C-INCB018424/g for plasma and < 51.1 ng equivalents ^{14}C-INCB018424/g for milk).
NA – Not applicable

2.6.5.9.a. In Vivo Metabolism in Mice and Rats

											Test Article: ruxolitinib			
Gender (M/F)/Number of animals:			**Mice: 21M/21F (3M/3F/timepoint)**				**Rats: 6M (plasma); 4M (excreta)**				**Rats: 4F**			
Feeding condition:			**Not fasted**				**Fasted**				**Fasted**			
Vehicle/Formulation:			**0.5% MC in water**				**0.5% MC in water**				**0.5% MC in water**			
Method of Administration:			**Gavage**				**Gavage**				**Gavage**			
Dose (mg/kg):			**50**				**25 (plasma); 50 (excreta)**				**25**			
Radionuclide: ^{14}C														
Specific Activity:			**5.25 x 10^6 Bq/mg**				**5.25 x 10^6 Bq/mg**				**5.45 x 10^6 Bq/mg**			
Study Number	[INCYTE-DMB-08.154.1]						[INCYTE-DMB-08.169.1] (plasma) [INCYTE-DMB-08.61.1] (excreta)				[INCYTE-DMB-09.84.1]			
	Mice (Male)			**Mice (Female)**			**Rat (Male)**				**Rat (Female)**			
Species	**Pl**	**Ur**	**Fe**	**Pl**	**Ur**	**Fe**	**Pl**	**Ur[a]**	**Bile[a]**	**Fe[a]**	**Pl**	**Ur**	**Bile**	**Fe**
Sampling Time or Period	0.25 hr	4-8 hr	8-24 hr	0.25 hr	4-8 hr	8-24 hr	1 hr	0-96 hr[b]	0-96 hr[b]	0-96 hr[b]	1 hr	0-8 hr	0-8 hr	0-24 hr
% of Dose in Sample	-	15.4	16.4	-	22.6	10.3	-	52.0	36.7	11.9	-	40.1	36.8	18.6
% Radioactivity in Sample														
Parent	64.2	ND	2.8	60.1	ND	2.7	6.7	0.3	ND	4.6	34.5	0.1	0.1	1.4
M1								10.1				2.6	4.3	
M1-B												0.9	1.8	
M2												1.1	2.5	
M2-B												2.5	1.4	

2.6.5.9.a. In Vivo Metabolism in Mice and Rats (cont'd)

											Test Article: ruxolitinib			
	Mice (Male)			Mice (Female)			Rat (Male)				Rat (Female)			
Species	Pl	Ur	Fe	Pl	Ur	Fe	Pl	Ur[a]	Bile[a]	Fe[a]	Pl	Ur	Bile	Fe
M3								3.3				5.2		
M4								4.6				3.5		3.7
M5									7.1					
M6									7.4					
M7	15.3	22.8	11.5	19.9	27.4	14.8	10.0	30.5		33.7	4.2	5.0	3.4	14.2
M8	2.3	6.8	10.1	ND	6.9	10.3	18.9				20.2	17.8		29.4
M9	1.3	2.8	ND	1.3	3.5	ND	14.0	29.0	9.2	8.8	2.3	5.1	1.7	2.6
M11	ND	7.8	3.7	1.6	7.5	2.3	18.2			21.8	11.3	19.1	6.6	5.6
M15									11.6				9.2	
M16	ND	5.9	5.3	0.7	5.1	5.7	6.8	4.1		6.5	3.8		1.3	5.7
M17									31.4				0.7	
M18	5.3	1.8	4.9	4.3	1.9	6.4	1.7	2.4			3.7			1.0
M19												12.1	4.7	4.9
M20								3.8				2.5		
M21								3.2				2.2		
M25													12.7	
M26												0.2	3.7	
M27		3.5	2.8		2.1	2.1					3.4	5.7		12.3
M28												2.9	22.0	
M34													0.5	

2.6.5.9.a. In Vivo Metabolism in Mice and Rats (cont'd)

											Test Article: ruxolitinib			
	Mice (Male)			Mice (Female)			Rat (Male)				Rat (Female)			
Species	Pl	Ur	Fe	Pl	Ur	Fe	Pl	Ur[a]	Bile[a]	Fe[a]	Pl	Ur	Bile	Fe
M37		1.0	8.2		1.5	6.2								
M40		4.6	2.7		3.7	2.3								
M41		ND	9.2		5.9	11.0	7.1							
M42		11.3			5.3									
M43							2.9							
M48		1.5	1.4		1.9	3.4	4.8				4.1	2.2		2.8
M49												0.5		
M50	4.1		0.8	2.8		0.3						0.5	1.9	
M50-B											2.3	2.9		
M52							1.7							
M53							2.7						3.6	
M54											1.1			
Unknown peaks[c]			19.2			15.9					1.6	0.9	4.4	11.9
Others[d]											9.7	1.2	13.4	3.1

Additional Information:

[a] In some cases, multiple metabolites co-eluted in male rat urine, bile and feces due to the shorter run time performed for these matrices.
[b] ~ 30% of each sample was pooled from each time point such that 90% of the excreted dose from one rat was contained in a single sample.
[c] represents metabolite peak(s) where metabolite structure could not be elucidated or those containing an undefined mixture of metabolites.
[d] In female rats, multiple mono-, di-, tri- and quad- oxygenated metabolites and in some cases, subsequent conjugation, were also observed, each constituting ≤ 5% of radioactivity in the corresponding matrix.
MC – methylcellulose
ND – not detected
In male rats, metabolite profiles in excreta after iv dosing were similar to oral dosing.

2.6.5.9.b. In Vivo Metabolism in Dogs and Humans

							Test Article: ruxolitinib		
Gender (M/F)/Number of animals:	**Dogs: 2M**			**Dogs: 2F**			**Humans: 6M**		
Feeding condition:	**Not fasted**			**Not fasted**			**Fasted**		
Vehicle/Formulation:	**Solution / 0.5% MC in water**			**Solution / 0.5% MC in water**			**Solution / Water**		
Method of Administration:	**Gavage**			**Gavage**			**Oral**		
Dose (mg/kg):	**3**			**3**			**25 mg**		
Radionuclide: ^{14}C									
Specific Activity:	**5.25 x 10^6 Bq/mg**						**5.45 x 10^6 Bq/mg**		
Study Number	[INCYTE-DMB-08.149.1]						[INCYTE-DMB-08.153.1]		
	Dog (Male)			**Dog (Female)**			**Human**		
Species	**Plasma**	**Urine**	**Feces**	**Plasma**	**Urine**	**Feces**	**Plasma**	**Urine**	**Feces**
Sampling Time or Period (hr)	1 hr	0-4 hr	0-24 hr	1 hr	0-4 hr	0-24 hr	1 hr	0-8 hr	24-48 hr
% of Dose in Sample	-	10.3	46.8	-	10.8	45.5	-	45.1	9.7
Mean % Radioactivity in Sample									
Parent	27.3	4.6	19.8	31.0	2.6	25.1	74.1	0.5	1.2
M7	1.3	ND	0.8	1.9	ND	1.0	3.8	14.3	9.9
M8	2.5	6.5	4.2	3.4	2.7	6.4	3.6	17.8	22.1
M9	2.7	4.1[a]	3.3	3.2	2.3[a]	1.2	ND	ND	ND
M11	1.1	ND	0.9	1.5	ND	1.2	3.7	23.6	ND
M16	2.9	2.9	[b]	5.0	5.9	[b]	2.3	5.2	4.5
M18	12.5	5.8	2.8	16.3	9.6	4.2	7.3		6.3
M23			3.2			5.2			
M24			2.8			3.2			
M25		5.2			6.4				
M26		2.0			7.1				
M27	[c]	8.4	3.6	[c]	10.2	3.9		18.9	14.5
M28		2.8			2.5			1.3	

2.6.5.9.b. In Vivo Metabolism in Dogs and Humans (cont'd)

							Test Article: ruxolitinib		
	Dog (Male)			Dog (Female)			Human		
Species	**Plasma**	**Urine**	**Feces**	**Plasma**	**Urine**	**Feces**	**Plasma**	**Urine**	**Feces**
M29			4.5			4.6			
M30		4.3			ND				
M31	2.6	2.0	3.5	3.6	2.9	4.0			4.6
M32	7.3			6.3					
M34	1.9			1.3					
M35		5.5			8.8				
M37	5.9	22.9	14.7[d]	5.0	22.4	7.2[d]		1.4	
M38		4.8			3.8		1.6		
M39	22.6			10.6					
M43									6.2
M45									2.3
M49							2.2	11.0	15.6
M51								1.9	
Unknown peak			11.4			10.8			

Additional Information:
[a] In dog urine, M14 co-eluted with M9
[b] In dog feces, M16 co-eluted with M11
[c] In dog plasma, M27 co-eluted with stereoisomer, M16
[d] In dog feces, M37 co-eluted with M36
MC - methylcellulose

26.5.13.a. Excretion in Rats After a Single Oral Dose

									Test Article: ruxolitinib			
Species:	**Rat**				**Rat**				**Rat**			
Gender (M/F)/Number of animals:	**4M**				**4M**				**4F**			
Feeding condition:	**Fasted**				**Fasted**				**Fasted**			
Vehicle/Formulation:	**Solution / 0.5% methylcellulose in water**				**Solution / 10% DMAC, 30% of SBE-beta-cyclodextrin aqueous solution (50% w/v), and 60% saline**				**Solution / 0.5% methylcellulose in water**			
Method of Administration:	**Oral**				**Intravenous**				**Oral**			
Dose (mg/kg):	**50**				**10**				**25**			
Analyte:	**TRA[a]**				**TRA[a]**				**TRA[a]**			
Assay:	**LSC**				**LSC**				**LSC**			
Excretion route:	**Urine**	**Bile**	**Feces**	**Total**	**Urine**	**Bile**	**Feces**	**Total**	**Urine**	**Bile**	**Feces**	**Total**
Time												
0 – 24 hr	51.6	36.5	12.6	101	48.3	36.3	12.9	97.5	44.8	39.1	18.6	102
0 – 48 hr	51.9	36.6	13.1	102	48.6	36.5	14.1	99.2	45.2	39.3	19.8	104
0 – 72 hr	52.0	36.7	13.3	102	48.7	36.5	14.2	99.4	45.3	39.5	20.0	105
0 – 96 hr	52.0	36.7	13.5	102	48.8	36.5	14.4	99.7	45.4	39.5	20.1	105
Study number	[INCYTE-DMB-08.61.1]								[INCYTE-DMB-09.82.1]			
Additional Information: Cage wash also examined, but not shown [a] Total radioactivity; percent recovery, ^{14}C												

2.6.5.13.b. Excretion in Dogs and Humans After a Single Oral Dose

							Test Article: ruxolitinib		
Species:	**Dog**			**Dog**			**Human**		
Gender (M/F)/Number of animals:	**2M**			**2F**			**6M**		
Feeding condition:	**Not fasted**			**Not fasted**			**Fasted**		
Vehicle/Formulation:	**Solution / 0.5% methylcellulose in water**			**Solution / 0.5% methylcellulose in water**			**Solution / water**		
Method of Administration:	**Oral**			**Oral**			**Oral**		
Dose (mg/kg):	**3**			**3**			**25 mg**		
Analyte:	**TRA[a]**			**TRA[a]**			**TRA[a]**		
Assay:	**LSC**			**LSC**			**LSC**		
Excretion route:	**Urine**	**Feces**	**Total**	**Urine**	**Feces**	**Total**	**Urine**	**Feces**	**Total**
Time									
0 – 24 hr	32.1	46.8	78.9	32.8	45.5	78.3	69.9	0.5	70.4
0 – 48 hr	33.9	53.1	87.0	35.8	51.9	87.7	73.0	9.9	82.9
0 – 72 hr	34.3	54.5	88.8	36.2	57.3	93.5	73.4	16.1	89.5
0 – 96 hr[b]	34.4	54.7	89.1	36.3	57.9	94.2	73.5	18.8	92.3
0 – 192 hr[b]							73.6	21.9	95.5
Study number	[INCYTE-DMB-08.62.2]						[INCYTE-DMB-08.150.1]		

Additional Information:

[a] Total radioactivity; percent recovery, ^{14}C

[b] For dogs, samples collected up to 96 hr. Human samples collected up to 144 hr for four subjects, 168 hr for one subject and 192 hr for one subject.

In cases when there was no sample for a given interval from an individual subject (ie 0-24 h feces), the TRA for the individual sample was assigned a value of 0 for calculations.

Cage wash for dog also examined, but not shown.

6 General Toxicology

Overall toxicology summary

General toxicology: Single- and repeat-dose toxicology studies up to 6 months in duration in rats and up to 12 months in duration in dogs were conducted. Single doses of ruxolitinib up to 100 mg/kg in rats and 40 mg/kg in dogs were well tolerated; these were the highest doses tested in these studies. No ruxolitinib-related acute toxicity was determined in the single dose studies. Of note single-dose studies were non-GLP, only limited dose levels were used and minimal toxicity evaluations were performed. Repeated dosing in rats and dogs resulted in toxicities mainly in the hematopoietic system including reduced levels of circulating leukocytes, especially lymphocytes in rats and dogs. Lymphoid depletion was noted in multiple lymph nodes and spleen.

Genetic toxicology: Ruxolitinib was not mutagenic in the bacterial mutagenicity assay or clastogenic in the *in vitro* chromosome aberration assay or in the *in vivo* micronucleus assay in rats.

Carcinogenicity: Ruxolitinib was not carcinogenic in a 26-week study in Tg.rasH2 mice. A 2-year rat carcinogenicity study is ongoing.

Reproductive toxicology: In embryo-fetal developmental studies in the rat and rabbit, ruxolitinib was not teratogenic, but was associated with increased late resorptions (rabbit only) and fetal toxicity (reduced fetal weights in rats and rabbits). The No-Observed-Adverse-Effect-Level (NOAEL) dose for the rat and rabbit study was 30 mg/kg/day. In a pre- and post-natal development study, there were no adverse findings for fertility indices and maternal and embryo-fetal survival, growth, and developmental parameters. A placental transfer and lactation study in rats given ruxolitinib demonstrated that low concentrations of ruxolitinib cross the placenta, and parent compound is eliminated rapidly resulting in limited fetal exposure. In lactating rats, ruxolitinib derived radioactivity was excreted into the milk with a concentration that was 13-fold higher than the corresponding maternal plasma concentration.

Special toxicology: no study conducted

6.1 Single-Dose Toxicity

Some single-dose studies were reviewed by Dr. Babara Hill under IND 77101, the results are not presented here. In conclusion, the toxicities associated with ruxolitinib were similar to those observed in the repeated-dose studies.

6.2 Repeat-Dose Toxicity

Study title: 6-month oral (gavage) toxicity study of INCB018424 in rats with a 6 week recovery period

Study no.:	(b) (4)-519048 Sponsor study No.: T07-10-06
Study report location:	Electronic submission, M4. pages 1-4625
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1 October 2007
GLP compliance:	yes
QA statement:	yes (X) no ( )
Drug, lot #, and % purity:	INCB018424, SS-IN3-139, 99.9%

Key Study Findings

- No treatment-related death was observed at doses up to 60 mg/kg/day for 26 weeks;
- Lower body weights were noted in a dose-related manner for treated males and lower food consumption was noted for the 60 mg/kg/day group males;
- Reduced levels of circulating leukocytes were noted at all dose levels along with lower spleen weights in both sexes.
- Lymphoid depletion was noted in most spleen sections and in several mandibular lymph nodes at the 60 mg/kg/day dose level;
- Treatment-related changes were generally reversible.

Methods

Doses:	5,10,30, 60 mg/kg* * The dose levels were stated to be based on previous studies; no other specific information was provided
Frequency of dosing:	once daily for 182 days, followed by a 42-day recovery period (recovery group)
Route of administration:	Oral gavage
Dose volume:	10 mg/mL
Formulation/Vehicle:	0.5% methylcellulose in reverse osmosis-treated water
Species/Strain:	Rat/Crl:CD(SD)
Number/Sex/Group:	See table below
Age:	approximately 35 days old
Weight:	ranged from 199 g to 286 g for males and from 139 g to 199 g for females
Satellite groups:	5/sex for control, 11/sex/group for treatment group
Unique study design:	none
Deviation from study protocol:	none

Number of Animals/sex/group- Rising Dose					
	Vehicle 0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	30 mg/kg/day	60 mg/kg/day
Main	15	15	15	15	15
Recovery	8	8	8	8	8

Observations and Results

OBSERVATIONS AND TIMES:

Mortality	Two times daily
Clinical examinations	Two times daily
Detailed physical examinations	weekly
Body weights	weekly
Food consumption	weekly
Ophthalmoscopy	Weeks -1, 25, 32
Clinical Pathology:	week 12 (hematology and serum chemistry), and prior to sacrifice (week 26 or 32)
Gross pathology:	At death or at scheduled sacrifice
Organ weights:	At sacrifice
Histopathology:	At sacrifice or death Adequate Battery: yes (x), no ( ) , Peer review: yes (x), no ( )

Toxicokinetics:	prior to dosing and at 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after dose administration on study days 0, 27 and 181.

RESULTS:

Mortality

Animal	Sex	Dose (mg/kg/day)	Days of death	Type of death	Death cause
8177	M	0	57	Found dead	unknown
8078	M	5	162	Found dead	unknown
8198	M	30	155	Found dead	unknown
8222	M	30	166	Found dead	unknown
8331	F	60	101	Found dead	unknown

Summary: None of the early death are considered as treatment-related deaths.

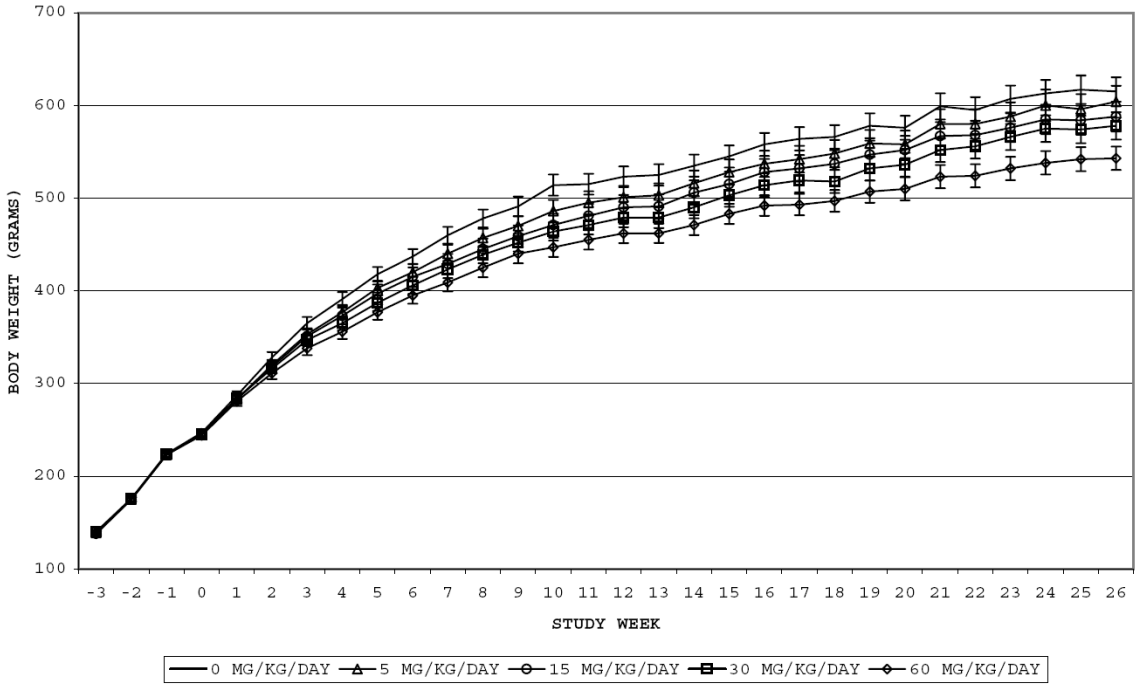
Clinical Signs

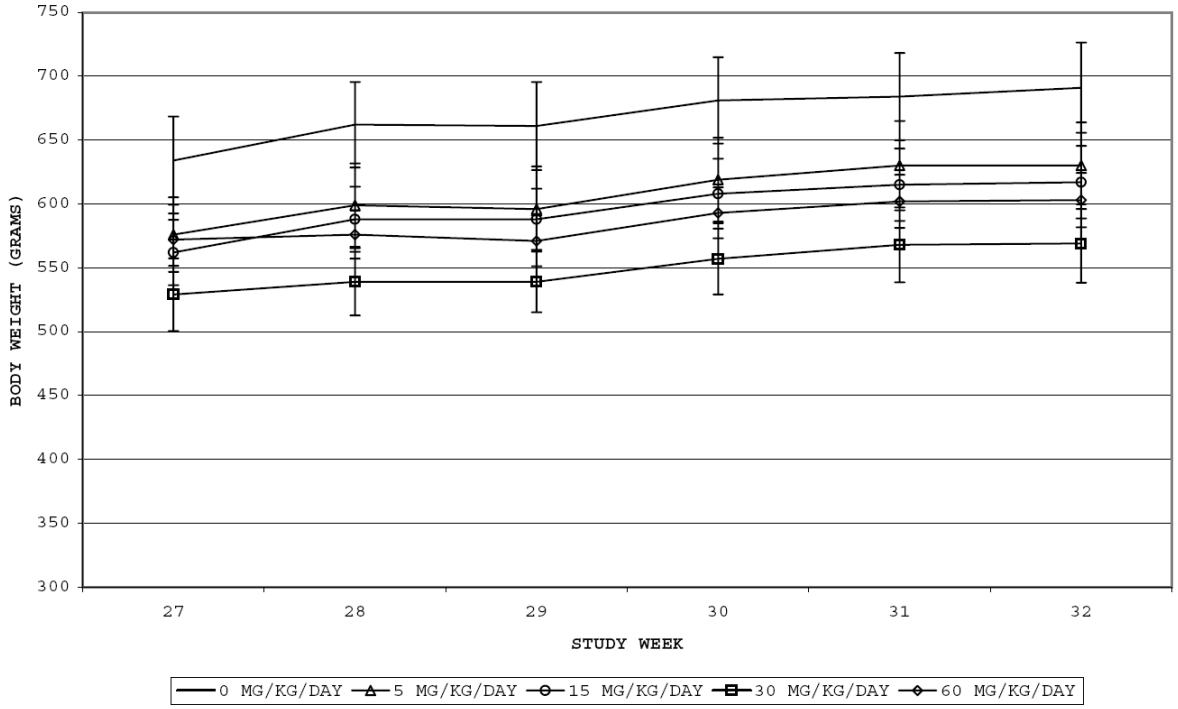
Early death animals	• unremarkable
5 mg/kg/day	• unremarkable
10 mg/kg/day	• unremarkable
30 mg/kg/day	• unremarkable
60 mg/kg/day	• wet clear and wet/dry red material around the mouth, male and female

Body Weights

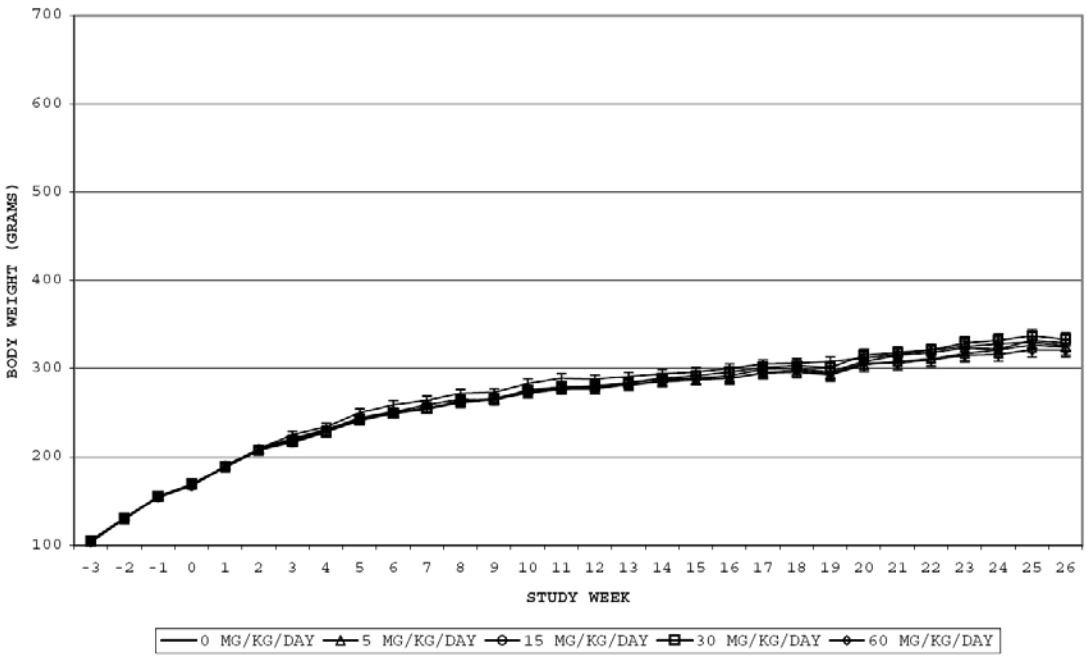
The following figures are excerpted from the Applicant's submission, the data are verified by the reviewer.

Summary of body weight (g) (dosing period- males)

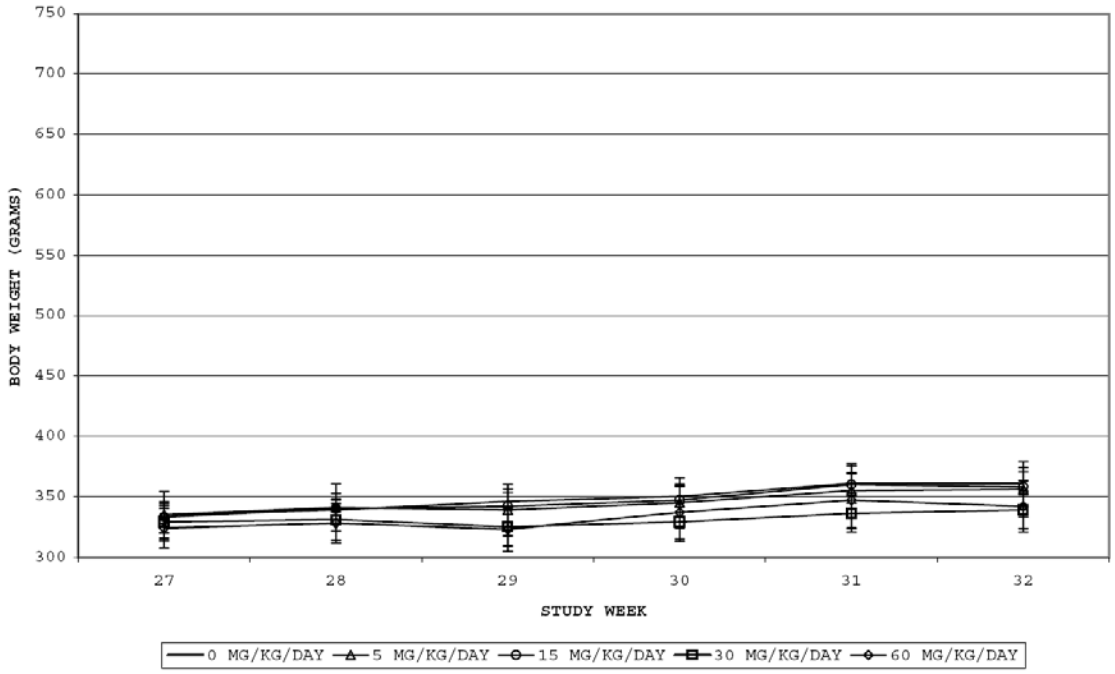




Summary of body weight (g) (dosing period- Females)



Summary of body weight (g) (dosing period- females)



Summary:

- During the dosing period, decreased body weights were observed in test article-treated group males as early as the second week of dosing. Mean body weights at the end of the dosing period were 1.8%, 4.4%, 6.0% and 11.7% lower than control values for the 5, 15, 30 and 60 mg/kg/day group males, respectively. Lower weight changes were partially recovered by the end of the recovery period.
- No significant body weight changes were observed in females.

Feed Consumption

Lower food consumption (occasionally statistically significant) was noted for the 60 mg/kg/day group males (↓0- 21%). A similar trend was not observed for the 60 mg/kg/day group females.

Ophthalmoscopy

No test-article related changes

Hematology

MALES	**Percent Change From Vehicle Control**											
Doses (mg/kg/day)	5			15			30			60		
Assessment week	12	26	32	12	26	32	12	26	32	12	26	32
Hemoglobin										↓7	↓4	
hematocrit										↓7	↓3	
WBC	↓12	↓5	↓18	↓30	↓16		↓29	↓24	↓18	↓32	↓32	↓14
Neutrophil ($E^3/\mu l$)	↓9	↓6	↓23	↓8		↑39	↑15		↓12	↑45	↑12	↓6
Lymphocyte ($E^3/\mu l$)	↓13	↓5	↓16	↓33	↓20	↓8	↓36	↓31	↓19	↓45	↓43	↓16
Monocyte ($E^3/\mu l$)	↑10		↓20		↑18	↑10	↓5	↓5	↓23	↑10	↓14	↓13
Eosinophil ($E^3/\mu l$)	↑8		↓21	↓25	↓8	↓7	↓42	↑23		↑25	↓8	↑7
Basophil ($E^3/\mu l$)	↓33		↓50	↓33			↓33	↓57	↓14	↓63	↓57	↓14
LUC ($E^3/\mu l$)	↓12	↓28		↓38	↓14		↓63	↓44		↓25	↓57	↓13
FEMALES	**Percent Change From Vehicle Control**											
Doses (mg/kg/day)	5			15			30			60		
Assessment week	12	26	32	12	26	32	12	26	32	12	26	32
Hemoglobin										↓4	↓2	
WBC	↓6	↓17	↑8	↓21	↓10		↓19	↓21		↓39	↓38	
Neutrophil ($E^3/\mu l$)	↑21	↓6	↓8		↑30	↓6	↑22	↓8	↓8	↑44		↓8
Lymphocyte ($E^3/\mu l$)	↓9	↓19	↑12	↓24	↓18		↓25	↓23		↓50	↓46	↑10
Monocyte ($E^3/\mu l$)		↓25		↓14	↓10		↑7	↓30	↓15	↓7	↓40	↓20
Eosinophil ($E^3/\mu l$)			↑33		↓11	↑11		↓22		↓43	↓44	
Basophil ($E^3/\mu l$)	↓50		↓50				↓50	↓100		↓50		↓100
LUC ($E^3/\mu l$)	↓20	↓40		↓40	↓40	↑33	↓40	↓60	↑33	↓60	↓60	

Blank cells: unremarkable changes.
Week 32 represents the recovery data.
LUC: large unstained cell.

Summary:

- Lower white blood cell counts and lower counts of absolute, lymphocyte, monocyte, basophil, and large unstained cells (LUC) were noted at study weeks 12 and 26 in male and female animals at all dose levels of INCB018424. Hemoglobin and hematocrit were slightly lower in the male and female groups at 60 mg/kg/day. These changes were reversible.

Clinical Chemistry
No test-article related changes

Urinalysis
No test-article related changes

Gross Pathology
Unremarkable

Organ Weights
The following table is excerpted from the Applicant's application. The data on the table are verified by the reviewer.

Toxicologically Relevant Final Body Weight and Organ Weight Changes At Study Week 26

Dose Group (mg/kg/day):	5	15	30	60
Final Body Weight (male)	↑3.2	↑0.2%	↑0.2%	↓11.2%*
Final Body Weight (female)	↓2.0%	0.0%	↑3.9%	↓1.6%
Adrenal Gland (Male)				
Absolute	↓12.2%**	↓15.0%**	↓23.1%**	↓26.7%**
Relative-to-Body	↓10.0%**	↓10.0%**	↓20.0%**	↓10.0%**
Relative-to-Brain	↓12.1%**	↓14.4%**	↓23.4%**	↓24.8%**
Spleen (Male)				
Absolute	↓11.0%	↓17.1%**	↓23.2%**	↓39.0%**
Relative-to-Body	↓13.9%**	↓16.7%**	↓23.6%**	↓31.3%**
Relative-to-Brain	↓11.4%	↓17.0%**	↓23.3%**	↓37.9%**
Spleen (Female)				
Absolute	↓14.8%*	↓13.0%	↓16.7%**	↓27.8%**
Relative-to-Body	↓13.1%*	↓11.9%*	↓19.9%**	↓26.1%**
Relative-to-Brain	↓14.0%*	↓13.7%*	↓15.1%*	↓26.8%**

* = Significantly different from the control group at $p<0.05$ using Dunnett's test
** = Significantly different from the control group at $p<0.01$ using Dunnett's test

Toxicologically Relevant Final Body Weight and Organ Weight Changes At Study Week 32 (recovery)

Dose Group (mg/kg/day):	**5**	**15**	**30**	**60**
Final Body Weight (male)	↓9%	↓11%	↓18%	↓13%
Final Body Weight (female)			↓5%	↓5%
Adrenal Gland (Male)				
Absolute	↑4%	↓13%	↓2%	↓8%
Relative-to-Body	↑25%		↑25%	↑13%
Relative-to-Brain		↓13%		↓9%
Spleen (Male)				
Absolute	↑6%	↑12%		
Relative-to-Body	↑17%	↑25%**	↑20%	↑17%
Relative-to-Brain		↑13%		
Spleen (Female)				
Absolute		↓7%	↓5%	↓7%
Relative-to-Body		↓5%		
Relative-to-Brain		↓6%		

Note: Blank: unremarkable change

Summary:

- Test article-related changes include decreased weights of the spleen in both males and females, and decreased weights of adrenal gland in males. Organ weight changes were reversible.

Histopathology

At the study week 26, test article-related histologic changes were restricted to the 60 mg/kg/day group where minimal to mild lymphoid depletion was observed in the spleen and mandibular lymph nodes of both genders. Additionally, minimal cortical atrophy was observed in the adrenal glands of the 60 mg/kg/day group males. The incidence and severity of microscopic findings in the spleen, mandibular lymph nodes, and adrenal glands are summarized in the following table (from the submission). At the study week 32 (recovery necropsy), microscopic changes were not observed that were attributed to the test article, suggesting full recovery.

Incidence Of Selected Histopathologic Findings At The Study Week 26 Primary Necropsy

	Males					Females				
Dosage (mg/kg/day):	**0**	**5**	**15**	**30**	**60**	**0**	**5**	**15**	**30**	**60**
Adrenal Cortex [a]	**15**	**15**	**15**	**15**	**15**	**15**	**0**	**0**	**1**	**15**
Atrophy	0	0	0	0	7	0	0	0	0	0
Minimal	-	-	-	-	7	-	-	-	-	-
Mandibular lymph node [a]	**15**	**15**	**15**	**15**	**14**	**15**	**15**	**15**	**15**	**15**
Depletion, lymphoid	0	0	0	0	3	0	0	0	0	3
Minimal	-	-	-	-	2	-	-	-	-	2
Mild	-	-	-	-	1	-	-	-	-	1
Spleen [a]	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**14**	**15**
Depletion, lymphoid	1	0	1	1	15	0	0	0	0	11
Minimal	0	-	0	1	6	-	-	-	-	9
Mild	1	-	1	0	9	-	-	-	-	2

[a] = Number of tissues examined from each group.

Toxicokinetics: refer to PK section.

STUDY SUMMARY:

INCB018424 administered by oral gavage to Sprague Dawley rats at doses of 5, 15, 30 and 60 mg/kg/day for 26 weeks was well tolerated, with no test article-related deaths, ophthalmic findings or alterations in coagulation and urinalysis parameters. Test article-related clinical observations were limited to findings of clear and red material around the mouth noted for the 60 mg/kg/day group males and females. Lower body weights were noted in a dose-related manner for treated males and lower food consumption was noted for the 60 mg/kg/day group males. The mean body weight for the 60 mg/kg/day males was approximately 12% lower than the mean control value by the end of the dosing period and did not fully recover by the end of the 42-day recovery period. Reduced levels of circulating WBCs and lymphocytes were noted at all dose levels along with lower spleen weights in both sexes. Lymphoid depletion was documented in most spleen sections and in several mandibular lymph nodes at the 60 mg/kg/day dose level. Adrenal atrophy observed in histopathology correlated with reduced absolute and relative weight of adrenal glands. Clinical and anatomical pathology findings at the study week 32 recovery necropsy indicated that partial to full recovery was in progress in both genders administered all dose levels of INCB018424.

Study title: A 6-month oral (gavage) toxicity study with INCB018424 in beagle dogs with 6-week recovery period

Study no.:	(b) (4)-519049
	Sponsor study number: T07-10-07
Study report location:	M4.2.3.2.8, ncl-124-report.pdf, , page 1-779
Conducting laboratory and location:	(b) (4)
Date of study initiation:	26 September 2007
GLP compliance:	yes
QA statement:	yes (X) no ( )
Drug, lot #, and % purity:	INCB018424, SS-IN3-139, 99.9%

Key study findings:

- Unscheduled deaths were observed at 10 mg/kg;
- The causes of death were attributed to inflammatory lesions in the lung/thoracic cavity;
- All early death animals were also observed with bacterial infections of the lungs and/or the paws;
- Demodectic mange associated bacterial infections of the skin were observed in animals at 5 and 10 mg/kg groups;
- lower red blood cells, hematocrit and hemoglobin were observed in the 5 mg/kg/day group males and 10 mg/kg/day group males and females; higher absolute neutrophil and monocyte counts and lower absolute lymphocyte and eosinophil counts, was most prominent in the 10 mg/kg/day group males;
- Histopathology changes included lymph node depletion within follicular centers, granulomatous inflammation with parasitic mites in the regional lymph nodes, inflammatory cell infiltrates in the lymph nodes, and acute and/or subacute inflammation of the lung tissues at doses ≥5 mg/kg/day.

METHODS:

Doses:	0.5, 2.5, 5 and 10 mg/kg/day
Dose Justification:	Dosage levels were selected based on results from a previous 28-day dog study (Burns, 2007) in which 10 mg/kg/day was defined as the NOAEL dose
Controls:	0.5% methylcellulose in reverse osmosis-treated water
Species/strain:	Beagle dog

Number/sex/group:

Number of Animals/sex/group					
	Vehicle Control	**0.5 mg/kg/d**	**2.5 mg/kg/d**	**5.0 mg/kg/d**	**10mg/kg/d**
Main	5	5	3	3	5
Recovery	1-2	1-2	1-2	1 -2	1-2

Route, formulation, volume	Oral gavage. diluted in 0.5% methylcellulose in reverse osmosis-treated water, 10 mg/mL
Satellite groups used for toxicokinetics:	12/sex/group
Study design:	Male and female dogs were dosed daily via oral gavage for a total 6 months, followed by a 43 days recovery period (recovery groups)

OBSERVATIONS AND TIMES:

Mortality	Two times daily
Clinical examinations	daily
Detailed physical examinations	weekly
Body weights	weekly
Food consumption	Recorded daily, reported weekly
Hematology and coagulation	prior to randomization (study day -5) and on study days 27, 49, 86, 135 (hematology only), 174 and 224
Serum chemistry	prior to randomization (study day -5) and on study days 27, 49, 86, 174 and 224
Urinalysis	prior to randomization (study day -5) and on study days 27, 49, 86, 174 and 224
Urine metabolite profiling	on study days -8, 0 and 176
Ophthalmoscopy	during study weeks -2, 25 and 32
EKG	weeks -2, 25 and 31
Gross pathology:	All animals sacrifice
Organ weights:	All animals at sacrifice
Histopathology:	animals found dead or euthanized in extremis as well as all animals in the control (0 mg/kg/day) and high dose (10 mg/kg/day) groups. Adequate Battery: yes (x), no ( ) , Peer review: yes (x), no ( )
Toxicokinetics:	prior to dosing and at 0.5, 1, 2, 4, 8, 12 and 24 hours after dose administration on study days 0, 40 and 175

RESULTS:

Mortality: Three early deaths at 10 mg/kg/day.
1 female (# 4454): died on study day 126,
2 males: # 4411 was found dead on study day 179,
4430 was euthanized in extremis on study day 156.

Clinical signs:
Early death:
Male #4411: reddened facial area, clear to colored discharge from the eyes, soft feces and/or diarrhea were observed prior to death.
Male #4430: hypoactivity, prostration with labored respiration and swelling in the left hindlimb were noted. Other clinical observations noted prior to euthanasia included soft feces, diarrhea and clear and/or colored discharge from the eyes and warts on the left hindlimb.
Female #4454: Clinical observations prior to death included hypoactivity, increased respiration rate, labored respiration, diarrhea, swelling of the left and right forelimbs and hindlimbs and impaired use of the left and right hindlimbs.
Terminal death:
Test article-related clinical observations consisting of clear to colored discharge from the eyes, soft feces and/or diarrhea (females), reddened areas, swelling and hair loss on the limbs (which correlated to demodectic mange), were noted for the 5 and 10 mg/kg/day group males and females
Several animals in the 10 mg/kg/day group were also observed with interdigital cysts. Additionally, four animals at this dose had warts on the limbs and/or facial area.

Bodyweight: With the exception of the early death animals in the 10 mg/kg/day group which had some notable body weight changes (male #4430 lost 0.7 kg total; female #4454 lost 0.7 kg total), there were no obvious test article-related changes in mean body weights in any treated group when compared to the control group.
Food Consumption:
< week 19: unremarkable
19-26 weeks:
male: lower at 10 mg/kg, ↓8%-28% comparing to control group
female: dose-dependently reduced in all treatment groups
0.5 mg/kg-↓11%-22% comparing to control group
2.5 mg/kg- ↓4%-18% comparing to control group
5 mg/kg- ↓11%-17% comparing to control group
10 mg/kg--↓11%-28% comparing to control group
26-32 weeks:
male: lower at 10 mg/kg, ↓31%-51% comparing to control group
female: 0.5 mg/kg-↓10%-42% comparing to control group
2.5 mg/kg- ↓41%-↑9% comparing to control group
5 mg/kg- ↓12%-37% comparing to control group
10 mg/kg--↓42%-↑17% comparing to control group

Hematology:

MALES	Percent Change From Vehicle Control					
5 mg/kg/day						
Assessment Day	27	49	86	135	174	224
RBC	↓8	↓4	↓10	↓9	↓14	
Hemoglobin	↓6		↓10	↓9	↓16	
Hematocrit	↓7		↓10	↓9	↓15	
Reticulocyte	↓27	↓29	↓25	↓29	↑15	↓10
Neutrophils	↑29	↑23	↓6	↑5	↑9	↓5
Lymphocytes	↓13	↓11	↓11		↓14	
Monocytes	↑49	↑32	↓17	↑15	↑31	↑43
Eosinophils	↓17	↓18	↓59	↓35	↓36	
Basophils	↓50		↓50			↑50
10 mg/kg						
RBC	↓11	↓13	↓16	↓20	↓27	↓9
Hemoglobin	↓10	↓13	↓18	↓22	↓33	↓17
Hematocrit	↓11	↓14	↓17	↓21	↓30	↓16
Reticulocyte	↓32	↓17	↓30	↓12	↑22	↑11
Neutrophils	↑22	↑16	↑30	↑71	↑64	↓23
Lymphocytes	↓21	↓29	↓23	↓13	↑28	↓33
Monocytes	↑37	↑21	↑46	↑70	↑102	↑60
Eosinophils	↓48	↓53	↓64	↓30	↓59	↓29
Basophils	↓25	↓33	↓50	↑8		↑38
LUC			↑20	↑100	↑50	↓63
FEMALES	**Percent Change From Vehicle Control**					
5 mg/kg/day						
Assessment Day	27	49	86	135	174	224
RBC					↓8	
Hemoglobin				↓6	↓9	
Hematocrit				↓5	↓9	
Reticulocyte	↓18		↓18	↓30	↑47	↓21
Monocytes			↓10	↑12	↑20	↓26
Eosinophils	↓41	↓68	↓57	↓49	↓80	↑74
Basophils		↑67	↑67	↑100	↑50	↑100
LUC	↑85	↑71	↑63	↑113	↑83	
10 mg/kg/day						
RBC			↓6	↓7	↓14	↑8
Hemoglobin	↓5	↓ 8	↓11	↓11	↓19	
Hematocrit		↓6	↓9	↓10	↓17	
Reticulocyte	↑5	↓15	↓12	↓12	↑32	↑226
Neutrophil			↓10	↑11	↑10	↓15
Monocytes	↑16	↑23	↑17	↑42	↑76	↓27

Eosinophils	↓59	↓65	↓57	↓55	↓73	↑79
Basophils		↑33	↑33	↑33	↑50	
LUC	↑114	↑171	↑50	↑137	↑117	↓33

Day 224 represents the recovery data

Summary:

- Test article-related effects included lower red blood cell counts, hemoglobin and hematocrit in the 5 mg/kg/day group males and the 10 mg/kg/day group males and females. Absolute reticulocyte counts were also lower in spite of lower red cell mass, which suggested a nonregenerative anemia and appeared to be reflective of a test article-related suppression of erythropoiesis. The study results showed a trend over time toward higher absolute neutrophil and monocyte counts (suggesting inflammation) and lower absolute lymphocyte and eosinophil counts, most prominent in the 10 mg/kg/day group males. Full or partial recovery was evident in hematologic parameters on study day 224.

Clinical Chemistry: unremarkable
Urinalysis: unremarkable
Ophthalmoscopy: unremarkable
EKG: unremarkable
Organ Weight: unremarkable

Gross Pathology:

Early deaths:

Male #4411: Reddened areas of the gastrointestinal tract, thickened heart and over 300 mL of red fluid in the thoracic cavity were observed macroscopically.

Male #4430: Reddened areas in the gastrointestinal tract, dark red and white discolorations on the lungs, lungs not fully collapsed and enlarged thyroid glands were noted.

Female #4454: Reddened areas in the gastrointestinal tract, dark red and white discolorations on the lungs, small and pale spleen, pale thyroid glands and approximately 100 mL of red fluid in the thoracic cavity were noted

Terminal deaths: The following summary table was excerpted from the submission

Toxicologically Relevant Gross Necropsy Observations

Organ	Observation	Dosage Level(s) (mg/kg/day)	Sex	Interval(s)
Skin or Ears	Parasitic mite-related Scabbing/Discoloration or Swelling	5.0, 10 10	M F	PN, RN PN, RN
Skin or Ears	Papilloma-related Nodule(s)	10	M/F	PN
Lungs	Acute to subacute inflammation-related Discolorations	5.0, 10 5.0	M F	PN
Prostate	Small	10	M	PN
Female Reproductive Organs	Diestrus-related gross findings or comments	5.0, 10	F	PN

M = Males, F = Females, PN = Primary Necropsy, RN = Recovery Necropsy

Histopathology:

Early death animals:

Male #4411: Variable lymphoid depletion in lymphoid organs and parasitic mite-related changes in the skin and mandibular lymph node were noted microscopically. Acute inflammation within the thoracic cavity was considered to be the cause of death.

Male #4430: moderate acute inflammation in the lungs morphologically consistent with a bacterial bronchopneumonia, which was considered to be the cause of death, and variable lymphoid depletion in the lymphoid organs were noted.

Female #4454: lymphoid depletion was evident in most sections of lymphoid tissue. Moderate bone marrow hypocellularity was noted. The cause of death was attributed to multifocal necrosis (with acute inflammation and bacteria) in the lung. Acute inflammation, necrosis and hemorrhage were noted in all 4 paws. There was also evidence of bacterial colonization of areas of skin with inflammation.

Terminal animals: Minimal-to-moderate acute or subacute inflammation was noted in the lungs of occasional dogs administered ≥ 2.5 mg/kg/day. Parasitic mites without inflammation were noted in one 2.5 mg/kg/day female. Lymphoid depletion within follicular centers and/or more generalized lymphoid depletion, and granulomatous inflammation with parasitic mites in regional lymph nodes were noted in animals at 5 and 10 mg/kg groups. Areas of inflammation occasionally included the

presence of bacterial colonization. Squamous cell papilloma with basophilic intranuclear inclusion bodies in the skin was noted in 2 dogs (1 male and 1 female) administered 10 mg/kg/day of the drug.

In the reproductive organs, prostate hypoplasia/atrophy was noted in 1 dog administered 5 mg/kg/day and 2 dogs administered 10 mg/kg/day. In the female dogs, the diestrus stage of the estrous cycle was noted in 1, 1, and 3 females administered 0.5, 5 and 10 mg/kg/day, respectively. Estrus was noted in 1 female administered 0.5 mg/kg/day and in 1 female administered 5 mg/kg/day. All control group females were in either anestrus or proestrus. A lack of regressing/residual corpora lutea and minimal mammary gland development in most females suggested that these dogs were either sexually immature or in their first estrous cycle.

At the recovery examination, pyogranulomatous inflammation in the skin, typically associated with mite and/or bacterial infestation, was noted in males and females that had been administered 5 or 10 mg/kg/day, with extension of inflammation into the mandibular lymph node. One control group female and 5 of 7 females administered INCB018424 were in diestrus.

Toxicokinetics: refer to PK section

STUDY SUMMARY:

INCB018424 was administered to male and female dogs via oral gavage at doses of 0, 0.5, 2.5, 5, and 10 mg/kg/day daily for 6 months, followed by a 6-week recovery period. Systemic toxicity was observed at dosage levels of 5 and 10 mg/kg/day. Test article-related effects included 3 unscheduled deaths in the 10 mg/kg/day group animals with clinical findings of reddened areas, swelling and hair loss on the limbs, diarrhea and/or soft feces and clear and/or colored discharge from the eyes. The causes of death in all 3 dogs were attributed to inflammatory lesions in the lung/thoracic cavity (acute inflammation in both males and lung necrosis in the female). Generalized lymphoid depletion, granulomatous inflammation with parasitic mites, inflammatory cell infiltrates in the lymph nodes and acute and/or subacute inflammation of the lungs were noted microscopically in the ≥5 mg/kg/day group animals. Microscopic findings consisted of demodectic mange in one 2.5 mg/kg/day group animal.

Study title: 52-week oral gavage chronic toxicity and toxicokinetic study with INCB018424 in dogs with a 6-week recovery period

Study no.: 7456-271
Sponsor study number: T08-07-03
Study report location: Electronic submission, page 1-1106
Conducting laboratory and location: (b) (4)
Date of study initiation: 02 July 2008
GLP compliance: yes
QA statement: yes (X) no ( )
Drug, lot #, and % purity: INCB018424, BPR-07-101-B2-21, 97.8%

Key study findings:

- Unscheduled deaths were observed at 6 mg/kg;
- Dogs died of clinical demodicosis;
- Treatment with INC018424 resulted in reduced mean absolute lymphocytes, eosinophils, and red cell mass parameters;
- Histopathology changes included decreased lymphocytes in the gut-associated lymphoid tissue (GALT) of the ileum, in the cortex of the mandibular and mesenteric lymph nodes, and in the white pulp of the spleen;
- Treatment-related changes were reversible.

METHODS:

Doses:	0.75, 1.5, 3, and 6 mg/kg/day
Dose Justification:	Dosage levels were selected based on results from a previous 28-day dog study (Burns, 2007) in which 10 mg/kg/day was defined as the NOAEL dose
Controls:	0.5% methylcellulose in reverse osmosis-treated water
Species/strain:	Beagle dog
Number/sex/group:	

Number of Animals/sex/group					
	Vehicle Control	**0.75 mg/kg/d**	**1.5 mg/kg/d**	**3 mg/kg/d**	**6 mg/kg/d**
Main	5	5	5	5	5
Recovery	2	2	2	2	2

Route, formulation, volume	Oral gavage. diluted in 0.5% methylcellulose in reverse osmosis-treated water, 10 mg/mL
Satellite groups used for toxicokinetics:	none

Study design:	Male and female dogs were dosed daily via oral gavage for a total 52 weeks, followed by a 6-week recovery period (recovery groups). Note: male group at 6 mg/kg were terminated early (dosing phase week 29, dosing phase day 197)

OBSERVATIONS AND TIMES:

Mortality	Two times daily
Clinical examinations	daily
Detailed physical examinations	weekly
Body weights	predose phase, weekly during the dosing phase, on the first day of recovery phase, and weekly thereafter during the recovery phase.
Food consumption	1-week interval during the predose phase, weekly during the dosing phase, on the first day of recovery phase, and weekly thereafter during the recovery phase
Hematology and coagulation	prior to randomization (study day -5) and on study days 27, 49, 86, 135 (hematology only), 174 and 224
Serum chemistry	during the predose phase and once during dosing phase weeks 4, 12, 26, 39, and 52 (excluding Group 5 males) of the dosing phase additionally, a hematology sample was collected from Group 5 Male No. H49124 on dosing phase day 169 approximately 24 hours postdose (based on dosing time from dosing phase day 168) Blood and urine samples were collected prior to the early sacrifice of the surviving group 5 males on dosing phase day 197
Urinalysis	once during the predose phase and once during dosing phase weeks 4, 12, 26, 39, and 52 (excluding Group 5 males) Blood and urine samples were collected prior to the early sacrifice of the surviving Group 5 males on Dosing Phase Day 197
Urine metabolite profiling	Urine samples were collected once prior to dosing, on day 1, and during dosing phase weeks 4, 26, and 52 (excluding Group 5 males) of the dosing phase
Ophthalmoscopy	during the predose phase, during week 52 of the dosing phase (excluding Group 5 males),

	and during week 6 of the recovery phase (excluding Group 5 males)
EKG	Recorded once during the predose phase, during Week 52 of the dosing phase (excluding Group 5 males), and during week 6 of the recovery phase (excluding Group 5 males)
Gross pathology:	All animals at sacrifice
Organ weights:	All animals at sacrifice
Immunophenotype	all animals during Week 26 (in conjunction with the clinical pathology samples).
Histopathology:	All animals at sacrifice Adequate Battery: yes (x), no ( ) Peer review: yes (x), no ( )
Toxicokinetics:	day 1 and during weeks 4, 13, 26, and 52 (excluding Group 5 males) of the dosing phase within 1 hour of dosing and approximately 0.5, 1, 2, 4, 8, 12, and 24 hour postdose. Additionally, a sample was collected from Group 5 Male No. H49124 on Dosing Phase Day 169 approximately 24 hours postdose (based on dosing time from Dosing Phase Day 168). Surviving Group 5 males were bled on Dosing Phase Day 196 (Week 28) within 1 hour of dosing and approximately 0.5, 1, 2, 4, 8, 12, and 24 hours postdose. The 12 and 24 hour samples were collected from fasted dogs.

RESULTS:

Mortality: four early deaths at 6 mg/kg/day.
1 female: on study day 197; moribund sacrifice
3 males: on study day 197; moribund sacrifice
The moribund condition was attributed to inflammation of the skin/subcutis and footpad

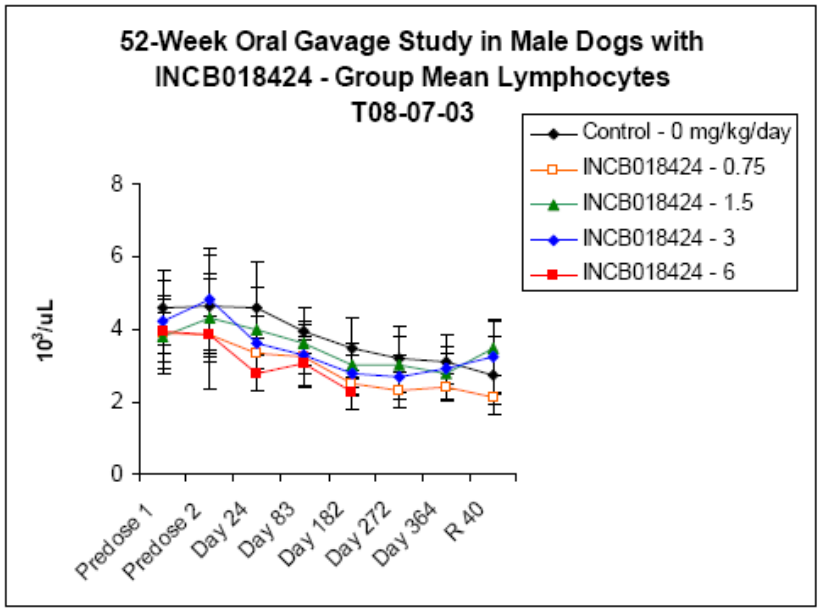
Clinical signs:
Early death: cage sores, alopecia,black skin, broken skin, dry skin, red skin, scabs, interdigital cysts, interdigital furunculosis, papillomatosis, and pododermatitis
Male #4411: reddened facial area, clear to colored discharge from the eyes, soft feces and or diarrhea were observed prior to death.
Male #4430: hypoactivity, prostration with labored respiration and swelling in the left hindlimb were noted. Other clinical observations noted prior to

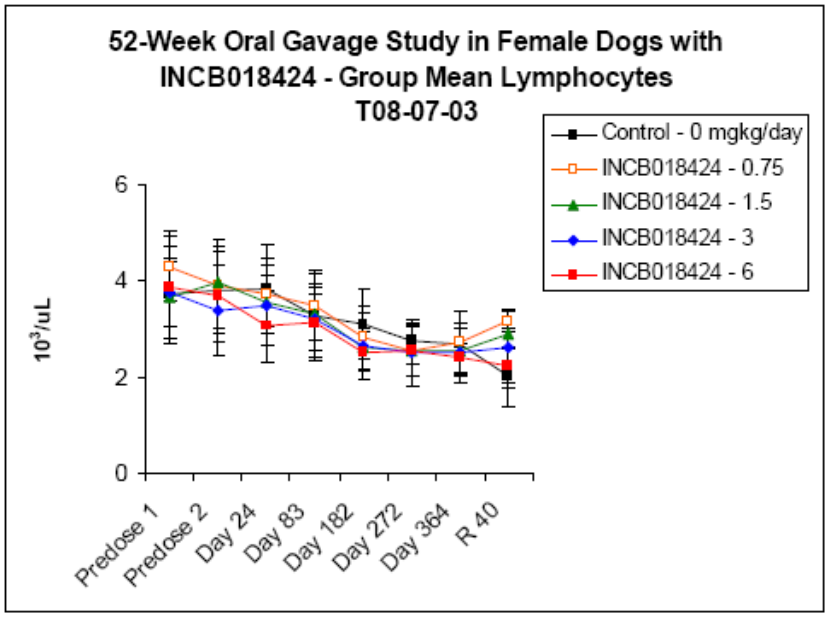
euthanasia included soft feces, diarrhea and clear and/or colored discharge from the eyes and warts on the left hindlimb.

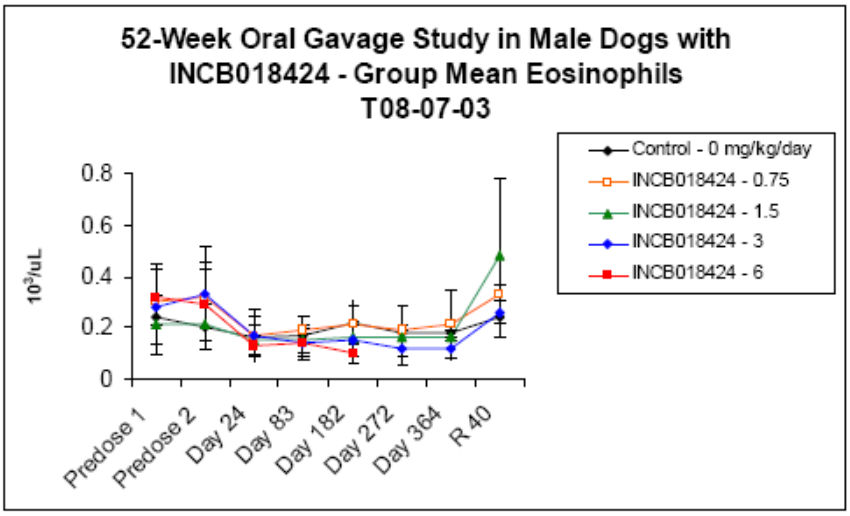
Female #4454: Clinical observations prior to death included hypoactivity, increased respiration rate, labored respiration, diarrhea, swelling of the left and right forelimbs and hindlimbs and impaired use of the left and right hindlimbs.

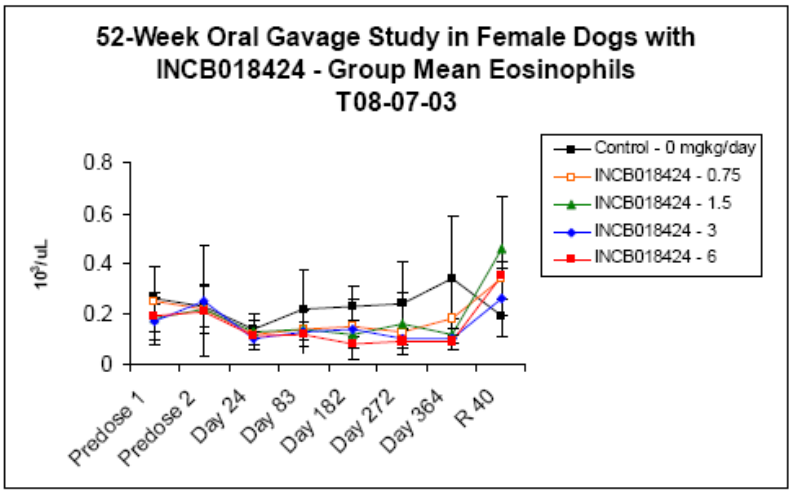
Terminal sacrifices: unremarkable

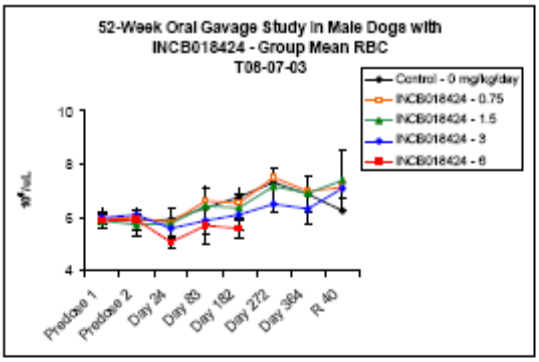
Bodyweight: unremarkable
Food Consumption: unremarkable
Hematology:

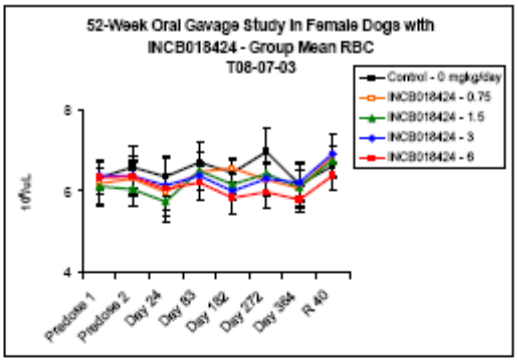


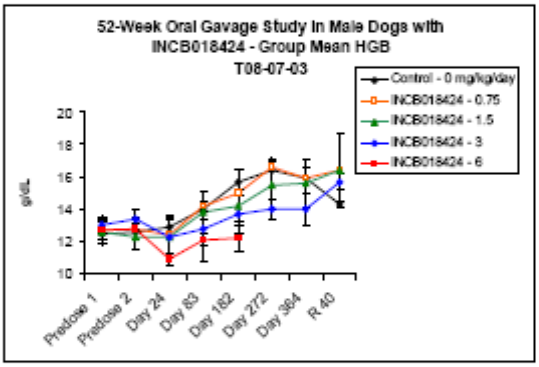


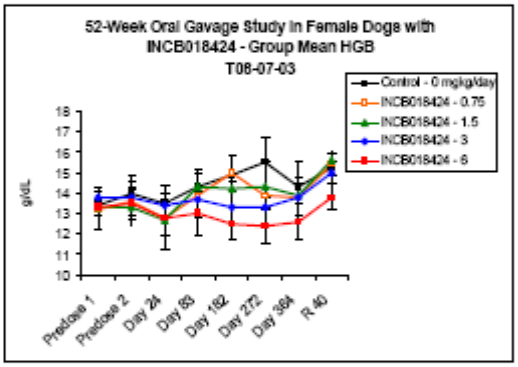

52-Week Oral Gavage Study in Male Dogs with
INCB018424 - Group Mean Eosinophils
T08-07-03
Control - 0 mg/kg/day
INCB018424 - 0.75
INCB018424 - 1.5
INCB018424 - 3
INCB018424 - 6
10³/uL
0.8
0.6
0.4
0.2
0
Predose 1
Predose 2
Day 24
Day 83
Day 182
Day 272
Day 364
R 40

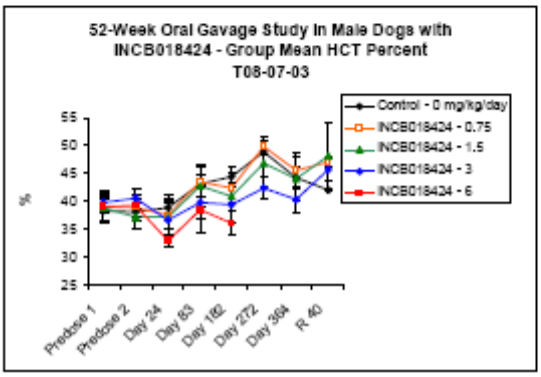

52-Week Oral Gavage Study in Female Dogs with
INCB018424 - Group Mean Eosinophils
T08-07-03
Control - 0 mgkg/day
INCB018424 - 0.75
INCB018424 - 1.5
INCB018424 - 3
INCB018424 - 6
10³/uL
0.8
0.6
0.4
0.2
0
Predose 1
Predose 2
Day 24
Day 83
Day 182
Day 272
Day 364
R 40

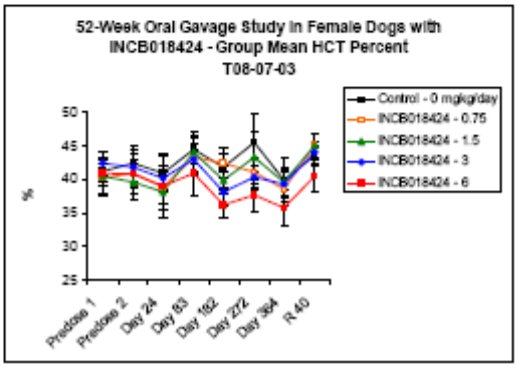












Summary:

- Decreased red cell parameters including decreased red cell count (↓up to 17%), hemoglobin concentration (↓up to 22%) , and/or hematocrit percentage (↓up to 18%) was observed in both males and females, which was generally present in males from Day 24 through Day 197 of the dosing phase, and in females starting from Day 24 throughout the dosing phase. There was recovering trends in females, but not fully recovered by the end of the recovery period (day 40 of recovery phase);
- Decreased eosinophil counts were observed in females dogs at all dose levels from Day 83 (↓up to 45%), and males at doses of ≥ 3 mg/kg/day from Day 182 (↓up to 55%). These changes in eosinophil counts were reversed by Day 40 of the recovery phase;
- Lymphocyte counts of males given 6 mg/kg/day were decreased from Day 24 through Day 197 of the dosing phase (up to↓35%);

- Other hematologic changes included an increase of neutrophil and monocyte counts (↑up to 40%) from Days 182 through 197 in males at 6 mg/kg/day, suggesting inflammation.

Notes:

1) Compensatory increase in reticulocytes was not observed;
2) Decreases in lymphocytes correlated with the microscopic observations of decreased lymphocytes in gut-associated lymphoid tissue, mandibular and mesenteric lymph nodes. Decreased lymphocyte counts also correlated with decreases in total T cells, CD4+ cells and CD8+ cells in males;
3) Increased neutrophil and monocyte counts of males from Days 182 through 197 correlated with the microscopic observation of cutaneous pyogranulomatous inflammation associated with demodex mange mites.

Clinical Chemistry: unremarkable
Urinalysis: unremarkable
Ophthalmoscopy: unremarkable
EKG: unremarkable
Organ Weight: unremarkable

Gross Pathology: Abrasion, discoloration, scaling, thickening, and/or alopecia of the skin/subcutis and footpad were observed in animals and females at doses ≥ 3 mg/kg, including early deaths. Lymph node enlargement (mandibular and other) was observed in two males given 6 mg/kg/day. Footpad abrasions were present at the end of the recovery phase, other findings were recovered.

Histopathology:

Early death animals: The microscopic findings in the four moribund animals were similar to those in the other animals given 6 mg/kg/day (see below), except minimal acute inflammation of the liver (Animal No. H49124), minimal hypocellularity (decreased neutrophil storage pool) of sternal bone marrow (Animal No. H49125), minimal acute inflammation of the mandibular lymph node (Animal No. H49128), and moderate acute fibrinous inflammation of the mesenteric lymph node (Animal No. H49162).

Note: Special histologic staining procedures for microorganisms (Periodic acid-Schiff and the Gram stains, Brown and Brenn and Brown and Hopps) did not reveal a cause for the inflammation in the mesenteric lymph node. Above findings in early dearth animals were considered secondary to the marked inflammation of the skin/subcutis and/or footpad present in these animals, which in the case of Animal Nos. H49124 and H49162 may have been accompanied by the systemic spread of opportunistic pathogens.

All animals including early death animals and scheduled sacrificed animals:

The following table is excerpted from the submission, the data have been verified by the reviewer.

Text Table 1: Significant Microscopic Findings, Dosing Phase (Including Unscheduled Deaths)
Incidence (Mean Severity)

Sex	Males					Females				
Number Examined	5	5	5	5	7	5	5	5	5	5
Dose Level (mg/kg/day)	0	0.75	1.5	3	6	0	0.75	1.5	3	6
Organ										
Ileum- Decreased Lymphocytes, Gut-Associated Lymphoid Tissue	-	-	-	5(1.0)	7(1.0)	-	-	-	1(0.2)	4(1.6)
Lymph node, Mandibular- Decreased Lymphocytes, Cortex	-	-	-	-	-	-	-	-	-	1(0.2)
Inflammation, Pyogranulomatous	-	-	-	-	2(0.9)	-	-	-	-	-
Lymph node, Other[a]- Inflammation, Pyogranulomatous	ne	ne	ne	ne	1(2.0)	ne	ne	ne	ne	ne
Spleen- Decreased Lymphocytes, White Pulp	-	-	-	-	-	-	-	-	-	1(0.2)
Lymph node, Mesenteric- Decreased Lymphocytes, Cortex	-	-	-	-	2(0.3)	-	-	-	-	1(0.2)
Skin/Subcutis- Inflammation, Pyogranulomatous	-	-	-	2(1.0)	6(2.7)	-	-	-	-	4(2.0)
Mites, Intrafollicular	-	-	-	1(P)	5(P)	-	-	-	-	4(P)
Hyperkeratosis	-	-	-	2(1.2)	-	-	-	-	1(1.6)	-
Foot/Footpad[a]- Inflammation, Pyogranulomatous	ne	ne	ne	1(3.0)	3(4.0)	ne	ne	-	3(3.3)	-
Mites, Intrafollicular	ne	ne	ne	1(P)	3(P)	ne	ne	-	1(P)	-
Rectum, Perianal Skin- Inflammation, Pyogranulomatous	-	-	-	-	2(0.6)	-	-	-	1(0.4)	3(1.2)
Mites, Intrafollicular	-	-	-	-	2(P)	-	-	-	-	-

a = Not required by protocol (the number examined ranged from 1-3)
- = Not present
ne = Not examined
( ) = Grading scale: 1 = minimal; 2 = slight; 3 = moderate, 4 = marked, 5 = severe, P = Present

Recovery animals:

The following table from the submission, the data have been verified by the reviewer.

Text Table 2: Significant Microscopic Findings, Recovery Phase (Including Unscheduled Deaths)
Incidence (Mean Severity)

Sex	Males					Females				
Number Examined	2	2	2	2	0	2	2	2	2	2
Dose Level (mg/kg/day)	0	0.75	1.5	3	6	0	0.75	1.5	3	6
Organ										
Ileum- Decreased Lymphocytes, Gut-Associated Lymphoid Tissue	-	-	-	1(0.5)	ne	-	-	-	1(0.5)	1(0.5)
Lymph node, Mandibular- Decreased Lymphocytes, Cortex	-	-	-	-	ne	-	-	-	-	-
Inflammation, Pyogranulomatous	-	-	-	-	-	-	-	-	-	-
Lymph node, Other[a]- Inflammation, Pyogranulomatous	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
Spleen- Decreased Lymphocytes, White Pulp	-	-	-	-	ne	-	-	-	-	-
Lymph node, Mesenteric- Decreased Lymphocytes, Cortex	-	-	-	-	ne	-	-	-	-	1(0.5)
Skin/Subcutis- Inflammation, Pyogranulomatous	-	-	-	-	ne	-	-	-	-	-
Mites, Intrafollicular	-	-	-	-	ne	-	-	-	-	-
Hyperkeratosis	-	-	-	-	ne	-	-	-	-	-
Foot/Footpad[a]- Inflammation, Pyogranulomatous	ne	ne	ne	1(4.0)	ne	-	-	1(3.0)	1(3.0)	2(3.5)
Mites, Intrafollicular	ne	ne	ne	-	ne	-	-	-	1(P)	1(P)
Rectum, Perianal Skin- Inflammation, Pyogranulomatous	-	-	-	-	ne	-	-	-	1(1.0)	1(1.0)
Mites, Intrafollicular	-	-	-	-	ne	-	-	-	-	-

a = Not required by protocol (the number examined ranged from 1-3)
- = Not present
ne = Not examined
( ) = Grading scale: 1 = minimal; 2 = slight; 3 = moderate, 4 = marked, 5 = severe, P = Present

Summary:

- A test article-related decrease in lymphocytes was noted microscopically in some organs of males and females given >3 mg/kg/day. The decrease in lymphocytes was most consistently evident in the gut-associated lymphoid tissue (GALT) of the ileum. Pyogranulomatous inflammation of the skin/subcutis (including the perianal skin present with the section of rectum) and footpad affected most of the animals given 6 mg/kg/day and some of those given 3 mg/kg/day. Decreased lymphocytes and pyogranulomatous inflammation of the skin/subcutis were reversible, but not fully recovered at the end of recovery phase.

Toxicokinetics: refer to PK section

STUDY SUMMARY:

INCB018424 caused death when it was given to dogs daily at the dose of 6 mg/kg. Treatment lowered mean absolute lymphocytes, reduced eosinophil counts and decreased red cell parameters. Histopathology changes included decrease of lymphocytes in the gut-associated lymphoid tissue (GALT) of the ileum, in the cortex of the mandibular and mesenteric lymph nodes, and in the white pulp of the spleen. The development of generalized demodicosis, a secondary effect based of INCB018424, was considered adverse at dose levels of 3 and 6 mg/kg/day.

Histopathology inventory

Study	(b) (4)-519048	(b) (4)-519049	7456-271
Species	**Rat**	**Dog**	**Dog**
Adrenals	x*	x*	x*
Aorta	x	x	x
Bone Marrow smear	x	x	x
Bone (femur)	x	x	x
Brain	x*	x*	x*
Cecum	x	x	x
Cervix	x	x	x
Colon	x	x	x
Duodenum	x	x	x
Epididymis	x	x	x
Esophagus	x	x	x
Eye	x	x	x
Fallopian tube			
Gall bladder			
Gross lesions	x	x	x
Harderian gland	x	x	x
Heart	x*	x*	x*
Ileum	x	x	x
Injection site	x		
Jejunum	x	x	x
Kidneys	x*	x*	x*
Lachrymal gland	x	x	x
Larynx		x	x
Liver	x*	x*	x*
Lungs	x*	x*	x*
Lymph nodes, cervical			
Lymph nodes mandibular	x	x	x
Lymph nodes, mesenteric	x	x	x
Mammary Gland			
Nasal cavity			
Optic nerves	x		
Ovaries	x	x	x
oviducts	x	x	x
Pancreas	x	x	x
Parathyroid	x		
Peripheral nerve			
Peyer's patches	x		

Pharynx		x	x
Pituitary	x*	x*	x*
Prostate	x	x	x
Rectum	x	x	x
Salivary gland	x	x	x
Sciatic nerve	x	x	x
Seminal vesicles	x	x	x
Skeletal muscle	x	x	x
Skin	x	x	x
Spinal cord	x	x	x
Spleen	x*	x*	x*
Sternum		x	x
Stomach	x	x	x
Teeth			
Testes	x	x	x
Thymus	x*	x*	x*
Thyroid	x*	x*	x*
Tongue	x	x	x
Trachea	x	x	x
Urinary bladder	x	x	x
Uterus	x	x	x
Vagina	x	x	x
Zymbal gland			

X, histopathology performed
*, organ weight obtained

7 Genetic Toxicology

***In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)**

Study title: INCB018424: *Salmonella* plate incorporation mutagenicity assay
- reviewed by Dr. Barbara Hill under IND 77,455

Key findings: INCB01824 (free base) was not mutagenic in the *Salmonella* plate incorporation assay, under the conditions of this experiment.

Study no.: AB22ZU.501.BTL
Sponsor study no.: T06-01-electronic submission
Conducting laboratory: (b) (4)
Date of study initiation: 1-16-06
GLP compliance: No
QA reports: No
Drug, lot #, and % purity: INCB01824 (free base), Lot# 003
Vehicle: DMSO
Methods
Strains/species/cell line: *Salmonella typhimurium* strains TA98 and TA100

Concentrations used in definitive study: 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg/plate (±S9; S9 derived from Aroclor 1254 induced rat liver homogenate); 2 plates/dose

Basis of concentration selection: No range finding study was performed. No appreciable toxicity or precipitate were noted at concentrations used in the definitive study.
Negative controls: DMSO

Positive controls:
1) TA 98 strain – 2-Nitrofluorene (-S9, 1 µg/plate) and 2-aminoanthracene (+S9, 1 µg/plate)
2) TA 100 strain – Sodium azide (-S9, 1 µg/plate) and 2-aminoanthracene (+S9, 1 µg/plate)
Incubation and sampling times: Plates were incubated at 37 ± 2ºC for 2 days (*S. typhimurinum*) after treatment. Plates were counted for colony formation by hand after completion of the incubation period.

Results
Study validity:

A test article was considered to be positive if it produced at least a 2-fold increase in the mean revertants per plate for tester strains TA98 or TA100 and this increase in the mean number of revertants per plate had to be accompanied by a dose response to increasing concentrations of the test article.

Solvent control mean reversion frequencies fell within established ranges. Positive control results were appropriate showing a mean reversion frequency that was three times or more greater than the mean reversion frequency of the solvent control

plates. The dose range selected for the definitive study was appropriate according to ICH guidelines.

Study outcome:

The test article produced a negative response in the presence and absence of S-9 activation. All of the tester strains treated with the test article exhibited a mean reversion frequency that was similar to the corresponding solvent control.

Study title *Salmonella-Escherichia coli*/mammalian-microsome reverse mutation assay with a confirmatory assay

- reviewed by Dr. Barbara Hill under IND 77,455

Key findings: INCB01824 phosphate was not mutagenic in the Ames test, under the conditions of this experiment.

Study no.: 7456-188
Sponsor study no.: T06-08-01
Volume #, and page #: electronic submission
Conducting laboratory: (b) (4)
Date of study initiation: 7-24-06
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: INCB01824 phosphate, Lot# 009
Vehicle: DMSO
Methods

Strains/species/cell line: *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537; *Escherichia coli* strain WP2 *uvr*A

Concentrations used in definitive study: 33.3, 100, 333, 1000, 2500 and 5000 µg/plate (±S9; S9 derived from Aroclor 1254 induced rat liver homogenate) in all tester strains; 3 plates/dose. A confirmatory assay was conducted using the same doses used in the definitive study.

Basis of concentration selection: The range finding study conducted with 6.67, 10.0, 33.3, 66.7,100, 333, 337, 1000, 3330 and 5000 µg/plate for tester strainsTA98 and WP2*uvr*A (±S9; S9 derived from Aroclor 1254 induced rat liver homogenate); 1 plate/dose. No precipitate was noted at any concentration. Very slight toxicity was noted at 5000 µg/plate in TA98 and no toxicity was noted at 5000 µg/plate in WP2*uvr*A. The maximum concentration used in the definitive study was 5000 µg/plate for tester strains based on the results of the range finding study.

Negative controls: DMSO

Positive controls: Provided in following table

Test strain	S9 mix	Positive control	Dose (μg/plate)
TA 98	+	Benzo[a]pyrene	2.5
TA 98	–	2-nitrofluorene	1.0
TA 100	+	2-aminoanthracene	2.5
TA 100	–	Sodium azide	2.0
TA 1535	+	2-aminoanthracene	2.5
TA 1535	–	Sodium azide	2.0
TA 1537	+	2-aminoanthracene	2.5
TA 1537	–	ICR-191	2.0
WP2*uvr*A	+	2-aminoanthracene	25
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0

Incubation and sampling times: Plates were incubated at 37 ± 2ºC for 2 days (*S. typhimurinum*) or 3 days (*E. coli*) after treatment. Plates were counted for colony formation by automated colony counter and/or by hand after completion of the incubation period.

Results

Study validity:

A test article was considered to be positive if it produced at least a 2-fold increase in the mean revertants per plate for tester strains TA98, TA100 or WP2*uvr*A or if it produced at least a 3-fold increase in mean revertants per plate for tester strains TA1535 or TA1537. This increase in the mean number of revertants per plate had to be accompanied by a concentration-response to increasing concentrations of the test article. Solvent control mean reversion frequencies fell within established ranges. Positive control results were appropriate showing a mean reversion frequency that was three times or more than the mean reversion frequency of the solvent control plates. The c range selected for the definitive study was appropriate according to ICH guidelines.

Study outcome:

The test article produced a negative response in the presence or absence of S-9 activation. All tester strains treated with the test article exhibited a mean reversion frequency that was similar to the corresponding solvent control.

***In Vitro* Assays in Mammalian Cells**

Study title INCB018424: Chromosomal aberrations in cultured human peripheral blood lymphocytes

reviewed by Dr. Barbara Hill under IND 77,455

Key findings: INCB01824 was negative in the human blood peripheral lymphocyte assay with or without S9 activation, under the conditions of this assay.

Study no.: 7456-187
Sponsor study no.: T06-08-02
Volume #, and page #: electronic submission

Conducting laboratory: (b) (4)
Date of study initiation: 7-24-06
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: INCB01824 phosphate, Lot# 009
Vehicle: DMSO
Methods
Strains/species/cell line: Human peripheral blood lymphocytes

Doses used in definitive study:

In the initial chromosomal assay, concentrations of 5.43, 7.75, 11.1, 15.8, 22.6, 32.3, 46.1, 65.9, 94.1, 134, 192, 274, 392, 560 and 800 µg/ml INCB01824 phosphate were used for a 3-hr incubation (± S9); 2/concentration. Cultures treated with concentrations of 22.6, 32.3, 46.1 and 65.9 µg/ml INCB01824 phosphate without metabolic activation and 15.8, 22.6, 32.3 and 94.1 µg/ml INCB01824 phosphate with metabolic activation were analyzed for chromosomal aberrations.

In the confirmatory chromosomal assay, concentrations of 1.25, 2.50, 5.00, 10.0, 20.0, 40.0, 65.0, 80.0, 95.0 and 110 µg/ml INCB01824 phosphate were used for a 22-hr incubation (-S9) and concentrations of 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 65.0, 80.0, 95.0, 110, 125 and 150 µg/ml INCB01824 phosphate were used for a 3 hr incubation (+S9); 2/concentration. Cultures treated with concentrations of 10.0, 20.0, 50.0 and 65.0 µg/ml INCB01824 phosphate without metabolic activation and 10.0, 20.0, 50.0 and 95.0 µg/ml INCB01824 phosphate with metabolic activation were analyzed for chromosomal aberrations.

Basis of concentration selection:
The concentrations selected for analysis of chromosomal aberrations was based on the level of toxicity noted for the incubations. The high concentration was selected so that at least 50% reduction in the mitotic index relative to the solvent control was noted. For the initial chromosomal assay, the 65.9 µg/ml INCB01824 phosphate concentration exhibited a 56% mitotic index reduction for the 3-hour incubation (-S9) and the 94.1 µg/ml INCB01824 phosphate concentration exhibited a 51% mitotic index reduction for the 3-hour incubation (+S9). For the confirmatory chromosomal assay, the 65.0 µg/ml INCB01824 phosphate concentration exhibited a 55% mitotic index reduction for the 22-hour incubation (-S9) and the 95.0 µg/ml INCB01824 phosphate concentration exhibited a 54% mitotic index reduction for the 3-hour incubation (+S9).

Negative controls: DMSO

Positive controls: Mitomycin C (-S9): 0.75, 1.0 and 1.5 µg/ml for 3 hour exposure, 0.2, 0.3, and 0.4 µg/ml for 22 hour exposure.
Cyclophosphamide (+S9): 20, 25 and 40 µg/ml for 3 hour exposure

Incubation and sampling times:

Cell cultures were incubated with test article ± S9 for 3 hours and harvested 22 hours after treatment initiation. Cell cultures were incubated with test article –S9 for 22

hours and then harvested for analysis. Slides were prepared from the harvested cultures and stained with 5% Giemsa solution for the analysis of mitotic index and chromosomal aberrations.

Results
Study validity:

A test article was considered to be positive for inducing chromosomal aberrations if a significant increase (the difference was considered significant when $p \leq 0.05$) in the number of cells with chromosomal aberrations is observed at one or more concentrations and a linear trend test demonstrates a concentration-response relationship. Solvent control mean reversion frequencies fell within established ranges. Positive control results were appropriate for this assay. The range of concentrations selected for the initial and confirmatory assays were appropriate according to ICH guidelines.

Study outcome:
No significant increase in cells with chromosomal aberrations was noted in the analyzed cultures.

***In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)**

Study title INCB018424: In vivo rat bone marrow micronucleus assay
reviewed by Dr. Barbara Hill under IND 77,455

Key findings: INCB01824 was negative in the in vivo rat micronucleus assay, under the conditions of this experiment.

Study no.: 7456-209
Sponsor study no.: T06-10-02
Volume #, and page #: electronic submission
Conducting laboratory: (b) (4)
Date of study initiation: 9-27-06
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: INCB01824 phosphate, Lot# SS-IN2-175
Vehicle: 0.5% methylcellulose
Methods
Strains/species/cell line: Sprague-Dawley rats; 9 weeks; males: 266-304 g; females:196-233 g

Doses used in definitive study:
Single oral (gavage) doses of 0 (vehicle), 62.5, 125, 250 mg/kg INCB01824 phosphate; 10 ml/kg; 5/sex/dose/timepoint (an additional 3 high-dose males and 12 high-dose females were dosed as potential replacements)

Basis of dose selection: Preliminary toxicity study was conducted with single oral (gavage) doses of 250, 300 and 350 mg/kg INCB01824 phosphate, 10 ml/kg, 3/sex/dose. Mortality was noted in the mid- (1 male, 2 females) and high-dose groups (1 male, 2 females). Clinical signs of toxicity were noted in low-, mid- and high-dose groups which included squinted eyes, hypoactivity, ataxia, labored, irregular or audible respiration, lacrimation, body cold to touch, flattened posture, pale appearance and hunched posture. The high-dose was selected as 250 mg/kg based on the results of the preliminary toxicity study.

Negative controls: 0.5% methylcellulose

Positive controls: Cyclophosphamide (60 mg/kg), oral (gavage)

Incubation and sampling times: Single oral (gavage) doses of INCB01824 phosphate or cyclophosphamide were administered to rats. Bone marrow for analysis of nucleated cells was obtained from control and high-dose INCB01824 phosphate treated rats at 24 and 48 hours (5/sex/group/timepoint) after dose administration and at 24 hr after dose administration in low-dose, mid-dose, and positive control animals (5/sex/group).

Stained bone marrow slides were scored for micronucleus and the PCE (polychromatic erythrocytes) to NCE (normal chromatic erythrocytes) cell ratio. The micronucleus frequency (expressed as percent micronucleated cells) was determined by analyzing the number of micronucleated PCEs from at least 2000 PCEs per animal. The PCE:NCE ratio was determined by scoring the number of PCEs and NCEs observed while scoring at least 500 erythrocytes per animal.

Results

Study validity:

A test article was considered to be positive if a statistically significant increase in micronucleated PCEs for at least one dose level, and a statistically significant dose-related response were observed.

Solvent control mutant frequencies fell within established ranges. Positive control results were appropriate. Dose range selected for the definitive study was appropriate according to ICH guidelines.

Study outcome:

INCB01824 phosphate did not induce any statistically significant increases in micronucleated PCEs at any of the doses tested in this study. A high level of mortality was noted in high-dose females (16/25). No mortality was noted in low- or mid-dose animals or high-dose males. No clinical signs of toxicity were noted in low-dose animals. Clinical signs of toxicity noted in mid-dose animals included squinted eyes, ataxia, hypoactivity and/or reddened skin on the paws, ears and/or nose. Clinical signs of toxicity noted in high-dose animals included squinted eyes, ataxia, hypoactivity, reddened skin on the paws, ears, and/or nose, irregular or labored respiration, lacrimation, hyperactivity, convulsions, body that was cold to touch, and/or lateral recumbency. In addition, bone marrow toxicity was noted in the high dose group (20%

decrease in PCE/NCE ratio compared to concurrent vehicle control). Due to the unexpected mortality in the high dose females, no slides were prepared for this group for the 48 hour timepoint.

8 Carcinogenicity

The carcinogenicity study was reviewed by Dr. Miyun Tsai-Turton.

Study title: **INCB018424: 26 week repeated dose oral carcinogenicity study in Tg.rasH2 mice**

Adequacy of the carcinogenicity study and appropriateness of the test model: Yes. The Applicant followed eCAC recommendations.

Key study findings:

- Ruxolitinib was given to mice once daily at 0, 15, 45, and 125 mg/kg for 26 weeks via oral gavage. TK group was included in this study. Urethane (1000 mg/kg; three i.p. injection on Week 1) was used as positive control whereas 0.5% methylcellulose was used as vehicle control.
- In main study animals, there were deaths in low-dose (1 M and 1 F) and mid-dose (3 M and 4 F) groups but not in high-dose group. In TK study animals, there was one death in high-dose (1 F) group.
- No ruxolitinib related clinical sign was noted.
- There was a decrease in body weight gain in high-dose animals (11 % in male and 15% in female) but there were no signs of toxicity associated with ruxolitinib. Food consumption was decreased in treated males (all dose groups) and females (mid-dose/high-dose groups).
- Decreased absolute and/or relative organ weights were noted (*male*: brain/kidneys/liver/testes and *female*: brain/kidneys/liver/heart/spleen). These were probably attributed to decreased body weights and no correlation to gross histological findings.
- Neoplastic findings: ruxolitinib did not increase the incidence of neoplastic lesions. There were lesions/tumors in lungs (i.e. multiple adenomas and/or carcinoma in control/treated animals) and in spleen (i.e. hemangiosarcomas in treated animals), or in multiple organs such as skin/skeletal muscle (i.e. hemangiosarcomas/hemangiomas in control/treated animals, sarcoma in treated animals). Other tumors observed in control/treated animals included Harderian gland adenoma, liver adenoma, thyroid follicular adenoma, nasal adenocarcinoma, stomach papilloma, and thymus thymoma. All findings, however, either had low incidence rates or fell within historical control ranges established by (b) (4).
- Non-neoplastic findings: ruxolitinib increased the incidence/severity of non-neoplastic inflammatory lesions of the nasal cavity (i.e. minimal to moderate exudative inflammation).

Study no.: T09-02-03
Volume #, and page #: eCTD 4.2.3.4

Conducting laboratory and location: (b) (4)
Date of study initiation: Feb 17th (male) and Feb 19 (female) 2009
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: INCB018424, Lot No. BPR-07-101-B2-21, 99.6% purity
CAC concurrence: Yes on SPA (special protocol assessment for dose selection and test model)

Methods

- *Doses:* 0, 15, 45, and 125 mg/kg/day INCB018424

Group	Dose levels (mg/kg/day)	Dose Concentration (mg/mL)	Number of Animals			
			Main Study (Tg.rasH2)		TK Study (CByB6F1)**	
			Male	Female	Male	Female
Group 1 (Vehicle)	0	0	25	25	10	10
Group 2 (Positive Control)	1000 (urethane)*	100	25	27[1]	-	-
Group 3	15	1.5	25	25	44	44
Group 4	45	4.5	25	25	44	44
Group 5	125	12.5	25	25	44	44
Total			125	127	142	142

*The Positive Control animals were administered a total of 3 intraperitoneal (i.p.) injections on Study Days (SD) 1, 3, and 5.
**Extra TK animals (4/sex for Group 1 and 2/sex for Groups 3-5) were used to reasonably ensure adequate animals for TK bleeding. Extra TK animals in Group 1 remaining after the Day 1 TK bleed remained on study for use during urine collection.
[1] = Two additional animals were added as replacement.

- *Basis of dose selection:* MTD
- *Species/strain:* hemizygous Tg.rasH2 mice
- *Number/sex/group (main study):* 25/sex/group
- *Route, formulation, volume:* oral gavage
- *Frequency of dosing:* daily for 182 to 183 consecutive days
- *Satellite groups used for toxicokinetics or special groups:* TK (44/sex/group) with CByB6F1 mice
- *Age:* 8 weeks
- *Animal housing:* environmentally controlled room with temp of 69-75 °F and a relative humidity of 30-70% with a 12 hr light/12 hr D cycle
- *Restriction paradigm for dietary restriction studies*: *ad libitum* access to drinking water and diet
- *Drug stability/homogeneity:* stability (prepared in 0.5% methylcellulose at 0.005 to 1000 mg/ml was confirmed for 24 hrs at RT and 14 days under refrigerated conditions. Homogeneity was confirmed with the means of each location (top,

middle, bottom) were within 7% of the overall mean and all replicates were with 5% of their respectively mean. The mean concentration results were within 10% of the theoretical concentration and all replicates were within 5% of their respective means.

- *Dual controls employed:* Yes, vehicle control was 0.5% methylcellulose and the positive control was 1000 mg/kg urethane (via i.p. injection on Days 1, 3, and 5).
- Interim sacrifices:
- *Deviations from original study protocol:* Yes but these were not expected to affect the outcome of the study.

Observation times

- *Mortality:* twice daily
- *Clinical signs:* twice daily
- *Body weights:* once weekly from Day 1 to Week 13 and biweekly thereafter
- *Food consumption:* weekly
- *Organ weight:* organs (i.e. adrenal glands, brain, heart, kidneys, liver, spleen, and testes/ovaries) were collected and weighted on scheduled necropsy
- *Histopathology: Peer review: yes,* organ tissues (see below) were collected on scheduled necropsy for microscopic evaluation.

Adrenal glands	Ovaries
Aorta	Pancreas
Bone (femur and sternum)	Parathyroid glands
Bone marrow (femur and sternum)	Pituitary gland
Brain	Prostate gland
Epididymides	Salivary gland
Esophagus	Sciatic nerve
Eyes	Seminal vesicles
Gall bladder	Skeletal muscle (thigh)
Gross lesions	Small intestine duodenum, jejunum, and ileum)
Harderian gland	Spinal cord (cervical, thoracic, and lumbar)
Heart	Spleen
Kidneys	Stomach
Large intestine (cecum, colon, rectum)	Testes
Liver	Thymus
Lungs and bronchi	Thyroid glands
Lymph nodes (mesenteric, mediastinal, and mandibular)	Trachea
	Urinary bladder
Mammary gland with adjacent skin	Uterus
Nasal cavity	Vagina

- *Toxicokinetics:* blood samples were collected from 3 animals/sex/dose/timepoint on Days 1-2 and 176-177 (predose, 0.5, 1, 2, 4, 8, and 24 hr post-dose for ruxolitinib-treated TK animals). Urine samples of designated TK animals (3 animals/cage) were collected during week 26 for metabolite identification.
- *Serum antibody analysis*: blood collection from designated animals (10 M/10F predose, 10M/10F Group1 and surviving M/F Group 5 on Day 183) were collected via retroorbital sinus for possible future analysis (non-GLP).

Results

Mortality: mortalities (main study) were seen in 1 low-dose (LD) and 3 mid-dose (MD) male rats, 1 LD and 4 MD female rats (compared to 12 males and 12 females with urethane-treated animals). Mortality (TK study) was seen in 1 high-dose (HD) female.

Clinical signs: there were no treatment-related effects on clinical observations. Clinical sings were either transient, with no dose-response, or appeared in a small number in treated animals (compared to clinical signs such as ataxia, decreased motor activity, labored breathing, hunched posture, hyperactivity, and hypothermia in urethane-treated animals).

Body weights: there were decreases (but no dose-related effects) on body weights or body weight gains in LD and MD males. There was also 11% (males) and 15% (females) decreases in absolute body weight gain from Day 1 to Day 183 in HD group.

Food consumption: There was a decrease in total food consumption from Day 1 to Day 183 in all treated males when compared to the vehicle control. There was also a significant decrease in total food consumption in MD and HD females when compared to the vehicle control.

Organ weights: There were decreases in several absolute and relative organ weights (i.e. male: brain/kidneys/liver/testes and female: brain/kidneys/liver/heart/spleen). Such decreases (with no histological correlates) were considered to be secondary to decreased body weight.

[Absolute Male Organ Weights] Group 3-5 Vs. Group 1

Parameter	Group No.	Significant Difference compared to Control (Group 1) ↑ or ↓
Brain	5	↓ DR
Kidneys	4 & 5	↓ DR
Liver	3, 4 & 5	↓ DR
Testes	5	↓

↑ = Statistically significantly ($p < 0.05$) increased compared to Group 1.
↓ = Statistically significantly ($p < 0.05$) decreased compared to Group 1.
DR = Dose Related

Nominal Dose: Group 1 – 0 mg/kg/day Group 3 – 15 mg/kg/day
Group 4 – 45 mg/kg/day Group 5 – 125 mg/kg/day

[Relative Male Organ Weights] Group 3-5 Vs. Group 1

Parameter	Group No.	Significant Difference compared to Control (Group 1) ↑ or ↓
Kidneys	5	↓ DR
Liver	3, 4 & 5	↓

[Absolute Female Organ Weights] Group 3-5 Vs. Group 1

Parameter	Group No.	Significant Difference compared to Control (Group 1) ↑ or ↓
Brain	4 & 5	↓ DR
Heart	3, 4 & 5	↓
Kidneys	4 & 5	↓ DR
Liver	3, 4 & 5	↓ DR
Spleen	4 & 5	↓ DR

[Relative Female Organ Weights] Group 3-5 Vs. Group 1

Parameter	Group No.	Significant Difference compared to Control (Group 1) ↑ or ↓
Heart	3 & 4	↓
Kidneys	5	↓
Liver	3, 4 & 5	↓ DR
Spleen	4 & 5	↓ DR

↑ = Statistically significantly ($p < 0.05$) increased compared to Group 1.
↓ = Statistically significantly ($p < 0.05$) decreased compared to Group 1.
DR = Dose Related

Nominal Dose: Group 1 – 0 mg/kg/day Group 3 – 15 mg/kg/day
Group 4 – 45 mg/kg/day Group 5 – 125 mg/kg/day

Gross pathology: There was no treatment-related effect on macroscopic findings in either sex (compared to the pulmonary and splenic lesions noted in urethane-treated animals)

Histopathology: see appendix for detailed microscopic findings provided by the sponsor.

- *Non-neoplastic:* Minimal to moderate exudative inflammation of the nasal cavity was observed.

Nasal cavity

Mostly minimal to mild inflammation of the submucosa of the nasal cavities was considered a background lesion. These were characterized by infiltration of mostly neutrophils and fewer lymphocytes in the submucosal glands and the interglandular spaces. The incidence of such lesion was increased slightly in the ruxolitinib treated mice in both sexes.

Exudative inflammations of the nasal cavities were noted. These were characterized by accumulation of degenerate neutrophils, rare lymphocytes and macrophages, fibrin, and sloughed cells. This was only occasionally noted in the vehicle control mice. However, this incidence and severity of this inflammation were higher in the HD group but comparable in the LD and MD groups when compared to vehicle control in both sexes.

Additional changes present in both lesion and inflammation include dilation of glands, presence of eosinophilic droplets, and hyperplasia of submucosal glands or lining epithelium (mostly minimal intensity). These changes were considered to be part of the inflammatory process.

Adenocarcionoma of the nasal cavity was seen in 1 Group 3 male and 2 Group 3 females. This appeared to arise from the deep submucosal glands. The incidence of this nasal adenocarcinoma was low (with no dose relationship) and comparable with historical control range established by the (b) (4)

Male				
	Group 1	Group 3	Group 4	Group 5
Inflammation, submucosa				
Minimal	16	21	21	24
Mild	3	0	0	1
Inflammation, exudative				
Minimal	2	1	6	16
Mild	0	1	3	7
Moderate	0	0	1	2
Adenocarcinoma	0	1	0	0
Female				
	Group 1	Group 3	Group 4	Group 5
Inflammation, submucosa				
Minimal	12	19	21	25
Mild	1	0	0	0
Inflammation, exudative				
Minimal	4	1	3	6
Mild	0	1	2	11
Moderate	0	0	0	5
Adenocarcinoma	1	2	0	0

Nominal Dose: Group 1 – 0 mg/kg/day Group 3 – 15 mg/kg/day
Group 4 – 45 mg/kg/day Group 5 – 125 mg/kg/day

Others

A variety of other non-neoplastic lesions (i.e. minimal brain inflammation, mild aorta proliferation, minimal heart periarteritis, and minimal liver necrosis) were noted but all were considered to be background/spontaneous, incidental, and/or their incidences were comparable across the dose groups.

Based on these findings, the nasal cavity was considered to be a target organ for non-neoplastic inflammatory changes.

- *Neoplastic:* There was no significant increase in the incidence of neoplasia (i.e. lesions in lung, spleen, hemangiomas/hemangiosarcomas in spleen/other organs, sarcomas in multiple organs, non-vascular/sarcomas/pulmonary tumors in multiple organs).

Neoplastic lesions in lungs

The incidence of single or multiple adenomas, carcinomas, and that of combined incidence of all pulmonary tumors in vehicle and ruxolitinib treated mice was comparable and fell within the historical control range established by (b) (4)

There was significant increase in the incidence of pulmonary tumors in urethane-treated mice when compared to control mice, proving the validity of the test system.

MALE						
	Group 1	Group 2	Group 3	Group 4	Group 5	HCR
Adenoma, single	1	0	2	3	2	0-6
Adenoma, multiple	1	25	0	0	0	0-1
Carcinoma	0	8	0	0	0	0-2
All Lung Tumor	2	25*	2	3	2	0-6
FEMALE						
	Group 1	Group 2	Group 3	Group 4	Group 5	HCR
Adenoma, single	3	0	3	1	1	0-6
Adenoma, multiple	0	25	0	0	0	0-1
Carcinoma	0	18	1	0	0	0-1
All Lung Tumor	3	25*	4	1	1	0-6

Nominal Dose: Group 1 – 0 mg/kg/day Group 2 – Positive Control Group 3 – 15 mg/kg/day

Group 4 – 45 mg/kg/day Group 5 – 125 mg/kg/day

HCR: Historical Control Range (See Appendix I).

*: Multiple adenomas and/or carcinomas were present in the same animal in Urethane treated mice

Neoplastic lesions in spleen

The incidence of splenic hemangiosarcomas was low and comparable between ruxolitinib treated dose groups and fell within the historical control range. There were no splenic hemangiosarcomas seen in the vehicle control mice of both sexes.

There was significant increase in the incidence of splenic hemangiosarcomas in urethane-treated mice when compared to control mice, proving the validity of the test system.

MALE						
	Group 1	Group 2	Group 3	Group 4	Group 5	HCR
Hemangiosarcoma	0	24	0	1	1	0-4
FEMALE						
	Group 1	Group 2	Group 3	Group 4	Group 5	HCR
Hemangiosarcoma	0	24	2	2	0	0-4

Nominal Dose: Group 1 – 0 mg/kg/day Group 2 – Positive Control Group 3 – 15 mg/kg/day
Group 4 – 45 mg/kg/day Group 5 – 125 mg/kg/day
HCR: Historical Control Range (See Appendix I)

Hemangiomas and hemangiosarcomas in multiple organs including spleen (under the general category of mesenchymal tumors)

There was a non significant increase in the incidence of hemangiomas and hemangiosarcomas in multiple organs of Groups 4/5 males (i.e. spleen, skin, and skeletal muscle) and Groups 3/4 females (i.e. spleen and skin). However such increase was not dose-dependent. The incidence of all hemangiomas and hemangiosarcomas in multiple organs in vehicle as well as ruxolitinib treated dose groups fell within the historical control range established at (b) (4)

MALE					
	Group 1	Group 3	Group 4	Group 5	HCR
Hemangiosarcomas					
Spleen	0	0	1	1	0-4
Testes	1	0	0	0	0-1
Skin	0	0	1	0	NR
Hemangiomas					
Skeletal muscle	0	0	0	1	NR
Combined incidence	1	0	2	2	0-4
FEMALE					
	Group 1	Group 3	Group 4	Group 5	HCR
Hemangiosarcomas					
Spleen	0	2	2	0	0-4
Skin	0	1	0	0	0-1
Combined incidence	0	3	2	0	0-4

Nominal Dose: Group 1 – 0 mg/kg/day Group 3 – 15 mg/kg/day
Group 4 – 45 mg/kg/day Group 5 – 125 mg/kg/day
HCR: Historical Control Range (See Appendix I)
NR: Not recorded in our historical control data base since no findings were found.

Sarcomas in multiple organs (under the general category of mesenchymal tumors)

The incidence of these sarcomas was low and involved random organs and these were not present in control Group 1.

Male					
	Group 1	Group 3	Group 4	Group 5	HCR
Skin, sarcoma	0	0	1	0	0-1
Skeletal muscle, sarcoma	0	1	0	0	NR
Female					
	Group 1	Group 3	Group 4	Group 5	HCR
Multicentric, sarcoma	0	0	1	0	NR

Nominal Dose: Group 1 – 0 mg/kg/day Group 3 – 15 mg/kg/day
Group 4 – 45 mg/kg/day Group 5 – 125 mg/kg/day
HCR: Historical Control Range (See Appendix I)
NR: Not recorded in our historical control data base since no findings were found.

Other tumors (non vascular, non-sarcomas, and non pulmonary) in multiple organs

There was a non-dose dependent increase in the incidence of thymomas in Group 4 females. However, they were not seen in vehicle control and were not recorded in the historical control ranges.

The incidence of all remaining tumors involved isolated organs and/or fell within the historical control ranges.

Male					
	Group 1	Group 3	Group 4	Group 5	HCR
Harderian gland, adenoma	2	0	0	1	0-1
Thyroid, follicular adenoma	0	1	0	0	NR
Nasal, Adenocarcinoma	0	1	0	0	0-2*
Liver, adenoma	0	0	1	0	NR
Female					
	Group 1	Group 3	Group 4	Group 5	HCR
Harderian gland, adenoma	2	0	0	2	0-4
Stomach, papilloma	1	0	0	0	0-1
Thymus, thymoma	0	0	3	0	NR
Nasal, adenocarcinoma	1	2	0	0	0-1

Nominal Dose: Group 1 – 0 mg/kg/day Group 3 – 15 mg/kg/day
Group 4 – 45 mg/kg/day Group 5 – 125 mg/kg/day
HCR: Historical Control Range in vehicle control mice (See Appendix I)
*: Nasal cavity adenomas are reported in the HCR but no adenocarcinomas.
NR: Not recorded in our historical control data base since no findings were found.

Based on these findings, the lung, spleen, skin, skeletal muscle were apparent to be target organs for neoplastic changes/lesions. However, ruxolitinib did not increase the incidence of these lesions. Most changes identified occurred with low incidences and with no dose-response, or fell within historical control ranges established by (b) (4)

Toxicokinetics: TK analysis showed that there was minimal gender difference in CByB6F1 hybrid mice. The C_{max} values did not always increase proportionally with dose and were similar between Day 1 and Day 176 in males, but decreased slightly (30-

50%) in females on Day 176. The AUC values increased in a greater than dose proportionally and were generally similar between Day 1 and Day 176, except a slight decrease (39%) in HD females on Day 176. The t ½ values were higher in HD groups when compared to LD and MD groups.

Summary of Toxicokinetics in a 26-Week Carcinogenicity Study in Tg.rasH2 Mice [T09-02-03]

Gender	Day	Parameter	15 mg/kg	45 mg/kg	125 mg/kg
Male	1	C_{max} (μM)	4.51	12.3	28.6
		T_{max} (h)	0.500	0.500	0.500
		AUC_{0-24} (μM*h)	3.83	16.4	69.3
		$t_{1/2}$ (h)	0.487	1.01	2.33
	176	C_{max} (μM)	4.02	13.9	24.4
		T_{max} (h)	0.500	0.500	0.500
		AUC_{0-24} (μM*h)	4.55	18.5	67.8
		$t_{1/2}$ (h)	0.787	1.07	1.39
Female	1	C_{max} (μM)	4.73	13.0	26.6
		T_{max} (h)	0.500	0.500	0.500
		AUC_{0-24} (μM*h)	5.76	23.1	127
		$t_{1/2}$ (h)	1.19	1.01	3.62
	176	C_{max} (μM)	3.30	7.68	13.1
		T_{max} (h)	0.500	0.500	0.500
		AUC_{0-24} (μM*h)	5.14	22.5	78.0
		$t_{1/2}$ (h)	1.08	1.20	3.91

9 Reproductive and Developmental Toxicology

Fertility and Early Embryonic Development

Study title: Oral gavage study of fertility and early embryonic development to implantation with INCB018424 in rats

Study no.:	8212204
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	6/22/2009
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	INCB018424, lot#BPR-07-101-B2-21, 97.8%

Key Study Findings

Males

- Reduction in body weight was observed at ≥ 30 mg/kg of the drug; NOAEL is 10 mg/kg
- Doses up to 60 mg/kg did not affect male reproductive function (no effects on sperm count, concentration, or motility)

Females

- Doses up to 60 mg/kg did not result in toxicities in females; a NOAEL was not identified
- At doses up to 60 mg/kg, there were no treatment-related effects on the estrous cycling, number of pregnancies or copulation rates
- A treatment-related increase in post-implantation loss was noted in the 60 and 30 mg/kg/day groups. In the 60 mg/kg/day group, this increase was significant and was attributed to an increase in the number of late resorptions

Conclusions:

- Reproductive function and fertility were not affected in males or females at doses up to 60 mg/kg;
- Embryo-fetal viability was reduced at ≥ 30 mg/kg (not affected at 10 mg/kg).
- The dose of 30 mg/kg/day and 60 mg/kg/day result in an AUC_{0-24} approximately 34% and 2-fold the human AUC_{0-24}, respectively, at the maximum human dose of 25 mg twice daily (the human AUC_{0-24} being 8.8 μM.h).

Methods

Doses:	0, 10, 30, 60 mg/kg at dose volume of
Frequency of dosing:	
Dose volume:	10 mL/kg
Route of administration:	oral gavage
Formulation/Vehicle:	0.5% (w/v) methylcellulose (400 cps), in reverse osmosis/deionized water
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	22/sex/dose
Satellite groups:	None
Study design:	All males were dosed for at least 10 weeks and included at least 28 days prior to mating and throughout the mating phase. Females were dosed for at least 14 days prior to mating (premating phase), throughout the mating phase, and through Gestation Day 7 (GD 7). Treated males were paired with treated females during the mating phase. On GD 13, females were necropsied and the uterus of each was examined for the number of live and dead fetuses and resorptions. Ovaries were examined for the number of corpora lutea. All males were necropsied and evaluated for reproductive capacity.
Deviation from study protocol:	none

Observations and Results

Parameters and endpoints evaluated: mortality, clinical observations, body weight and food consumption data, necropsy findings, cesarean section data, and reproductive organ weight data, estrous cycle, and sperm evaluations

Statistical evaluations: Levene's test was done to test for variance homogeneity. One-way analysis of variance [ANOVA] was used to analyze data. If the ANOVA was significant ($p < 0.05$), Dunnett's t-test was used for control versus treated group comparisons. The sperm motility and total count was compared using the Kruskal-Wallis nonparametric ANOVA test. If a significant result was obtained at the 5% probability level, the Wilcoxon (Mann-Whitney U) test served as the post-hoc group comparison test (at the 5% one-tailed probability level).

Mortality

Two females were sacrificed moribund (10 mg/kg on day 34 and 60 mg/kg on day 30. Note: As the moribundity of these animals did not display a dose-dependency, no clinical signs were noted with the exception of moribundity (pale appearance) and resorptions (red vaginal discharge), and there were no treatment related necropsy findings, these unscheduled deaths were not considered treatment-related.

Clinical Signs

Early death: Prior to death, both of these animals were noted with clinical signs of a pale appearance to the entire body, red vaginal discharge, red fluid in the pan, and/or both eyes pale.

Scheduled death (males and females): unremarkable

Body Weight

Early death: unremarkable

Scheduled death:

Male: Treatment-related decreases in mean body weight were observed in all treatment groups.

10 mg/kg: ↓3 to 5%, compared to control from Day 35 to the end of study, which also correlated with a decrease in mean body weight gain from Day 35 to the end of study.

30 mg/kg: ↓4-9%, beginning on Day 17, lasting to the end of study on Day 71, statistically significant compared to control from Day 38 until the end of the study.

60 mg/kg/day: ↓3-11%, beginning on Day 24, lasting to the end of study on Day 71; statistically significant compared to control from Day 38 until the end of the study.

Female: unremarkable

Feed Consumption

Male: 9% increase compared to control in mean food consumption was noted in the 60 mg/kg/day males from Days 0 to 28.

Female: unremarkable

Necropsy

Male: There were no treatment-related effects on average organ weights or relative organ weights, sperm count, sample, concentration and motility.

Female: There were no treatment-related effects on the estrous cycling of females. There were no treatment-related effects on pregnancy or copulation rates. Preimplantation loss was slightly increased in the 60 mg/kg/day group. This was mainly due to litter effects (Animal Nos. B55161 and B55163), and as the remaining 18 litters in the 60 mg/kg/day group were similar to controls. A treatment-related increase in postimplantation loss was noted in the 60 and 30 mg/kg/day groups. In the 60 mg/kg/day group, this increase was significant and was attributed to a 100% litter involvement in resorptions and an increase in the number of late resorptions compared to control.

Overall reproductive function data are presented in the following table (excerpted from the application, data have been verified by the reviewer).

	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	60 mg/kg/day
No. of males paired	22	22	22	22
No. of animals mated (Male/Female Copulation Index)	22	21	21	21
Male/Female Copulation Index (%)	100	95	95	95
No. of Females Pregnant	22	20	21	21
Male/Female Fertility Index (%)	100	91	95	95

Toxicokinetics
TK: not conducted in this study. The Applicant used data from study T07-10-14 (GLP study, Oral Administration of INCB018424 via Gavage: Dose Range Study for Effects on Embryo-Fetal Development in Sprague Dawley Rats) to compare the plasma exposure (AUCs) in rats and humans.

The following table is from the Applicant's submission (Study# T07-10-14).

Summary of INCB018424 Toxicokinetics in Pregnant Sprague-Dawley Rats on Gestation Day 13 Given Daily Oral Doses of 15, 30, 60 or 120 mg/kg INCB018424

Parameter	Dose			
	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
C_{max} (µM)	0.375	1.02	3.35	9.56
T_{max} (h)	1.00	1.00	1.00	1.00
AUC_{0-t} (µM*h)	0.712	2.59	18.3	57.6
AUC_{0-24} (µM*h)	0.750	2.98	19.0	57.6
$t_{1/2}$ (h)	2.42	3.98	2.17	2.09

Embryo-fetal Development

Study title: Oral Administration of INCB018424 via Gavage: Definitive Study for Effects on embryo-fetal Development in Sprague Dawley rats

Study no.: (b) (4) study # 1603-07595
Incyte Study# T07-12-04
Study report location: Module 4
Conducting laboratory and location: (b) (4)
Date of study initiation: December 3, 2007
GLP compliance: Yes
QA statement: yes (x) no ( )
Drug, lot #, and % purity: INCB018424, Lot #: BPR-07-101-B2-21, Purity: 98.9%

Key Study Findings

- Maternal mortality was observed at 60 mg/kg;
- Statistically significant decreased fetal weights (up to 9%) were observed at 60 mg/kg, the dose that resulted in mortality in 2 dams.
- No maternal or embryo-fetal developmental effect was seen at 30 mg/kg/day dose (NOAEL=30 mg/kg/day)
- The dose of 60 mg/kg results in an AUC_{0-24} of 19 μM.hr which is 2 times the human AUC_{0-24} at the highest dose of 25 mg, twice daily (50 mg/day).

Methods

Doses:	15*, 30, 60 mg/kg/day *Due to a typographical error, the actual dose level was 14 mg/kg/day Dose justification: Dose selection was based on results from previous developmental dose-range finding toxicity study.
Frequency of dosing:	once daily
Dose volume:	10 mL/kg
Route of administration:	oral gavage
Formulation/Vehicle:	0.5% methylcellulose
Species/Strain:	Female Crl:CD®(SD)IGS BR rats 201-236 g and 8-10 weeks at mating
Number/Sex/Group:	25 animals/group
Study design:	daily dosing from gestation days (GD) 7-20*, Necropsy/cesarean on gestation day 22 *the day of confirmation of mating was considered GD1
Deviation from study protocol:	none

Observations and Results

Parameters and endpoints evaluated:

Procedure	Frequency of Testing
Cageside Observations	≥ 2 Daily
Clinical Observations	GD 7, 10, 13, 16, 20, and 22
Postdose Observations	30 minutes (± 5 minutes) after each dose
Body Weight	GD 7, 10, 13, 16, 20, and 22
Food Consumption	GD 7−10, 10−13, 13−16, 16−20, and 20−22

Reproductive parameters: the number and placement of uterine implantation sites, the number of live and dead fetuses, the number of early or late resorptions, any abnormalities of the

placenta or embryonic sac, and the number of corpora lutea on each ovary.

Fetal examination: Each fetus (live or dead) was sexed, weighed, examined for external malformations/variations, visceral/skeletal malformations/variations).

Statistical evaluations:

Quantitative data (body weights, body weight changes, food consumption, uterine weights, and uterine data) were analyzed using the Kolmogorov-Smirnov test for normality, the Levene Median test for equal variance, and by one-way Analysis of Variance (ANOVA). If either the normality or equal variance test failed, then the analysis was continued using the non-parametric Kruskal-Wallis ANOVA on rank transformed data. For parametric data, if the ANOVA indicated statistical significance among experimental groups then the Dunnett's t-test was used to delineate which groups (if any) differed from the control. For non-parametric data, if the ANOVA indicated statistical significance among experimental groups then the Dunn's test was used to delineate which groups (if any) differed from the control. The probability value of less than 0.05 (two-tailed) was used as the critical level of significance for all tests. The litter was used as the experimental unit.

TK: not conducted in this study. The Applicant used data from study T07-10-14 (GLP study, Oral Administration of INCB018424 via Gavage: Dose Range Study for Effects on Embryo-Fetal Development in Sprague Dawley Rats) to compare the plasma exposure (AUCs) in rats and humans.
The following table is from the Applicant's submission (Study# T07-10-14).

Summary of INCB018424 Toxicokinetics in Pregnant Sprague-Dawley Rats on Gestation Day 13 Given Daily Oral Doses of 15, 30, 60 or 120 mg/kg INCB018424

Parameter	Dose			
	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
C_{max} (μM)	0.375	1.02	3.35	9.56
T_{max} (h)	1.00	1.00	1.00	1.00
AUC_{0-t} (μM*h)	0.712	2.59	18.3	57.6
AUC_{0-24} (μM*h)	0.750	2.98	19.0	57.6
$t_{1/2}$ (h)	2.42	3.98	2.17	2.09

In life observations:

Mortality: 2 early deaths at 60 mg/kg. DG18, GD22 (prior to the scheduled necropsy).

Clinical signs:

Early death

1 death at GD18: there were no abnormal observations prior to death.

1 death at GD22: brown vaginal discharge was noted starting on GD 20.

Scheduled death: unremarkable

Body weight: Body weights and body weight changes for treated animals were comparable to those of controls throughout the study.

Food consumption: Overall, there were no differences between the groups in the total amount of food consumed (GD 7-22).

Terminal and necropsy evaluations:

Dams:

Necropsy evaluation:

Early death*:*

1 death on GD 18: a discolored uterus with brown amoniotic fluid was noted at necropsy.

1 death on GD22: unremarkable

Scheduled death: unremarkable

Uterine and Ovarian parameters

Changes in uterine weight: unremarkable

Embryo-fetal data:

Changes of mean fetal body weight

Index	Fetal weight (g)				Percentage deviation from control			
Group (mg/kg)	0	15	30	60	0	15	30	60
Male	5.6	5.8	5.6	5.3**	-	-	-	-5**
Female	5.5	5.5	5.3	5.0**	-	-	-4	-9**
Male+ female	5.7	5.6	5.4	5.2**	-	-	-5	-9**

** p≤0.01

Fetal morphological data: unremarkable

Conclusion: Under the conditions of this study, INCB018424 was not teratogenic in rats; the maternal no-observed-adverse-effect level (NOAEL) and the fetal NOAEL are 30 mg/kg.

Study title: Oral Administration of INCB018424 via Gavage: Definitive Study for Effects on Embryo-Fetal Development in New Zealand White Rabbits

Study no.:	(b) (4) study # 1603-07597 Incyte Study# T07-12-05
Study report location:	
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 10, 2007
GLP compliance:	yes
QA statement:	yes (x) no ( )
Drug, lot #, and % purity:	INCB018424 Lot #: BPR-07-101-B2-21 Purity: 98.9%

Key Study Findings

- Two unscheduled mortalities were observed at 60 mg/kg;
- The following were noted in one unscheduled death: moderate appetite loss, low posture, limping, hypersensitivity to touch, languid behavior, and decreases in body weight and food consumption were noted
- Treatment with INCB018424 resulted in a significantly higher number of late resorptions at 60 mg/kg/day, a dose that resulted in mortalities in does. Increased late resorption contributed to increased total post-implantation loss at 60 mg/kg/day dose
- INCB018424 at 60 mg/kg caused lower fetal weights;
- No maternal or embryo-fetal developmental effect was seen at 30 mg/kg/day dose (NOAEL=30 mg/kg/day)
- The dose of 60 mg/kg results in an AUC_{0-24} of 606 nM.hr of which is 7% the human AUC_{0-24} at the highest dose of 25 mg twice daily (50 mg/day), the human AUC_{0-24} being 8.8 µM.h.

Methods

Doses:	10, 30, 60 mg/kg/day Dose justification: Dose selection was based on results from previous developmental dose-range finding toxicity study.
Frequency of dosing:	once daily
Dose volume:	10 mL/kg
Route of administration:	oral gavage
Formulation/Vehicle:	0.5% methylcellulose
Species/Strain:	Female New Zealand white rabbits 3000-3873 g and 5-6 weeks at mating
Number/Sex/Group:	20 animals/group
Satellite groups:	3 animals/group
Study design:	daily dosing from gestation days (GD) 8-21*, Necropsy/cesarean on gestation day 30 *the day of confirmation of mating was considered GD1.
Deviation from study protocol:	None

Observations and Results

Parameters and endpoints evaluated:

Procedure	Frequency of Testing	
	Main Phase Animals	Toxicokinetic Animals
Cageside Observations	≥ 2 Daily	≥ 2 Daily
Clinical Observations	GD 8, 11, 14, 17, 21, 24, 27, and 30	GD 8, 11, 14, 17, and 21[a]
Body Weight	Daily	GD 8, 11, 14, 17, and 21[b]
Food Consumption	Daily	Not required

Reproductive parameters: the number and placement of uterine implantation sites, the number of live and dead fetuses, the number of early or late resorptions, any abnormalities of the placenta or embryonic sac, and the number of corpora lutea on each ovary.

Fetal examination: Each fetus (live or dead) was sexed, weighed, examined for external malformations/variations, visceral/skeletal malformations/variations).

Statistical evaluations:

Quantitative data (body weights, body weight changes, food consumption, uterine weights, and uterine data) were analyzed using the Kolmogorov-Smirnov test for normality, the Levene Median test for equal variance, and by one-way Analysis of Variance (ANOVA). If either the normality or equal variance test failed, then the analysis was continued using the non-parametric Kruskal-Wallis ANOVA on ranktransformed

data. For parametric data, if the ANOVA indicated statistical significance among experimental groups then the Dunnett's t-test was used to delineate which groups (if any) differed from the control. For non-parametric data, if the ANOVA indicated statistical significance among experimental groups then the Dunn's test was used to delineate which groups (if any) differed from the control. The probability value of less than 0.05 (two-tailed) was used as the critical level of significance for all tests. The litter was used as the experimental unit.

TK: prior to dosing, and 0.5, 1, 2, 4, 8, and 24 hours after dosing on GD 8-9 and 21-22

Results

In life observations:

Mortality: one death at 60 mg/kg on GD21, and one became moribund on GD21.

Clinical signs:

Early death

1 death at GD21: there were no abnormal observations prior to death.

1 moribund at GD21 (animal#4359): moderate appetite loss, low posture, limping, hypersensitivity to touch, and moderate languid behavior were noted

Scheduled death: unremarkable

Body weight: Body weights and body weight changes for treated animals were comparable to those of controls throughout the study, except for the 1 moribund (animal #4359) which had notable weight loss from GD19.

Food consumption: Treatment with INCB018424 did not affect food consumption, except animal No. 4359 had reduced food consumption from GD 9 onwards until becoming moribund on GD 21.

Terminal and necroscopic evaluations

Doe:

Necropsy evaluation:

Early death:

1 death on GD 21: failure of the lung to collapse.

1 moribund at GD21: red vaginal fluid was noted

Scheduled death: unremarkable

Uterine and Ovarian parameters

Changes of uterine weight*:* unremarkable

Other uterine or ovarian parameters:

Doses (mg/kg/day)	0	10	30	60
Corpora lutea/rabbit	9.8	10.7	10.6	10.2
Implantation sites/rabbit	9.3	9.8	9.9	9.8
% pre-implantation loss/group (mean%)	5.5	7.9	6.7	4.8
Total post-implantation loss/group (mean%)	12 (6.8)	25 (11.7)	12 (6.9)	27 (15)
Total resorptions/rabbit	0.6	1.3	0.6	1.3
Late resorptions/rabbit	0.3	0.5	0.5	1.1*
Early resorptions/rabbit	0.3	0.8	0.2	0.3

* $p < 0.05$

Note: Calculated values do not include animals which were not pregnant or did not survive to the scheduled termination.

Summary: Treatment with INCB018424 at 60 mg/kg/day resulted in a significantly higher number of late resorptions (1.1 per dam at 60 mg/kg/day vs. 0.3 per control dam).

Embryo-fetal data:

Mean fetal body weight

Index	Fetal weight (g)				Percentage deviation from control			
Group (mg/kg)	0	10	30	60	0	10	30	60
Male	40	40	39	36*	-	-	-	-10*
Female	39	40	39	35	-	-	-	-10
Male+ female	39	40	38	36*	-	-	-	-8

** $p \leq 0.01$

Summary: Treatment with INCB018424 resulted in lower fetal weights at 60 mg/kg.

Fetal morphological data: unremarkable

Toxicokinetics: The following tables are excerpted from the study report. Toxicokinetic parameters for 10 mg/kg were not calculated as plasma concentrations were below the quantitation limit (0.01 µM) in all but three samples.

Summary of INCB018424 toxicokinetics in pregnant rabbits given daily oral dose of 30 or 60 mg/kg INCB018424

		Mean ± SD	
GD	Parameter	30 mg/kg	60 mg/kg
8	C_{max} (μM)	0.0984 ± 0.029	0.336 ± 0.042
	T_{max} (h)	0.833 ± 0.29	0.667 ± 0.29
	AUC_{0-t} (μM*h)	0.0891 ± 0.066	0.440 ± 0.20
	AUC_{0-24} (μM*h)	0.122 ± 0.066	0.479 ± 0.19
	$t_{1/2}$ (h)	NC	0.474[b]
21	C_{max} (μM)	0.0872 ± 0.054	0.380 ± 0.34
	T_{max} (h)	0.500 ± 0.0	0.500 ± 0.0
	AUC_{0-t} (μM*h)	0.0572 ± 0.040	0.556 ± 0.44
	AUC_{0-24} (μM*h)	0.0684 ± 0.043	0.606 ± 0.48
	$t_{1/2}$ (h)	NC	1.33 ± 0.38

GD: Gestation Day; GD 8 was the first day of dosing
NC: Not calculated due to insufficient time points to define the terminal phase
a: Toxicokinetic parameters for 10 mg/kg not calculated as plasma concentrations were below the quantitation limit of 0.010 μM in all but three samples
b: N=2; not calculated for one rabbit due to insufficient time points to define the terminal phase

Summary:

- The mean plasma Cmax and AUC values for INCB018424 on GD 8 and 21 increased in a greater than dose proportional manner;
- Cmax and AUC values on GD 21 were similar to those on GD 8;
- Absorption and elimination were rapid
- The AUC_{0-24} at 60 mg/kg dose (606 nM.hr) is 7% the human AUC at the human dose of 50 mg/day

Conclusion: Under the conditions of this study, INCB018424 was not teratogenic in rabbits; the maternal no-observed-adverse-effect level (NOAEL) and the fetal NOAEL are 30 mg/kg.

Prenatal and Postnatal Development

Study title: Oral Gavage Study for Effects on Pre- and Post-natal Development, Including Maternal Function with INCB018424 in Rats

Study no.:	1001237
Study report location:	electronic submission
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	02/18/2010
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	INCB018424, lot# BPR-09-134-B1-12, 98.9%

Key Study Findings

- Adverse effects included a slightly prolonged gestation period, reduced number of implantation sites, and reduced number of pups delivered in the 30 mg/kg/day F0 females;
- Reduced body weights were observed in the F1 pups at the highest maternal dose of 30 mg/kg/day. However, this effect seemed to be due to reduced initial weights on LD0 and lower early weight gain.
- The AUCs $_{0\text{-}24}$ at dose of 30 mg/kg/day were 4 μM*h on GD13, and 2.7 μM*h on lactation day 10 (LD10).
- Overall, no toxicologically significant drug-related effects were observed in F1 or F2. The highest dose tested (30 mg/kg/day) results in an $AUC_{0\text{-}24}$ that is approximately 34% the human $AUC_{0\text{-}24}$ at the maximum human dose of 25 mg twice daily (the human $AUC_{0\text{-}24}$ being 8.8 μM.h).

Methods

Doses:	0, 5, 15, 30 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mg/mL
Route of administration:	oral gavage
Formulation/Vehicle:	0.5% (w/v) methylcellulose (400 cps) in reverse osmosis/deionized water
Species/Strain:	Female Crl:CD(SD) rats
Number/Sex/Group:	25 F0 females/group
Satellite groups:	6/group (3/group for control)
Study design:	F0 in main study were dosed once daily beginning on GD 6 and continuing through LD 20F0 in TK study were dosed once daily beginning on GD 6 and continuing through LD 10
Deviation from study protocol:	none

Parameters and endpoints evaluated:

F0: Mortality, clinical signs, body weight during gestation and lactation, food consumption during gestation, natural delivery and litter data and necropsy data

F1: Maturation phases based on mortality, clinical signs, body weights, sexual development, locomotor activity, learning ability, and estrous cycle data during the postweaning;
Mortality, clinical signs, body weights, natural delivery and litter data and necropsy data on F1 females during the mating and gestation phases;
Mortality, clinical signs, body weight, breeding behavior, and necropsy data on F1 males

F2: Survival and body weight on lactation day 1 (LD 1).

Toxicokinetics: from F0 females via a jugular vein on GD 13 and LD 10 prior to dosing and 1, 2, 4, 8, and 24 hours postdose as follows: Three animals in Group 5 (control) were bled prior to dosing and at 1 hour postdose. The first three animals in Groups 6, 7, and 8 were bled prior to dosing and at 2 and 8 hours postdose. The second three animals in Groups 6, 7, and 8 were bled at 1, 4, and 24 hours postdose.

Results:

F0 dams

In-life observations:

Gestation

Mortality: Three F0 animals died at an unscheduled interval: one control female (B61091) was sacrificed on LD 1 after total litter death, one 5 mg/kg/day female (B61116) was sacrificed on GD 27 and was determined to be not pregnant, and one 30 mg/kg/day female (B61185) died as a result of a dosing-related accident on LD 8.

Note: The Applicant stated that above deaths are not considered treatment-related, the treatment is not considered to have adverse effect on survival. The reviewer agrees.

Clinical signs: unremarkable
Body weight: unremarkable
Food consumption: unremarkable

Natural Delivery, Litter, and Lactation

- No INCB018424-related effects on maternal survival, F1 survival, clinical signs, or maternal body weights were observed.
- Although the effect was slight, the mean gestation duration of 30 mg/kg/day animals was statistically significantly longer compared to control (22.1 versus 21.7 days, respectively) and greater than that seen in historical data (21.6 to 21.9 days; (b) (4), 2005-2009).
- While not significant, the mean number of implantation sites and mean number of pups delivered at 30 mg/kg/day were also less than those found in historical data (11.76 versus 11.83-14.44 and 11.52 versus 11.62-13.21, respectively).

- The mean number of live pups/litter with live pups at 30 mg/kg/day was also lower than control on LD 0 and precull LD 4 (11.12 versus 11.48 and 10.52 versus 11.38, respectively).

Toxicokinetics: report INCYTE DMB-08.25.1

Summary of INCB018424 Toxicokinetics in Female Sprague-Dawley Rats Given Daily Oral Doses of INCB018424 from Gestation Day 6 through Lactation Day 20

Day[a]	Parameter	5 mg/kg	15 mg/kg	30 mg/kg
GD13	C_{max} (μM)	0.0888	0.474	1.04
	T_{max} (h)	1.00	1.00	1.00
	AUC_{0-t} (μM*h)	0.207	0.911	3.25
	AUC_{0-24} (μM*h)	0.241	1.14	4.01
	$t_{1/2}$ (h)	1.21	1.52	2.09
LD10	C_{max} (μM)	0.0695	0.312	0.863
	T_{max} (h)	1.00	1.00	1.00
	AUC_{0-t} (μM*h)	0.0920	0.848	2.28
	AUC_{0-24} (μM*h)	0.137	0.928	2.68
	$t_{1/2}$ (h)	NC	1.41	1.78

a: GD = Gestation day; LD = Lactation day
NC: Not calculated due to insufficient timepoints

Note: the above summary table is excerpted from the application

Summary:

1) The plasma Cmax and AUC values generally increased in a greater than dose proportional manner;
2) The plasma Cmax and AUC values on GD 13 were generally comparable to those on LD 10;
3) Tmax and T1/2 are similar between DG13 and LD10;
4) AUC0-24 (μM*h) at 30 mg/kg: GD 13 = 4; LD 10 = 2.7.

Terminal and necropsy evaluations: unremarkable

F1 offspring

In-life observations: including the evaluation for F1 Postweaning (Maturation) and F1 Breeding, Gestation, Resting Phase

Mortality: none

Clinical signs: unremarkable

Body weight: With the exception of males on LD 7, the mean covariate-adjusted body weight of 30 mg/kg/day F1 pups was statistically lower, compared to control, on LDs 0, 4 (pre and postcull), 7, 14, and 21. The lower mean covariate body weights for both males and females on LD 7, 14, and 21 reflect the low body weight on LD 0 and lower early body weight gains rather than a continuing body weight effect.

The table below is excerpted from the application

Select F1 Pup Body Weight Data

MALES	Dose Level (mg/kg/day)				
	0	5	15	30	
	Mean Covariate Body Weight				
LD 0	6.70	6.52	6.57	6.24	**
LD 4, precull	10.01	9.61	9.65	8.98	**
LD 4, postcull	10.02	9.67	9.65	9.02	*
LD 7	16.02	15.28	15.33	14.45	
LD 14	33.22	32.07	31.33	29.98	**
LD 21	53.44	53.49	51.71	49.05	*
	Percentage Mean Covariate Body Weight Gain				
LD 7 to 14	107.4	109.9	104.4	107.5	
LD 14 to 21	60.9	66.8	65.0	63.6	

FEMALES	Dose Level (mg/kg/day)				
	0	5	15	30	
	Mean Covariate Body Weight				
LD 0	6.27	6.17	6.26	5.88	**
LD 4, precull	9.39	9.07	9.20	8.49	*
LD 4, postcull	9.46	9.10	9.27	8.62	*
LD 7	15.41	14.73	14.59	13.64	**
LD 14	31.97	31.02	30.21	28.61	**
LD 21	51.40	51.11	49.49	47.03	*
	Percentage Mean Covariate Body Weight Gain				
LD 7 to 14	107.5	110.6	107.1	109.8	
LD 14 to 21	60.8	64.8	63.8	64.4	

* = P≤0.05 ** = P≤0.01
LD = lactation day

Food consumption: unremarkable
Terminal and necropsy evaluations: unremarkable

F2 Pups
No treatment-related changes

10 Special Toxicology Studies none

11 Other toxicology studies

Genotoxic potential for identified impurities

The (b) (4) actual and potential impurities, together with the (b) (4) starting materials and (b) (4) intermediates (a total of (b) (4) were evaluated using in silico platforms designed to predict mutagenic liability potential. The in silico systems utilized included ToxCheck (v. 4.0), DEREK (v. 12.0.0), and MCASE (v. 2.1.0.18). Of the (b) (4) impurities evaluated, (b) (4) were identified to have structure alert for potential mutagenicity. The potential mutagenicity of the (b) (4) was further assessed using an in vitro Ames test, and the study reports for the conducted Ames assays were submitted with this application. Of note, the Ames assays were not conducted in compliance with GLP. Under the testing conditions used and applying standard mutagenicity criteria, none of the six impurities demonstrated evidence of

mutagenicity. [redacted] (b) (4) was present in the 6-month Tg.rasH2 carcinogenicity study at 0.17%.
Refer to Appendix/Attachments section for the review of the submitted study reports.

12 Integrated Summary and Safety Evaluation

TOXICOLOGY TABULATED SUMMARY

Repeat Dose Toxicity Studies		
Title	**6-month** GLP	**6-month** GLP
Species	Rat	Dog
Test System	Oral by gavage	Oral by gavage
Schedule Dose (mg/kg)	Daily 5,10,30,60 mg/kg/day	Daily 0.5, 2.5, 5, 10 mg/kg/day
Dose (mg/m^2)	30, 60, 180, 360 mg/m^2	10, 50, 100, 200 mg/m^2
Mortality	No treatment-related death	At HD, 2/7 males, 1/7 females
Clinical sign	wet clear and wet/dry red material around the mouth, male and female at HD	Early deaths: reddened facial area, clear to colored discharge from the eyes, soft feces and or diarrhea, hypoactivity, prostration with labored respiration, increased respiration rate. Schedule deaths: clear to colored discharge from the eyes, soft feces and/or diarrhea (females), reddened areas, swelling and hair loss on the limbs at MH, and HD
Body weight	During treatment period, ↓ in males, dose dependent During the recovery period, the mean body weights for the test article-treated males remained low compared to control values, the mean body weight gain were similar between the treatment groups and control group	Early deaths: ↓ Schedule deaths: unremarkable
Food consumption	↓ in males at HD (↓0- 21%)	< week 19: unremarkable 19-26 weeks: male: ↓ HD(↓8%-28%); female: ↓dose-dependent, LD-HD(↓4%-28%);

			26-32 weeks: male: ↓ HD(↓31%-51%); female: ↓LD-HD(↓10%-42%); ↑at some timepoints and some animals	
Ophthalmoscopy	unremarkable		unremarkable	
EKG	unremarkable		unremarkable	
Hematology	Male: ↓ Hbg: (↓4-7%), HD ↓hematocrit(↓3-7%), HD ↓WBC (↓5-32%), LD-HD Neut: -↓LD, LM(↓6-23%), -↑MH, HD(↑12-45%) ↓Lymphocyte (↓5-45%), LD-HD ↑Monocyte (↑10-20%), LD-HD except on week26 at HD ↓eosinophil (↓7-42%), LD-HD Except D26 at MH (↑)and week 12 at HD (↑) ↓basophil (↓14-57%), LD-HD ↓LUC (↓12-63%), LD-HD All reversible	Female: ↓ Hbg: (↓2-4%), HD ↓WBC (↓6-39%), all doses ↑Neutrophil: (↑21-44)all doses Except week 26, LD, MH (↓6-8%) ↓Lymphocyte: (↓9-50%), LD-HD ↑Monocyte (↑10-20%), LD-HD except on week26 at HD ↓eosinophil (↓11-44%), LMD-HD ↓basophil (↓50-100%), LMD-HD ↓LUC (↓20-60%), LD-HD All reversible	Male: ↓ RBC: (↓4-27%), MHD, HD ↓Hgb:(↓6-33%), MHD, HD ↓hematocrit: (↓7-30%), MHD, HD reticulocyte: ≤day135, ↓17-32%, MHD, HD, Day 174, ↑15-22%, MHD, HD ↑NEUT(↑5-71%), MHD, HD ↓LYM(↓11-33%), MHD, HD ↑MONO(↑15-102%), MHD, HD ↓eosinophil (↓17-64%), MHD, HD ↓basophil (↓25-50%), MHD, HD ↑LUC(↑20-100%), HD All reversible	Female: ↓ RBC: (↓6-14%), MHD, HD ↓Hgb:(↓6-19%), MHD, HD ↓hematocrit: (↓5-17%), MHD, HD reticulocyte: ≤day135, ↓5-30%, MHD, HD, Day 174, ↑10-47%, MHD, HD ↑NEUT(↑10-11%), HD ↑MONO(↑12-76%), MHD, HD ↓eosinophil (↓41-80%), MHD, HD ↑basophil (↑33-100%), MHD, HD ↑LUC(↑50-171%), MHD, HD All reversible, except for baso at MHD
Clinical chemistry	unremarkable		unremarkable	

Urilysis	unremarkable	unremarkable
Organ weight	↓spleen, male and female, ↓adrenal gland, male reversible	unremarkable
Gross Pathology	*unremarkable*	*Early death*: hemorrhage, necrosis, ulceration, bacterial colonies in tonsil (cause of death), aorta, GI, hemorrhage in heat, esophagus, thymus, diaphragm, decreased cellularity in lymph nodes, spleen, bone marrow, atrophy in testes, degenerate spermatogenic cells in epididymides *Schedule death*: MDH, HD, parasitic mite-related scabbing/discoloration or swelling, papilloma-related nodule in skin or ears, acute or subacute inflammation-related discoloration in lungs in male and female, small prostate in male, diestrus-related findings in female reproductive organs *Recovery: ,* parasitic mite-related scabbing/discoloration or swelling, papilloma-related nodule in skin or ears, all others recovered
Histopathology	HD, minimal to mild lymphoid depletion in the spleen and mandibular lymph nodes, male and female; HD, minimal cortical atrophy in the adrenal glands, male	*Early death*: Variable lymphoid depletion in lymphoid organs, bone marrow hypocellularity, parasitic mite-related changes in the skin and mandibular lymph node, parasitic mite-related , Acute inflammation within the thoracic cavity, prostate hypoplasia/atrophy (death cause) *Schedule death*: MDH, HD, scabbing/discoloration or swelling, papilloma-related nodule in skin or ears, acute or subacute inflammation-related discoloration in lungs in male and female, small prostate in male, diestrus-related findings in female reproductive organs *Recovery:* pyogranulomatous inflammation in the skin, all others recovered

Genetic Toxicology Studies

Strains/Species/Cell line	Route	Treatment Duration	Dose	Significant Findings
TA98, TA100,	*In vitro*	2 days	TA98, TA100: 1.5, 5.0, 15, 50, 150, 500, 1500 and	"-"

TA1535, TA1537; *Escherichia coli* strain WP2 *uvr*A	incubation		5000 µg/plate late TA1535, TA1537; *Escherichia coli* strain WP2 *uvr*A : 33.3, 100, 333, 1000, 2500 and 5000 µg/plate	
Human peripheral blood lymphocytes	*In vitro* incubation	With S-9: 3 hrs Without S-9: 22 hrs	1.25, 2.50, 5.00, 10.0, 20.0, 40.0, 65.0, 80.0, 95.0 and 110 µg/ml	"-"
rat	oral	Single dose	62.5, 125, 250 mg/kg (375, 750, 1500 mg/m^2)	"-"
Carcinogenicity studies				
Tg.rasH2 mice	oral	Once daily for 26 weeks	15, 45, and 125 mg/kg (45, 135, 375 mg/m^2)	"-"
Reproductive and Developmental Toxicology Studies				
Rat	oral	Daily for at least 14 days(prior to mating to GD7)	10, 30, 60 mg/kg (60, 180, 360 mg/m^2)	fertility and early embryonic development • Male NOAEL= 10 mg/kg, Female NOEL for maternal toxicity = 60 mg/kg; • Male and female NOEL for reproductive and fertility = 60 mg/kg; • NOEL for embryo/fetal viability = 10mg/kg
Rat	oral	Daily (7-20)	15, 30, 60 mg/kg (90, 180, 360 mg/m^2)	Embryofetal development • Mortality was observed at 60 mg/kg; • A transient increase in food

				consumption was observed at 60 mg/kg during GD 7-13. • Decreased fetal weights were observed at 60 mg/kg;
Rabbit	oral	Daily (G8-21)	10, 30, 60 mg/kg (120, 360, 720 mg/m^2)	Embryofetal development • Mortality at 60 mg/kg; • Moderate appetite loss, low posture, limping, hypersensitivity to touch, languid behavior, and decreases in body weight and food consumption were noted • higher number of late resorptions at 60 mg/kg/day; • lower fetal weights at 60 mg/kg
rat	oral	Daily (GD 6-LD 20	5, 15, 30 mg/kg (30, 90, 180 mg/m^2)	Prenatal and postnatal development • slightly prolonged gestation period,

				reduced number of implantation sites, and reduced number of pups delivered at 30 mg/kg/day F0 females; • decreased mean initial body weights, mean body weight gain at 30 mg/kg/day F1 pups; • The NOAEL for fertility indices and all maternal and embryofetal survival, growth, and development parameters is 30 mg/kg/day

13 Appendix/Attachments

Study title: AMES test

Key findings: Under the study condition, (b) (4) is not mutagenic to test strains TA98, TA100, TA1535, TA97a and TA102 in the Ames reverse-mutation assay.

Study no.: 1012574

Volume #, and page #: electronic submission, page 1-20
Conducting laboratory and location: (b) (4)

Date of study initiation: May 28, 2010
GLP compliance: no, following SOP
QA reports: yes (x) no ( )
Drug, lot #, and % purity: (b) (4)
Lot # 5035-197-2
Purity 99.9%

Methods: plate incorporation
Strains: TA98, TA 100, TA1535, TA97a, and TA102
Concentrations used in definitive study:
TA98 and TA100: 15, 50, 150, 500, 1500, and 5000 μg/plate
TA1535, TA97a and TA102, ±S9: 15, 50, 150, 500, 1500, and 5000 μg/plate
Bacteriotoxicity was evident at 5000 μg/plate using strains TA97a –S9 and TA102 +/- S9.
Basis of concentration selection: bacteriotoxicity or ICH S2 criteria for the highest concentration of 5000 μg/plate

Metabolic activation system: Liver S9 mix from male rats, Aroclor 1254-pretreated, 25 μl/plate
Negative controls: DMSO 100 μl/plate
Positive controls:

Tester Strain	Positive controls
TA97a	2-aminoanthracene 9-Aminoacridine
TA98	2-aminoanthracene Benzo(a)pyrene 2-nitrofluorene
TA100	2-aminoanthracene sodium azide
TA102	2-aminoanthracene Mitomycin C
TA1535	2-aminoanthracene sodium azide

Incubation and sampling times: 3 days

Results

Study validity:

No. of replicates: 3

Counting method: not provided

Criteria for positive results: The positive controls induced a response equal to twice (or more) the negative control incidence, which is formally the criterion for a mutagenic activity of a compound. Exception is strain TA102 for which a factor of 1.5 over the concurrent negative control value is considered as positive result.

Selection of bacteria tester strains was adequate based upon Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A). Positive controls produced expected responses. Drug concentration selection was adequate based upon observed bacteriotoxicity or ICH S2 criteria.

Study outcome:

Mean revertant counts in the presence of metabolic activation

Test article	Conc. (μg/plate)	Bacteria strain and number of revertant colonies per plate				
		TA98	TA100	TA1535	TA97a	TA102
(b) (4)	15	32	136	15	255	447
	50	34	127	18	249	596
	150	32	132	22	210	586
	500	31	125	13	174	458
	1500	27	136	12	288	410
	5000	31	133	17	243	*
Positive control	2aminoanthracene Benzo(a)pyrene 2-nitrofluorene	1324 324	2073	228	3811	2176
DMSO	-	46	170	16	244	465

Mean revertant counts in the absence of metabolic activation

Test article	Conc. (µg/plate)	Bacteria strain and number of revertant colonies per plate				
		TA98	TA100	TA1535	TA97a	TA102
(b) (4)	15	18	114	13	256	495
	50	24	96	19	234	527
	150	24	102	21	308	539
	500	30	104	17	334	315
	1500	38	126	23	200	348
	5000	21	123	18	182*	*
Positive control	2-nitrofluorene sodium azide 2aminoanthracene Mitomycin C	118	850	1261	4027	2520
DMSO	-	25	114	20	318	463

* Bacteriotoxicity was evident at 5000 µg/plate using strains TA97a –S9 and TA102 +/- S9

- The mean mutant numbers of negative control plates lay within the range of acceptable negative control values
- The positive controls induced a response equal to twice (or more) the negative control incidence, which is formally the criterion for a mutagenic activity of a compound
- Treatment with the test item did not increase the number of revertants above the corresponding negative control values

Comments and conclusions: The study was valid under the conditions of this study. (b) (4) is not mutagenic to test strains TA98, TA100, TA1535, TA97a and TA102 in the Ames reverse-mutation assay

Study title: AMES test

Key findings: Under the testing condition, (b) (4) is not mutagenic to test strains TA98, TA100, TA1535, TA97a and TA102 in the Ames reverse-mutation assay.

Study no.: 1012575

Volume #, and page #: electronic submission, page 1-23
Conducting laboratory and location: (b) (4)

Date of study initiation: May 28, 2010
GLP compliance: no, following SOP
QA reports: yes (x) no ( )
Drug, lot #, and % purity: (b) (4)
Lot # 5035-194-4
Purity 99.9%
Methods: plate incorporation

Strains: TA98, TA 100, TA1535, TA97a, and TA102

Concentrations used in definitive study:
TA98 and TA100: 15, 50, 150, 500, 1500, and 5000 µg/plate
TA1535, TA97a and TA102, ±S9: 15, 50, 150, 500, 1500, and 5000 µg/plate
Bacteriotoxicity was evident at 5000 µg/plate using strain TA102 –S9 and at ≥ 1500 µg/plate using strain TA102 +S9

Basis of concentration selection: bacteriotoxicity or ICH S2 criteria for the highest concentration of 5000 µg/plate
Metabolic activation system: Liver S9 mix from male rats, Aroclor 1254-pretreated, 25 µl/plate
Negative controls: DMSO 100 µl/plate
Positive controls:

Tester Strain	Positive controls
TA97a	2-aminoanthracene 9-Aminoacridine
TA98	2-aminoanthracene Benzo(a)pyrene 2-nitrofluorene
TA100	2-aminoanthracene sodium azide
TA102	2-aminoanthracene Mitomycin C
TA1535	2-aminoanthracene sodium azide

Incubation and sampling times: 3 days
Results
Study validity:
No. of replicates: 3

Counting method: not provided

Criteria for positive results: The positive controls induced a response equal to twice (or more) the negative control incidence, which is formally the criterion for a mutagenic activity of a compound. Exception is strain TA102 for which a factor of 1.5 over the concurrent negative control value is considered as positive result.

Selection of bacteria tester strains was adequate based upon Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A). Positive controls produced expected responses. Drug concentration selection was adequate based upon observed bacteriotoxicity or ICH S2 criteria.

Study outcome:

Mean revertant counts in the presence of metabolic activation

Test article	Conc. (μg/plate)	Bacteria strain and number of revertant colonies per plate				
		TA98	TA100	TA1535	TA97a	TA102
(b) (4)	15	46	142	20	293	610
	50	38	138	20	287	714
	150	40	140	17	266	576
	500	44	141	16	244	423
	1500	36	127	13	242	*
	5000	30	121	16	252	*
Positive control	2aminoanthracene Benzo(a)pyrene 2-nitrofluorene	1324 324	2073	228	3811	2176
DMSO	-	46	170	16	244	465

Mean revertant counts in the absence of metabolic activation

Test article	Conc. (μg/plate)	Bacteria strain and number of revertant colonies per plate				
		TA98	TA100	TA1535	TA97a	TA102
(b) (4)	15	17	109	20	197	513
	50	22	104	19	206	521
	150	26	86	23	204	490
	500	25	118	18	315	411
	1500	29	98	19	312	461
	5000	26	94	31	310	*
Positive control	2-nitrofluorene sodium azide 2aminoanthracene Mitomycin C	118	850	1261	4027	2520
DMSO	-	25	114	20	463	463

* Bacteriotoxicity was evident at 5000 μg/plate using strain TA102 –S9 and at ≥ 1500 μg/plate using strain TA102 +S9

- The mean mutant numbers of negative control plates lay within the range of acceptable negative control values;
- The positive controls induced a response equal to twice (or more) the negative control incidence, which is formally the criterion for a mutagenic activity of a compound;
- There were no relevant increases in the number of revertants above the corresponding negative control values under any of the test conditions used after treatment with the test item.

Comments and conclusions: The study was valid under the conditions of this study. (b) (4) is not mutagenic to test strains TA98, TA100, TA1535, TA97a and TA102 in the Ames reverse-mutation assay.

Study title: AMES test

Key findings: Under the testing condition, (b) (4) is not mutagenic to test strains TA98, TA100, TA1535, TA97a and TA102 in the Ames reverse-mutation assay.

Study no.: 1012576

Volume #, and page #: electronic submission, page 1-23
Conducting laboratory and location: (b) (4)

Date of study initiation: May 28, 2010
GLP compliance: no, following SOP
QA reports: yes (x) no ( )
Drug, lot #, and % purity (b) (4)
Lot # 5035-197-2
Purity 86.1%

Methods: plate incorporation

Strains: TA98, TA 100, TA1535, TA97a, and TA102
Concentrations used in definitive study:
TA98 and TA100, ±S9: 15, 50, 150, 500, 1500, and 5000 μg/plate
TA1535, TA97a and TA102, ±S9: 15, 50, 150, 500, 1500, and 5000 μg/plate
Bacteriotoxicity was evident at 5000 μg/plate using strains TA98 +/- S9, TA100 +/- S9, TA1535 +/- S9, TA97a –S9 and TA102 - S9 and at ≥ 1500 μg/plate using strains TA97a +S9 and TA102 +S9

Basis of concentration selection: bacteriotoxicity
Metabolic activation system: Liver S9 mix from male rats, Aroclor 1254-pretreated, 25 μl/plate
Negative controls: DMSO 100 μl/plate

Positive controls:

Tester Strain	Positive controls
TA97a	2-aminoanthracene 9-Aminoacridine
TA98	2-aminoanthracene Benzo(a)pyrene 2-nitrofluorene
TA100	2-aminoanthracene sodium azide

TA102	2-aminoanthracene Mitomycin C
TA1535	2-aminoanthracene sodium azide

Incubation and sampling times: 3 days

Results

Study validity:

No. of replicates: 3

Counting method: not provided

Criteria for positive results: The positive controls induced a response equal to twice (or more) the negative control incidence, which is formally the criterion for a mutagenic activity of a compound. Exception is strain TA102 for which a factor of 1.5 over the concurrent negative control value is considered as positive result.

Selection of bacteria tester strains was adequate based upon Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A). Positive controls produced expected responses. Drug concentration selection was adequate based upon observed bacteriotoxicity.

Study outcome:

Mean revertant counts in the presence of metabolic activation

Test article	Conc. (μg/plate)	Bacteria strain and number of revertant colonies per plate				
		TA98	TA100	TA1535	TA97a	TA102
(b) (4)	15	35	130	12	240	552
	50	31	122	20	243	640
	150	35	160	9	226	595
	500	30	151	20	303	458
	1500	43	131	20	*	*
	5000	*	*	*	*	*
Positive control	2aminoanthracene Benzo(a)pyrene 2-nitrofluorene	1324 324	2073	228	3811	2176
DMSO	-	46	170	16	244	465

Mean revertant counts in the absence of metabolic activation

Test article	Conc. (μg/plate)	Bacteria strain and number of revertant colonies per plate				
		TA98	TA100	TA1535	TA97a	TA102
(b) (4)	15	27	131	22	233	555
	50	27	126	29	236	546
	150	22	102	28	301	620
	500	25	129	17	244	543

	1500	32	122	28	236	680
	5000	*	*	*	182*	*
Positive control	2-nitrofluorene sodium azide 2aminoanthracene Mitomycin C	118	850	1261	4027	2520
DMSO	-	25	114	20	318	463

* Bacteriotoxicity was evident at 5000 µg/plate using strains TA98 +/- S9, TA100 +/- S9, TA1535 +/- S9, TA97a –S9 and TA102 - S9 and at ≥ 1500 µg/plate using strains TA97a +S9 and TA102 +S9

- The mean mutant numbers of negative control plates lay within the range of acceptable negative control values;
- The positive controls induced a response equal to twice (or more) the negative control incidence, which is formally the criterion for a mutagenic activity of a compound;
- There were no relevant increases in the number of revertants above the corresponding negative control values under any of the test conditions used after treatment with the test item.

Comments and conclusions: The study was valid under the conditions of this study. [redacted] (b) (4) is not mutagenic to test strains TA98, TA100, TA1535, TA97a and TA102 in the Ames reverse-mutation assay

Study title: AMES test

Key findings: Under the testing condition, [redacted] (b) (4) is not mutagenic to test strains TA98, TA100, TA1535, TA97a and TA102 in the Ames reverse-mutation assay.

Study no.: 1012577

Volume #, and page #: electronic submission, page 1-20
Conducting laboratory and location: [redacted] (b) (4)

Date of study initiation: May 28, 2010
GLP compliance: no, following SOP
QA reports: yes (x) no ( )
Drug, lot #, and % purity: [redacted] (b) (4)
Lot # 10022-192-Lot2
Purity 95.7%

Methods: plate incorporation

Strains: TA98, TA 100, TA1535, TA97a, and TA102

Concentrations used in definitive study:
TA98 and TA100, ±S9: 15, 50, 150, 500, 1500, and 5000 μg/plate
TA1535, TA97a and TA102, ±S9: 15, 50, 150, 500, 1500, and 5000 μg/plate
The test article precipitated at 5000 μg/plate under all test conditions. In strain TA102 +S9 precipitation was also seen at 1500 μg/plate. Bacteriotoxicity was not evident in this study; however, precipitation prevented colony counting.

Basis of concentration selection: solubility
Metabolic activation system: Liver S9 mix from male rats, Aroclor 1254-pretreated, 25 μl/plate
Negative controls: DMSO 100 μl/plate
Positive controls:

Tester Strain	Positive controls
TA97a	2-aminoanthracene 9-Aminoacridine
TA98	2-aminoanthracene Benzo(a)pyrene 2-nitrofluorene
TA100	2-aminoanthracene sodium azide
TA102	2-aminoanthracene Mitomycin C
TA1535	2-aminoanthracene sodium azide

Incubation and sampling times: 3 days
Results
Study validity:
No. of replicates: 3

Counting method: not provided

Criteria for positive results: The positive controls induced a response equal to twice (or more) the negative control incidence, which is formally the criterion for a mutagenic activity of a compound. Exception is strain TA102 for which a factor of 1.5 over the concurrent negative control value is considered as positive result.

Selection of bacteria tester strains was adequate based upon Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A). Positive controls produced expected responses. Drug concentration selection was adequate based upon observed solubility.

Study outcome:

Mean revertant counts in the presence of metabolic activation

Test article	Conc. (μg/plate)	Bacteria strain and number of revertant colonies per plate				
		TA98	TA100	TA1535	TA97a	TA102
(b) (4)	15	30	156	12	177	559
	50	20	144	16	189	549
	150	29	163	15	212	547
	500	27	139	14	239	464
	1500	34	141	23	247	410*
	5000	*	*	*	*	*
Positive control	2aminoanthracene Benzo(a)pyrene 2-nitrofluorene	1324 324	2073	228	3811	2176
DMSO	-	46	170	16	244	465

Mean revertant counts in the absence of metabolic activation

Test article	Conc. (μg/plate)	Bacteria strain and number of revertant colonies per plate				
		TA98	TA100	TA1535	TA97a	TA102
(b) (4)	15	40	138	24	189	532
	50	37	145	21	319	513
	150	38	135	21	213	517
	500	40	142	23	237	441
	1500	35	149	33	253	512
	5000	37*	169*	34*	*	*
Positive control	2-nitrofluorene sodium azide 2aminoanthracene Mitomycin C	118	850	1261	4027	2520
DMSO	-	25	114	20	318	463

* The test article precipitated at 5000 μg/plate under all test conditions. In strain TA102 +S9 precipitation was also seen at 1500 μg/plate. Bacteriotoxicity was not evident in this study; however, precipitation prevented colony counting.

- The mean mutant numbers of negative control plates lay within the range of acceptable negative control values;
- The positive controls induced a response equal to twice (or more) the negative control incidence, which is formally the criterion for a mutagenic activity of a compound;
- Treatment with the test item did not increase the number of revertants above the corresponding negative control values under any of the test conditions used.

Comments and conclusions: The study was valid under the conditions of this study. (b) (4) is not mutagenic to test strains TA98, TA100, TA1535, TA97a and TA102 in the Ames reverse-mutation assay.

Study title: AMES test

Key findings: Under the testing condition, (b) (4) is not mutagenic to test strains TA98, TA100, TA1535, TA97a and TA102 in the Ames reverse-mutation assay.

Study no.: 1012578

Volume #, and page #: electronic submission, page 1-27
Conducting laboratory and location: (b) (4) Glowienke, PhD, Basel/Switzerland site
Date of study initiation: May 28, 2010
GLP compliance: no, following SOP
QA reports: yes (x) no ( )
Drug, lot #, and % purity: (b) (4)
Lot # 10026-120-2 Lot 3
Purity 99.1%

Methods: plate incorporation

Strains: TA98, TA 100, TA1535, TA97a, and TA102
Concentrations used in definitive study:
TA98 and TA100, ±S9: 15, 50, 150, 500, 1500, and 5000 μg/plate
TA1535, TA97a and TA102, ±S9: 15, 50, 150, 500, 1500, and 5000 μg/plate

Basis of concentration selection: ICH S2 criteria for the highest concentration of 5000 μg/plate

Metabolic activation system: Liver S9 mix from male rats, Aroclor 1254-pretreated, 25 μl/plate
Negative controls: DMSO 100 μl/plate
Positive controls:

Tester Strain	Positive controls
TA97a	2-aminoanthracene 9-Aminoacridine
TA98	2-aminoanthracene Benzo(a)pyrene 2-nitrofluorene
TA100	2-aminoanthracene sodium azide
TA102	2-aminoanthracene

	Mitomycin C
TA1535	2-aminoanthracene sodium azide

Incubation and sampling times: 3 days

Results

Study validity:

No. of replicates: 3

Counting method: not provided

Criteria for positive results: The positive controls induced a response equal to twice (or more) the negative control incidence, which is formally the criterion for a mutagenic activity of a compound. Exception is strain TA102 for which a factor of 1.5 over the concurrent negative control value is considered as positive result.

Selection of bacteria tester strains was adequate based upon Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A). Positive controls produced expected responses. Drug concentration selection was adequate based upon ICH S2 criteria.

Study outcome:

Mean revertant counts in the presence of metabolic activation

Test article	Conc. (μg/plate)	Bacteria strain and number of revertant colonies per plate				
		TA98	TA100	TA1535	TA97a	TA102
(b) (4)	15	77	135	30	2437	501
	50	64	130	19	3511	529
	150	77	180	17	3706	516
	500	75	143	17	2340	492
	1500	77	134	20	2701	463
	5000	37	130	20	1892	363
Positive control	2aminoanthracene Benzo(a)pyrene 2-nitrofluorene	1324 324	2073	228	3811	2176
DMSO	-	46	170	16	244	465

Mean revertant counts in the absence of metabolic activation

Test article	Conc. (μg/plate)	Bacteria strain and number of revertant colonies per plate				
		TA98	TA100	TA1535	TA97a	TA102
(b) (4)	15	29	120	22	769	356
	50	28	125	23	986	418
	150	22	116	14	1541	471
	500	27	139	21	1041	440
	1500	31	151	17	1269	450

	5000	26	157	13	440	396
Positive control	2-nitrofluorene sodium azide 2aminoanthracene Mitomycin C	118	850	1261	4027	2520
DMSO	-	25	114	20	318	463

Repeat experiment on TA97a

[redacted] (b) (4)	Bacteria strain and number of revertant colonies per plate							
Dose (µg/plate)	39	78	156	313	625	1250	2500	5000
+S9								
No, colonies	242	268	264	232	240	contaminated	248	232
Positive cont	2aminoanthracene			3007				
Negative cont	DMSO			222				
-S9								
No, colonies	208	229	232	217	182	235	267	240
Positive cont	2aminoanthracene			3046				
Negative cont	DMSO			218				

Note: increased mean mutant numbers on TA97a were not concentration-dependent. In addition, they could not be confirmed in the repeated experiment. Thus, the test item was not considered to be mutagenic on TA97a.

- The mean mutant numbers of negative control plates lay within the range of acceptable negative control values
- The positive controls induced a response equal to twice (or more) the negative control incidence, which is formally the criterion for a mutagenic activity of a compound
- Treatment with the test item did not increase the number of revertants above the corresponding negative control values.

Comments and conclusions: The study was valid under the conditions of this study. [redacted] (b) (4) is not mutagenic to test strains TA98, TA100, TA1535, TA97a and TA102 in the Ames reverse-mutation assay

Study title: AMES test

Key findings: Under the testing condition, [redacted] (b) (4) is not mutagenic to test strains TA98, TA100, TA1535, TA97a and TA102 in the Ames reverse-mutation assay.

Study no.: 1012579

Volume #, and page #: electronic submission, page 1-20
Conducting laboratory and location: [redacted] (b) (4)

Date of study initiation: May 28, 2010
GLP compliance: no, following SOP

QA reports: yes (x) no ( )
Drug, lot #, and % purity: (b) (4)
Lot # 10026-120-1 Lot3
Purity: "not given"-stated in the study report

Methods: plate incorporation

Strains: TA98, TA 100, TA1535, TA97a, and TA102
Concentrations used in definitive study:
TA98 and TA100: 15, 50, 150, 500, 1500, and 5000 μg/plate
TA1535, TA97a and TA102, ±S9: 15, 50, 150, 500, 1500, and 5000 μg/plate
Bacteriotoxicity was evident at 5000 μg/plate using strain TA102 +/- S9.

Basis of concentration selection: Bacteriotoxicity or ICH S2 criteria for the highest concentration of 5000 μg/plate

Metabolic activation system: Liver S9 mix from male rats, Aroclor 1254-pretreated, 25 μl/plate
Negative controls: DMSO 100 μl/plate
Positive controls:

Tester Strain	Positive controls
TA97a	2-aminoanthracene 9-Aminoacridine
TA98	2-aminoanthracene Benzo(a)pyrene 2-nitrofluorene
TA100	2-aminoanthracene sodium azide
TA102	2-aminoanthracene Mitomycin C
TA1535	2-aminoanthracene sodium azide

Incubation and sampling times: 3 days
Results
Study validity:
No. of replicates: 3

Counting method: not provided

Criteria for positive results: The positive controls induced a response equal to twice (or more) the negative control incidence, which is formally the criterion for a mutagenic activity of a compound. Exception is strain

TA102 for which a factor of 1.5 over the concurrent negative control value is considered as positive result.

Selection of bacteria tester strains was adequate based upon Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A). Positive controls produced expected responses. Drug concentration selection was adequate based upon bacteriotoxicity or ICH S2 criteria.

Study outcome:

Mean revertant counts in the presence of metabolic activation

Test article	Conc. (µg/plate)	Bacteria strain and number of revertant colonies per plate				
		TA98	TA100	TA1535	TA97a	TA102
(b) (4)	15	21	128	13	172	543
	50	22	154	16	209	578
	150	23	153	17	211	582
	500	27	157	21	246	441
	1500	33	144	15	243	598
	5000	26	143	17	313	616*
Positive control	2aminoanthracene Benzo(a)pyrene 2-nitrofluorene	1324 324	2073	228	3811	2176
DMSO	-	46	170	16	244	465

Mean revertant counts in the absence of metabolic activation

Test article	Conc. (µg/plate)	Bacteria strain and number of revertant colonies per plate				
		TA98	TA100	TA1535	TA97a	TA102
(b) (4)	15	22	122	9	204	529
	50	19	127	14	243	633
	150	28	130	19	244	628
	500	28	122	17	308	551
	1500	27	130	19	301	607
	5000	27	123	21	307	*
Positive control	2-nitrofluorene sodium azide 2aminoanthracene Mitomycin C	118	850	1261	4027	2520
DMSO	-	25	114	20	318	463

* Bacteriotoxicity was evident at 5000 µg/plate using strain TA102 +/- S9.

- The mean mutant numbers of negative control plates lay within the range of acceptable negative control values;
- The positive controls induced a response equal to twice (or more) the negative control incidence, which is formally the criterion for a mutagenic activity of a compound;

- Treatment with the test item did not increase the number of revertants above the corresponding negative control values under any of the test conditions used.

Comments and conclusions: The study was valid under the conditions of this study. (b) (4) is not mutagenic to test strains TA98, TA100, TA1535, TA97a and TA102 in the Ames reverse-mutation assay.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The non-clinical studies adequately support the safety of ruxolitinib phosphate by oral route in myelofibrosis. See the EXECUTVE SUMMARY, Page 4, for an overall summary of nonclinical findings.

Signatures:

Reviewer Signature _____

Supervisor Signature_____

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

WEI CHEN
10/26/2011

HALEH SABER
10/27/2011

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 202192 **Applicant:** Incyte **Stamp Date:** June 3, 2011,

Drug Name: ruxolitinib phosphate **NDA/BLA Type:** new commercial

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		*Appears acceptable.
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		*Appears acceptable
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	n/a		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	x		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	x		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	n/a		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	x		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	*x		
11	Has the applicant addressed any abuse potential issues in the submission?	n/a		
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	n/a		

* Issues generally identified during review.

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ____yes____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Wei Chen, Ph.D 06/12/2012

Reviewing Pharmacologist Date

Saber Haleh, Ph.D

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

WEI CHEN
06/14/2011

HALEH SABER
06/14/2011