CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208434Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Alecensa (alectinib)

Date: December 4, 2015
To: File for NDA 208434
From: John K. Leighton, PhD, DABT
Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting and labeling reviews for Alecensa conducted by Drs. Sahalka and Ringgold, and secondary memorandum and labeling provided by Dr. Helms. Dr. Ramadevi Gudi also assisted with review of the genotoxicity data. I concur with Dr. Helms' conclusion that Alecensa may be approved for the proposed indication.

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/s/

JOHN K LEIGHTON
12/04/2015

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

PHARMACOLOGY/TOXICOLOGY NDA LABELING REVIEW

Application number:	208434
Supporting document/s:	2
Applicant's letter date:	6/19/2015
CDER stamp date:	7/06/2015
Product:	ALECENSA (Alectinib hydrochloride)
Indication:	Treatment of Non-Small Cell Lung Cancer (NSCLC)
Applicant:	Hoffmann-La Roche Inc. C/O Genentech Inc. 1 DNA Way South San Francisco, CA United States
Review Division:	Division of Hematology Oncology Toxicology (Division of Oncology Products 2)
Reviewers:	Eias Zahalka, PhD, MBA Kimberly Ringgold, PhD (Pharmacology, Genetic Toxicology, ADME)
Supervisor:	Whitney Helms, PhD
Division Director:	John Leighton, PhD, DABT (DHOT) Patricia Keegan, MD (DOP2)
Project Manager:	Gina Davis

Background:

Hoffmann-La Roche Inc. (the Applicant) submitted New Drug Application (NDA) 208434 for ALECENSA (alectinib hydrochloride) for the treatment of patients with locally advanced or metastatic Non-Small Cell Lung Cancer (NSCLC) whose disease has progressed on crizotinib.

FDA proposed labeling was submitted to the Applicant on November 5, 2015 for comments.

In response to FDA's proposed labeling sent on November 5, 2015, Hoffmann-La Roche Inc. submitted a revised label November 16, 2015, and provided rationale for changes that were not in agreement with FDA.

Changes related to pharmacology/toxicology data occurred in sections 5.5, 8.1, 8.3, 8.4, 12.1 and 13.1 of the label.

Applicant's Proposed Label Edits:

Red font and strikethrough represents Applicant's additions and deletions, respectively

5.5 Embryo-Fetal Toxicity

Based on findings from animal studies and its mechanism of action, ALECENSA ~~can~~ (b) (4) *cause fetal harm when administered to pregnant* (b) (4) *rats and* (b) (4) *rabbits during the period of organogenesis resulted in embryo-fetal toxicity and abortion at maternally toxic doses with exposures approximately 2.7-times those observed in humans with alectinib 600 mg twice daily. Advise pregnant women of the potential risk to a fetus.*

Advise females of reproductive potential to use effective contraception during treatment with ALECENSA and for 1 week following the final dose [see Use in Specific Populations (8.1 and 8.3) and Clinical Pharmacology (12.1)].

Applicant's rationale:
From Roche: Propose to keep (b) (4) because the data are from animals at maternally toxic doses with exposures approximately 2.7-times those observed in humans at the recommended dose.

FDA response: FDA does not agree to this change.
The proposed change of "can" to (b) (4) is not acceptable. "Can" is the appropriate regulatory language based on the literature of the role of ALK in neural development and the available animal data showing embryo-fetal loss at low clinical multiples of exposure. The change to "can" is applicable throughout the label.

8.1 Pregnancy

Risk Summary

Based on animal studies and its mechanism of action, ALECENSA (b) (4) ~~can~~ cause fetal harm when administered to a pregnant woman [see Clinical Pharmacology (12.1)]. There are no available data on ALECENSA use in pregnant women.

Administration of alecitinib to pregnant (b) (4) rats and (b) (4) -rabbits during the period of organogenesis resulted in embryo-fetal toxicity and abortion at maternally toxic doses with exposures approximately 2.7-times those observed in humans treated with alectinib at 600 mg twice daily [see Data]. Advise pregnant women of the potential risk to a fetus.

In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically-recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

Data

Animal Data

In a preliminary rabbit embryo-fetal study, administration of alectinib by oral gavage during the period of organogenesis resulted in abortion or complete embryo-fetal mortality at a maternally toxic dose of 27 mg/kg/day (approximately 2.9-fold the estimated area under the curve ($AUC_{0-24h,ss}$) (b) (4) in humans treated with alectinib 600 mg BID) in three of six pregnant rabbits (b) (4). The remaining three (b) (4) pregnant rabbits in this group had few live fetuses, decreased fetal and placental weights, and retroesophageal subclavian artery. In a rat preliminary embryo-fetal development study, administration of alectinib during organogenesis resulted in complete litter loss in all pregnant rats at 27 mg/kg/day (approximately 4.5-fold the estimated $AUC_{0-24h,ss}$ (b) (4) in humans treated with alectinib 600 mg BID). Doses greater than or equal to 9 mg/kg/day (approximately 2.7-fold the estimated human $AUC_{0-24h,ss}$ (b) (4) in humans treated with alectinib 600 mg BID), resulted in maternal toxicity as well as developmental toxicities including decreased fetal weight, dilated ureter, thymic cord, small ventricle and thin ventricle wall, and reduced number of sacral and caudal vertebrae.

Applicant's rationale:
From Roche: Please note that there are no (b) (4). Therefore the term "pregnant rabbits" is considered appropriate here.

FDA response: These changes are acceptable and do not change the meaning of the findings.

8.3 Females and Males of Reproductive Potential

Contraception

Females

ALECENSA (b) (4) ~~can~~ cause fetal harm when administered to a pregnant woman. Advise females of reproductive potential to use effective contraception during treatment with ALECENSA and for 1 week after the final dose [see Use in Specific Populations (8.1)].

Males

Based on genotoxicity findings, advise males with female partners of reproductive potential to use (b) (4) *effective contraception during treatment with ALECENSA and for 3 months following the final dose [see Non Clinical Toxicology (13.1)].*

(b) (4)

Applicant's rationale:
From Roche: Please see the accompanying response document as to why these effects were not considered related to treatment with alectinib and should not appear in the label – for a brief explanation see comment in Section 13.1.

FDA Response:

With the exception of "(b) (4)," these changes are acceptable. See section 13.1 for the justification for this agreement.

8.4 Pediatric Use

The safety and effectiveness of ALECENSA in pediatric patients have not been established.

~~*Animal Data*~~

~~*Juvenile animal studies have not been conducted using alectinib. In general toxicology studies, treatment of rats with doses of alectinib resulting in exposures greater than or equal to approximately 4.5 times those in humans treated with alectinib 600 mg twice daily resulted in changes in the growing teeth and bones. Findings in teeth included discoloration and changes in tooth size along with histopathological disarrangement of the ameloblast and odontoblast layers. There were also decreases in the trabecular bone and increased osteoclast activity in the femur and sternum.*~~

Applicant's rationale:
From Roche: **"**the applicant proposes not to include information about general toxicology studies conducted in adult rats under section 8.4 Pediatric Use. In accordance with 21 CFR 201.57(c)(9)(iv)(F) and FDA's guidance for Industry and Review Staff Pediatric Information Incorporated into Human Prescription Drug and Biological Products Labeling, the only language required in this section for drugs not seeking a pediatric indication is a statement explaining that the safety and effectiveness have not been established in the relevant pediatric population"

FDA response: FDA does not agree to this change.
The proposed change under section 8.4 (Pediatric Use) of the label is not acceptable. Data from the repeat-dose toxicology studies identified safety concerns that may be relevant for a pediatric population (dental and bone toxicity) and placing this information in this section is consistent with other labels for oncology products with similar findings in general toxicology studies. According to the FDA's guidance for Industry and Review Staff Pediatric Information Incorporated into Human Prescription Drug and Biological

Products Labeling (raw 314-316): If a specific risk has been identified for pediatric patients, this risk information must be described in the Pediatric Use subsection and, if appropriate, placed in the CONTRAINDICATIONS section or WARNINGS AND PRECAUTIONS section.

12.1 Mechanism of Action

Alectinib is a tyrosine kinase inhibitor that targets ALK and RET. In nonclinical studies, alectinib inhibited ALK phosphorylation and ALK-mediated activation of the downstream signaling proteins STAT3 and AKT and decreased in vitro tumor cell viability in- (b) (4) cell lines (b) (4). The major active metabolite of alectinib, M4, has shown similar in vitro potency and activity.

Alectinib and M4 demonstrated in vitro and in vivo activity against multiple mutant forms of the ALK enzyme, including some mutations identified in NSCLC tumors in patients who have progressed on crizotinib.

(b) (4)

In mouse models implanted with tumors carrying ALK fusions, administration of alectinib resulted in antitumor activity and prolonged survival, including in mouse models implanted intracranially with ALK-driven tumor cell lines.

Applicant's rationale:
From Roche:

1. (b) (4)" reworded to be more specific with regards to cell lines that were sensitive to alectinib inhibition.

2. *Applicant proposes to keep this statement:* (b) (4)
". Please see the accompanying response document for rational, Request 4.

FDA response: FDA does not agree to these changes.

1. The proposed change does not reflect accurately the submitted data.

FDA revised text:

In nonclinical studies, alectinib inhibited ALK phosphorylation and ALK-mediated activation of the downstream signaling proteins STAT3 and AKT, and decreased tumor cell viability in multiple cell lines harboring ALK fusions, amplifications, or activating mutations. The major active metabolite of alectinib, M4, showed similar in vitro potency and activity.

2. The P-gp data is already captured under section 12.3 under distribution (b) (4) In addition, the (b) (4)

(b) (4) is (b) (4) described (b) (4)

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity studies with alectinib have not been conducted.

Alectinib was not mutagenic in vitro in the bacterial reverse mutation (Ames) assay, but was positive with an increased number of micronuclei in a rat bone marrow micronucleus test. The mechanism of micronucleus induction was abnormal chromosome segregation (aneugenicity) and not a clastogenic effect on chromosomes.

No studies in animals have been performed to evaluate the effect of alectinib on fertility. No adverse effects on male and female reproductive organs were observed in general toxicology studies conducted in rats and monkeys. (b) (4)

Hoffmann-La Roche Inc. provided justification for the proposed edits under section 13.1:

REQUEST 6

Section 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

FDA included animal findings on (b) (4) in Section 13.1 (and in other sections of the USPI):

(b) (4)

COMPANY RESPONSE

Roche believes that the animal data do not point to a direct effect of alectinib on (b) (4) for the reasons pointed out below and should therefore not be listed in the final label.

(b) (4)

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/s/

EIAS A ZAHALKA
12/02/2015

KIMBERLY R RINGGOLD
12/04/2015

WHITNEY S HELMS
12/04/2015

MEMORANDUM

Date: November 6, 2015
From: Whitney S. Helms, PhD
Pharmacology Supervisor
Division of Hematology Oncology Toxicology for Division of Oncology Products 2
To: File for NDA # 208434
ALECENSA (alectinib)
Re: Approvability of Pharmacology and Toxicology

On July 6, 2015 Hoffman La-Roche completed its rolling submission of New Drug Application (NDA) 208434 for the use of alectinib for the treatment of patients with anaplastic lymphoma kinase (ALK)-positive, metastatic non-small cell lung cancer (NSCLC), whose disease has progressed on crizotinib. The product received breakthrough designation for the treatment of this patient population on June 23, 2013. Non-clinical studies examining the pharmacology and toxicology of alectinib provided to support NDA 208434 were reviewed in detail by Eias A. Zahalka, PhD, and Kimberly Ringgold, PhD, with additional consultation from Ramadevi Gudi, PhD on genotoxicity. The findings of these studies are summarized in the "Executive Summary" of the NDA review and reflected in the product label.

The established pharmacologic class of alectinib is kinase inhibitor. Alectinib is a small molecule administered orally at the recommended dose of 600 mg twice daily (BID). In the clinical trials used to support the approval of alectinib at this dose level, the Cmax at steady state was approximately 665 ng/mL (~1.28 μM) and the estimated AUC at steady state was approximately 14400 ng*hr/mL. Alectinib was ≥ 99% protein bound in all species tested. In biochemical screening assays alectinib was able to inhibit the anaplastic lymphoma kinase (ALK) and the Rearranged during transfection (RET) kinase as well as multiple point mutations of both kinases at concentrations of less than 30 nM. In the patient population intended for treatment with alectinib, tumors are screened for ALK translocation mutations—wild type ALK under the control of an inappropriate promoter resulting in altered expression of a the protein. These alterations in expression drive the initial oncogenic activity of the kinase. Additional point mutations in ALK have also been identified in patients whose tumors have become resistant to treatment with ALK inhibitors. ALK point mutations that tested positive for inhibition by alectinib included several that have been previously identified in patients who have progressed following treatment with criztoinib, including L1196M, C1156Y, 1151Tins, L1152R, and G1269A. In cellular assays incubation with alectinib resulted in inhibition of both ALK phospohorylation and activation of the downstream signaling proteins STAT3 and AKT. In mice subcutaneously implanted with ALK-driven lung, neuroblastoma, or anaplastic large cell lymphoma cell lines, alectinib also inhibited in vivo tumor growth, though the drug had no clear effect on tumors without ALK mutations.

The major target organs identified in general toxicology studies conducted in rats and monkeys included the gastrointestinal (GI) tract, adrenal gland, liver, and lungs, though findings in animals were generally minimal to mild in severity. In the GI tract and lungs, there were findings consistent with hemorrhage in one or both species. These findings correlated with

increases in APTT and PT at the highest dose level in rats. Interstitial lung disease and hepatotoxicity have been observed clinically and are included in the Warnings and Precautions section of the label for alectinib. Gastrointestinal toxicities were also frequent adverse events in clinical trials. Findings in rats of decreased trabecular bone and increased osteoclast activity in the metaphysis along with dental findings of whitened teeth and degeneration/necrosis of ameloblast, dilatation of capillary at papillary and odontoblast layers, and disarrangement of the ameloblast and odontoblast in the mid-region, while not seen in adults studied in the clinical trials for alectinib, are potentially relevant to a pediatric population and are included in section 8.4 of the label. Finally, to help support the use of the clinical formulation of alectinib intended for initial marketing which includes (b) (4) SLS ((b) (4)% w/w), the Applicant also conducted a nonclinical study in rats at a single dose level comparing the clinical and nonclinical formulations. (b) (4) SLS did not significantly affect the toxicity, including GI toxicity, or pharmacokinetics of alectinib in this study, supporting the safety of the initial clinical formulation intended for marketing.

In safety pharmacology studies, alectinib did not cause significant changes in CNS, respiratory, or gastrointestinal motor function. Alectinib did demonstrate some potential for QT prolongation in the in vitro hERG assay (IC_{50}=0.12 μM), though QT prolongation was not observed in in vivo cardiovascular studies and has not been reported clinically. At doses ≥ 20 mg/kg single dose administration of alectinib resulted in modest decreases in blood pressure; these decreases correlated with in vitro findings of inhibition of both Cav1.2 currents and aortic ring contractility at low concentrations, consistent with effects on vasodilation. In the repeat dose toxicology study, monkeys treated at the high dose level also displayed decreases in heart rate at the end of the dosing phase, consistent with bradycardia reported in clinical trials.

Carcinogenicity studies were not conducted to support the approval of alectinib and, in accordance with ICH S9, are not warranted to support the approval of a drug intended for the treatment of patients with advanced cancer. Alectinib was not mutagenic in the bacterial reverse mutation assay or clastogenic in the in vitro Chinese Hamster Lung (CHL) assay, but was positive in the in vivo micronucleus assay. The results of a second in vivo micronucleus test were supportive of an increase in numerical rather than structural aberations. Because of this finding, men with female partners of reproductive potential are advised to use contraception during and for 3 months following the final dose of alectinib.

Distribution studies in pregnant rats suggest that alectinib is able to cross the placenta and in pilot embryofetal development studies conducted in both rats and rabbits, alectinib was embryotoxic at maternally toxic doses. In rats treated at 9 mg/kg/day (approximately 2.7 times the $AUC_{ss,24}$ at the recommended human dose) maternal weight loss and developmental toxicities occurred, including decreased fetal weight, dilated ureter, thymic cord, small ventricle and thin ventricle wall, and reduced number of sacral and caudal vertebrae; at a dose of 27 mg/kg/day there was complete litter loss. In the rabbit embryofetal study, abortion or complete embryofetal mortality occurred in three of six rabbit litters at an oral dose of 27 mg/kg/day (approximately 2.9 times the estimated human $AUC_{ss,24}$ at the recommended dose of 600 mg BID). The remaining three litters in this group had few live fetuses, decreased fetal and placental weights, and retroesophageal subclavian artery. Importantly, ALK inhibition has also been reported to be associated with fetal toxicities including effects on neural development that might

have long term effects that would be difficult to detect in an embryofetal development study in animals[1- 2]. A warning for the risk of embryofetal toxicity is recommended. In addition, females of reproductive potential are advised to use effective contraception during treatment with alectinib and, based on a half-life of approximately 30 hours, for one week following the final dose of the drug.

Reproductive studies investigating the effects of alectinib on fertility or on post-natal development were not submitted and were not required for approval in this patient population. Findings of glandular atrophy in the prostate and seminal vesicles at the high dose (approximately 2.4 times the estimated AUC in humans at the recommended dose of 600 mg BID) in male rats as well as findings of interstial fibrosis of the testis in monkeys at the high dose level (approximately 0.2 times the estimated AUC in humans) suggest that alectinib may have an effect on male fertility. No clear findings of effects on female reproductive organs occurred in the 13-week general toxicology studies.

Metabolism of alectinib was similar between species and M4 was the major metabolite detected in both humans and animals. Metabolism was primarily CYP3A4-mediated. Alectinib and the M4 metabolite showed similar pharmacologic activity and pharmacokinetic properties (protein binding, elimination, distribution). Elimination of alectinib was primarily through the fecal route. Alectinib distributed to the red blood cell compartment and was widely distributed in animal tissues, including the brain (approximately 30-40% of the concentration in the blood at Cmax). In addition, administration of alectinib to mice intracranially implanted with an EML4-ALK-driven lung cancer tumor cell line resulted in improved survival compared to treatment with a vehicle control or crizotinib, supporting the ability of alectinib to penetrate the blood brain barrier.

Recommendations: I concur with the conclusion of Drs. Zahalka and Ringgold that the pharmacology and toxicology data support the approval of NDA 208434 for ALECENSA. There are no outstanding nonclinical issues related to the approval of ALECENSA for the treatment of patients with ALK-positive, metastatic NSCLC, whose disease has progressed on crizotinib. No nonclinical post-marketing studies are warranted at this time.

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[1] Iwahara, T. et. al. Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. Oncogene. 1997: 439-449.

[2] Yao S, Cheng M, Zhang Q, Wasik M, Kelsh R, et al. (2013) Anaplastic Lymphoma Kinase Is Required for Neurogenesis in the Developing Central Nervous System of Zebrafish. PLoS ONE 8(5): e63757. doi:10.1371/journal.pone.0063757

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/s/

WHITNEY S HELMS
11/06/2015

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
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PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	208434
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Applicant's letter date:	6/19/2015
CDER stamp date:	7/06/2015
Product:	ALECENSA (Alectinib hydrochloride)
Indication:	Treatment of Non-Small Cell Lung Cancer (NSCLC)
Applicant:	Hoffmann-La Roche Inc. C/O Genentech Inc. 1 DNA Way South San Francisco, CA United States
Review Division:	Division of Hematology Oncology Toxicology (Division of Oncology Products 2)
Reviewers:	Eias Zahalka, PhD, MBA Kimberly Ringgold, PhD (Pharmacology, Genetic Toxicology, ADME)
Supervisor:	Whitney Helms, PhD
Division Director:	John Leighton, PhD, DABT (DHOT) Patricia Keegan, MD (DOP2)
Project Manager:	Gina Davis

Disclaimer

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Any information or data necessary for approval of NDA 208434 that Hoffmann-La Roche Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 208434.

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1 Executive Summary

1.1 Introduction

Hoffmann-La Roche Inc. (the Applicant) has submitted New Drug Application (NDA) 208434 for ALECENSA (alectinib hydrochloride) for the treatment of patients with locally advanced or metastatic Non-Small Cell Lung Cancer (NSCLC) whose disease has progressed on crizotinib. Alectinib hydrochloride (alectinib) is a new molecular entity kinase inhibitor. The product received breakthrough designation for the treatment of the proposed patient population on June 23, 2013. The recommended human dose is 600 mg taken orally twice daily (BID). Nonclinical pharmacology, pharmacokinetic and toxicology studies have been submitted to support the approval of alectinib for the proposed indication.

1.2 Brief Discussion of Nonclinical Findings

Pharmacology studies demonstrated that alectinib is a reversible kinase inhibitor that targets the anaplastic lymphoma kinase (ALK) and the Rearranged during transfection (RET) kinase at clinically relevant concentrations (based on a Cmax of 665 ng/mL at the 600 mg BID dose and protein binding of greater than 99% in all species tested). Alectinib suppressed activation of the ALK kinase, demonstrated by inhibition of ALK phosphorylation, and prevented the phosphorylation of downstream signaling molecules, such as STAT3 and AKT. In both in vitro (cellular proliferation) and in vivo experiments, alectinib was able to inhibit multiple mutations of ALK that have been identified in patients whose disease has progressed following treatment with crizotinib. In addition, the Applicant investigated the activity of major metabolites of alectinib, including the M4 metabolite that has been identified at high levels in both humans and animals. M4 showed comparable inhibitory activity against ALK and RET as well as selected point mutations in each of these proteins. Similarly to their behavior in humans, alectinib and M4 also showed comparable distribution, protein binding, and elimination in the feces.

ALK has an important role during development, particularly in neural development, but has limited expression in normal cells. Translocations with the *ALK* gene have led to the expression of oncogenic fusion proteins resulting in dysregulated expression of, and increased signaling through this kinase. Thus, effects of alectinib on tumor cell lines with wild type ALK in the absence of a promotor translocation are not likely to be significant. Consistent with this mechanism, little inhibition was observed following alectinib treatment in either in vitro or in vivo NSCLC models with wild-type ALK. Alectinib treatment did result in tumor growth inhibition and tumor regression in the NSCLC, neuroblastoma and ALCL models carrying ALK fusions, confirming the anti-tumor activity in cancers with ALK gene amplifications or rearrangements. The Applicant also investigated the administration of alectinib in combination with other chemotherapeutic agents (cisplatin, paclitaxel, or gemcitabine) to mice implanted with a variety of tumor models driven by ALK rearrangements. In general, the results of these studies showed some improvements in anti-tumor activity of the combinations compared to monotherapy with little effect on body weight, suggesting that these

combination treatments did not result in major decrements in tolerability compared to monotherapy. Alectinib also showed greater inhibition of tumor growth than crizotinib in a mouse model implanted intracranially with the NCI-H2228 tumor cell line carrying an EML4-ALK fusion protein, suggesting that alectinib can cross the blood brain barrier and may have activity against brain metastases. Alectinib distributed to the red blood cell compartment at high levels and had broad tissue distribution including the brain; levels of radioactivity expressed in brain tissues were approximately 30-40% of those seen in the blood of animals administered radiolabelled alectinib, further supporting the penetration of alectinib across the blood brain barrier.

The Applicant conducted repeat-dose general toxicology studies (13 weeks) of alectinib in the rat and monkey. Exposures at the highest dose levels in rats exceeded the estimated AUC_{ss24} of 14400 ng*h/mL in humans at the clinically recommended dose of 600 mg BID, though top exposures in monkeys were approximately half of those at the clinically recommended dose. The predominant toxicities in both species were associated with the adrenal gland, gastrointestinal system (stomach, cecum, colon, rectum, ileum and jejunum), liver, reproductive system (testes, epididymis, seminal vesicles, and prostate) and respiratory system (lung and trachea), though findings in general were minimal to mild in nature. Clinical pathology changes correlated with macroscopic and microscopic findings in the liver (increases in creatinine, cholesterol, triglyceride, ALP isoenzymes, and globulin α2), and other target organs/systems (increases in reticulocytes, platelets, and neutrophils, and decreases in hematocrit, hemoglobin, along with abnormal red blood cell morphology), and with increased APTT and PT in rats. The reported increase in clotting time (APTT and PT) was consistent with the clinical observation of blackish feces in rats and decreased RBC parameters, dark-red gelatinous material in the cecum and colon, and hemorrhage in the mucosa of the ileum. Hemorrhage and intestinal perforation are serious adverse events that have occurred clinically as well. In the rat and monkey, changes in all organs showed recovery or a trend towards recovery. Cardiovascular assessments included in either the general toxicology study in monkeys or in stand-alone safety pharmacology studies suggested a potential for bradycardia and hypotension. Bradycardia as well as liver enzyme elevations, GI disorders, and anemia have been reported clinically.

In rats, additional target organs of interest included the growing bones and teeth. Findings included whitening as well as degeneration/necrosis of ameloblast, dilatation of capillary at papillary and odontoblast layers, disarrangement of ameloblast and odontoblast in the mid-region in the teeth and decreased trabecular bone with increased osteoclast activity in the metaphysis. These findings, while unlikely to be of concern in an adult patient population, may be relevant for a pediatric population. In addition, alectinib exhibited a phototoxic response in an in vitro cytotoxicity assay with Neutral Red uptake in Balb/c 3T3 mouse fibroblast cell line, consistent with phototoxicity reported clinically, and, while negative for mutagenicity in the Ames assay, was positive for clastogenicity in in vivo micronucleus assays. The effects on chromosomes were numerical rather than structural.

In addition to the standard toxicology studies, the Applicant also conducted a 28-day GLP-compliant comparative toxicity and toxicokinetic study of the clinical formulation of alectinib and the nonclinical formulation because of concerns (b) (4) ((b) (4)% w/w) of the excipient sodium lauryl sulfate (SLS) used to formulate the clinical product. The SLS formulation at 20 mg/kg/day (approximately 2.3 times the estimated human $AUC_{ss,24}$ at the 600 mg BID dose of alectinib) showed relatively comparable systemic exposure and toxicity profiles to the non-SLS containing formulation used in the long-term toxicology studies.

Alectinib did cross the placenta in distribution studies of radiolabelled drug administered to pregnant rats. In rabbit and rat embryofetal studies, administration of alectinib resulted in embryotoxicity at maternally toxic doses. In a rabbit preliminary embryofetal study, maternal toxicity was observed at an oral dose of 27 mg/kg/day (approximately 2.9 times the estimated human $AUC_{ss,24}$ *[14900 ng.hr/mL]* at the recommended human dose of 600 mg BID) and abortion or complete embryofetal mortality occurred in three of six rabbit litters at this dose. The remaining three litters in this group had few live fetuses, decreased fetal and placental weights, and retroesophageal subclavian artery. The developmental no observed adverse effect level (NOAEL) was 9 mg/kg/day (approximately 0.4-fold the estimated human $AUC_{ss,24}$ at the 600 mg BID dose). In a rat preliminary embryofetal study, maternal toxicity was observed at oral doses equal to or greater than 9 mg/kg/day (approximately 2.7 times the $AUC_{ss,24}$ at the recommended human dose). Complete litter loss was observed in all pregnant rats at 27 mg/kg/day. At 9 mg/kg/day maternal weight loss and developmental toxicities occurred, including decreased fetal weight, dilated ureter, thymic cord, small ventricle and thin ventricle wall, and reduced number of sacral and caudal vertebrae. The maternal and fetal no adverse effect level was 3 mg/kg/day (approximately equal to the estimated human $AUC_{ss,24}$ at the recommended human dose). Based on these data and data from the literature describing a role for ALK in neural development, a warning for risks to the developing fetus is warranted.

1.3 Recommendations

1.3.1 Approvability

There are no nonclinical findings that would preclude the approval of alectinib for the proposed indication.

1.3.2 Additional Non Clinical Recommendations

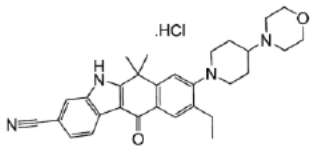
None

1.3.3 Labeling Recommendations

See final label. A separate labeling review will be conducted if warranted.

2 Drug Information

2.1 Drug:

Name:	Alectinib hydrochloride
Code Name:	RO5424802-002 ((b) (4) HCl salt) RO5424802-001 ((b) (4) HCl salt) RO5424802-000 (free base form)
Legacy Company Code:	CH5424802
Chemical Name:	9-ethyl-6,6-dimethyl-8-[4-(morpholin-4-yl)piperidin-1-yl]-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile hydrochloride
Molecular Formula:	$C_{30}H_{35}ClN_4O_2 \cdot HCl$
Molecular Weight:	519.08 g/mol (HCL salt), 482.62 g/mol (free base form)
Structure	**Alectinib HCl Structure**  *(Excerpted from Applicant's submission)*
Pharmacologic Class:	kinase inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs: IND 111723

2.3 Drug Formulation

Hard capsules size 1 containing 150 mg of alectinib.

Table 1: Composition of 150 mg Capsules

Components	Reference to Standards	Function	Quantity per Unit Dose (measure of wt/capsule)
Capsule Fill Mass			
Alectinib HCl	In-house specification	Active	161.33 mg[a]
Lactose Monohydrate	USP, Ph. Eur., JP		(b) (4)
Hydroxypropylcellulose	USP, Ph. Eur., JP		
Sodium Lauryl Sulfate	USP, Ph. Eur., JP		
Carboxymethylcellulose Calcium	USP, Ph. Eur., JP		
Magnesium Stearate	USP, Ph. Eur., JP		
(b) (4)	USP, Ph. Eur., JP		
Total Capsule Fill Weight	—		
Capsule Shell[c]			
Carrageenan	USP/NF, Ph. Eur., EEC, JPE		
Potassium Chloride	USP/NF, Ph. Eur., JP		
Titanium Dioxide (b) (4)	USP/NF, Ph. Eur., JP		
Carnauba Wax	USP/NF, Ph. Eur., JP		
Corn Starch	USP/NF, Ph. Eur., JP		
Hypromellose	USP/NF, Ph. Eur., JP		
Printing Ink[d]	—		
Capsule Shell Weight	—		
Total Capsule Weight	—	—	400.00 mg

(b) (4)

(Excerpted from Applicant's submission)

2.4 Comments on Novel Excipients

There were no novel excipients used in the manufacture of the drug product.

Sodium lauryl sulfate (SLS) was added as an excipient in the clinical formulation at a concentration of (b) (4)% (w/w SLS to API), however this excipient was not used in the formulations used in nonclinical studies. (b) (4) Applicant conducted a 28-day bridging repeat-dose study in rats to characterize the safety of the excipient (b) (4) Results of this study showed a comparable exposure/toxicity profile with both formulations.

2.5 Comments on Impurities/Degradants of Concern

There were no impurities present above the ICH qualification thresholds.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed alectinib hydrochloride regimen for the treatment of subjects with advanced or metastatic Non-Small Cell Lung Cancer is administration orally twice daily (BID) 600 mg (1200 mg/day).

2.7 Regulatory Background

Original IND (111723) was submitted to the FDA on September 30, 2011.

In type B meetings held on July 22, 2013 and November 17, 2014, the Applicant and the FDA discussed the proposed formulation (b) (4) in the drug product (b) (4) FDA recommendations for resolution were as follows:

- Identify other formulations.
- Provide adequate human clinical data to support safety.
- Conduct a nonclinical bridging study (28-day study comparing old toxicology formulation to current clinical formulation).
- Provide data demonstrating (b) (4) SLS.
- Provide data correlating rapid dissolution rate with bioavailability.

Following FDA's recommendation, the Applicant conducted a 28- day rat toxicology study comparing alectinib treatment with and without (b) (4)% SLS, the results of which are presented below in the current review. FDA agreed that the study was sufficient to address immediate concerns, but continued to encourage Roche to study alternate formulations of alectinib (b) (4) given previously documented toxicity data for SLS.

3 Studies Submitted

Study #	Study Title	Reviewed	
		Yes	No
Pharmacology Studies-Primary			
PHM09-0240S	Antitumor activity of CH5424802* in the A549 human lung cancer xenograft model	X	
PHM10-0020S	The effect of CH5424802* on ALK phosphorylation in NCI-H2228 xenograft tumors		X
PHM09-0241S	Long-term antitumor activity of CH5424802* in the NCI-H2228 human lung cancer xenograft model		X
PHM09-0242S	Antitumor activity of CH5424802* in the KARPAS-299 human ALCL xenograft model (including Amendment)	X	
PHM09-0220S	In vitro growth inhibition of CH5424802*-002 in human lymphoma and neuroblastoma cell lines	X	
PHM09-0239S	Antitumor activity of CH5424802* in the NCIH2228 human lung cancer xenograft model	X	
PHM09-0238S	Inhibition of spheroid formation by CH5424802* in human lung cancer cell lines	X	
PHM13-0023	*In vitro* evaluation of the inhibitory activity of CH5424802*-002 on various kinases: Part 3 (including Amendments)	X	
PHM13-0021	Evaluation of cell growth inhibitory activity by CH5424802-002* in RET-related cell lines		X
PHM13-0105	Evaluation of antitumor activity of CH5424802* in the LC-2/ad human lung cancer xenograft model		X
PHM13-0114	In vitro growth inhibition by CH5424802*-002 in Ba/F3 cells expressing mutated EML4-ALK	X	
PHM14-0078	Evaluation of antitumor activity of CH5424802* in mice model of Ba/F3 expressing EML4-ALK G1269A		X
PHM14-0079	Evaluation of antitumor activity of CH5424802* in mice model of Ba/F3 expressing EML4-ALK G1202R		X
PHM14-0080	Evaluation of antitumor activity of CH5424802* in mice model of Ba/F3 expressing EML4-ALK 1151Tins		X
PHM14-0081	Evaluation of antitumor activity of CH5424802* in mice model of Ba/F3 expressing EML4-ALK F1174L		X
PHM14-0082	Evaluation of antitumor activity of CH5424802* in mice model of Ba/F3 expressing EML4-ALK S1206Y		X
PHM14-0087	In vitro evaluation of the inhibitory activity of crizotinib and LDK378 on various kinases		X
PHM14-0077	In vitro evaluation of the inhibitory activity of the metabolite CH5468924 of CH5424802* on various protein kinases and ALK mutations	X	
PHM09-0237S	Inhibition of cellular ALK activity and ALK signaling pathway by CH5424802*	X	

PHM10-0025S	Antitumor activity of CH5424802* in combination with gemcitabine in the NCI-H2228 human lung cancer xenograft model	**X**	
PHM10-0026S	Antitumor activity of CH5424802* in combination with bevacizumab in the NCI-H2228 human lung cancer xenograft model		**X**
PHM12-0054	In vitro evaluation of the inhibitory activity of CH5424802*-002 on various kinases: Part 2	**X**	
PHM12-0105	Evaluation of cell growth inhibitory activity by CH5424802*-002 in spheroid cultured human non-small cell lung cancer cell lines	**X**	
PHM12-0067	In vitro growth inhibition by CH5424802-002 in human lymphoma cell lines	**X**	
PHM12-0140	Evaluation of antitumor activity of CH5424802-002 in a mouse model of Ba/F3 expressing EML4-ALK L1196M (including Amendment)		**X**
PHM12-0145	Evaluation of antitumor activity of CH5424802* after treatment of crizotinib in the NCI-H2228 human non-small cell lung cancer xenograft model		**X**
PHM12-0146	Efficacy of CH5424802* in an intracranial NCI-H2228 implantation model	**X**	
1056355	(b) (4) - Primary Screen Report		**X**
PHM12-0143	In vitro evaluation of the inhibitory activity of metabolites of CH5424802 on ALK (including Amendment 1)	**X**	
PHM12-0144	Evaluation of cell growth inhibitory activity by metabolites of CH5424802 in spheroid cultured NCI-H2228 human non-small cell lung cancer cell line	**X**	
PHM13-0062	Evaluation of antitumor activity of CH5424802* in an intracranial xenograft model using bioluminescence imaging		**X**
PHM13-0040	*In vitro* evaluation of the inhibitory activity of CH5424802*-002 on various kinases: Part 4	**X**	
PHM09-0213	In vitro evaluation of the inhibitory activity of CH5424802*-002 on various protein kinases		**X**
PHM09-0243S	Antitumor activity of CH5424802* in the NB-1 human neuroblastoma xenograft model (including Amendment)	**X**	
PHM10-0023S	Antitumor activity of CH5424802* in combination with cisplatin in the NCI-H2228 human lung cancer xenograft model	**X**	
PHM10-0024S	Antitumor activity of CH5424802* in combination with paclitaxel in the NCI-H2228 human lung cancer xenograft model	**X**	
Pharmacology Studies-Secondary			
8300820	In Vitro Pharmacology - Study of CH5424802*-000 (b) (4)		**X**
8300804	In Vitro Pharmacology - Study of CH5424802*-000, (b) (4)		**X**
8300790	In Vitro Pharmacology: General Safety Profile - Study of CH5424802*-000 (b) (4)		**X**

Pharmacokinetic / ADME			
ADM12-0142	Measurements of plasma concentration of CH5424802-000 and its metabolite CH5468924*-000 after single oral administrations of CH5424802*-002 in Wister Hannover rats		**X**
ADM12-0143	Measurements of plasma concentration of CH5424802*-000 and its metabolite CH5468924+-000 after single oral administrations of CH5424802*-002 in cynomolgus monkeys		**X**
ADM13-0065	In vitro study on inhibition of CH5424802* on OATP1B3 using HEK293 cells exogenously expressing OATP1B3		**X**
ADM13-0075	In vitro study on inhibitory properties of CH5424802* and its metabolite, CH5468924, on transport activity of MRP2 and BSEP using transporter-expressing vesicles		**X**
ADM14-0020	In vitro studies on CH5468924*-000 [CH5424802+ metabolite] transport of MDR1 and BCRP using Caco2 cells and BCRP expressing cells		**X**
1063003	RO5424802 (Alectinib) and RO5468924 (M4 Metabolite of RO5424802 [Alectinib]): assessment of the potential for active human hepatic uptake and substrate potential for OATP1B1 and OATP1B3 *in vitro*		**X**
ADM09-0146	Inhibitory Effects of CH5424802*-000 on Cytochrome P450 Enzyme Activities in Human Liver Microsomes		**X**
ADM12-0019	Determination of Inhibition Constant of CH5424802* on CYP2C8 Activity in Human Liver Microsomes		**X**
ADM12-0082	Enzyme Induction Study of CH5424802* Using Cryopreserved Human Hepatocytes		**X**
ADM12-0084	In vitro studies on transport and inhibition of P-glycoprotein for CH5424802* using Caco-2 cell monolayer		**X**
ADM12-0085	In Vitro studies on transport involving human BCRP protein and inhibition involving human BCRP, OATP1B1, OAT1, OAT3 and OCT2 proteins for CH5424802* using transporter expressing cells		**X**
ADM13-0011	Inhibitory Effects of CH5468924*-000 on Cytochrome P450 Enzyme Activities in Human Liver Microsomes		**X**
ADM13-0016	In vitro study on inhibition of CH5468924-000 [CH5424802* metabolite] on BCRP using MDCK cells exogenously expressing BCRP		**X**
ADM13-0040	In vitro study on inhibition of CH5468924 [CH5424802* Metabolite] on P-glycoprotein using Caco-2 cell monolayer		**X**
ADM09-0151	Identification of Cytochrome P450 Isozymes Involved in the Biotransformation of [14C]CH5424802*	**X**	
ADM09-0148	In vitro metabolic profiles of [14C]CH5424802* in mouse, rat, monkey, dog and human cryopreserved hepatocytes	**X**	

ADM12-0066	Identification of Cytochrome P450 Isoforms Involved in the Biotransformation of [14C]CH5424802* − Effect of cytochrome P450 inhibitors		**X**
1056535	Human In Vitro Metabolism of RO5424802: Role of Cytochrome P450 Enzymes		**X**
ADM13-0042	Exposure assessment of CH5468924+-000 and CH5507197‡-000 by using a qualified method in stored rat plasma samples from 'A 13-Week Repeated Dose Oral Gavage Toxicity Study of CH5424802*-002 in Rats Followed by a 8-Week Recovery Period'		**X**
ADM13-0043	Exposure assessment of CH5468924+-000 and CH5507197‡-000 by using a qualified method in stored monkey plasma samples from 'A 13-Week Repeated Dose Oral Gavage Toxicity Study of CH5424802*-002 in Cynomolgus Monkeys Followed by an 8- Week Recovery Period'		**X**
ADM13-0094	Determination of CH5424802*-000 and CH5468924-000 in Plasma after 1 Week Repeated Oral Administration of CH5424802-002 to Male Monkeys		**X**
1062030	Structure characterization of human unknown metabolites (Mia and Ml b) of alectinib*		**X**
1062527	Metabolism of 14C-RO5468924 (M4 Metabolite of RO5424802 [Alectinib]) by Recombinantly Expressed Individual Cytochrome P450 Enzymes		**X**
ADM14-0028	Identification of major component of Ml metabolite mixture in human hepatocytes incubation with [14C]CH5424802*		
ADM14-0139	Exploratory study on M1 metabolites in rat bile after an intravenous dosing of [14C]CH5424802*		**X**
ADM12-0064	Excretion and Metabolites Profiling in Bile and Enterohepatic Circulation after Single Intravenous Administration of [14C]CH5424802* to Rats	**X**	
ADM09-0149	Quantitative whole-body autoradiography in albino rats after single oral administration of [14C]CH5424802*		**X**
ADM09-0150	In vitro plasma protein binding and blood cell distribution of [14C]CH5424802*	**X**	
ADM13-0007	In Vitro Plasma Protein Binding and Blood Cell Distribution of CH5468924-000 [CH5424802* Metabolite]	**X**	
ADM12-0063	Quantitative Whole-Body Autoradiography in Pregnant Rats after Single Oral Administration of [14C]CH5424802* – Feto-Placental Transfer of Radioactivity	**X**	
ADM12-0067	Identification of [14C]CH5424802* Binding Protein in Human Plasma	**X**	
ADM13-0005	Quantitative Whole-Body Autoradiography in Pigmented Rats after Single Oral Administration of [14C]CH5424802* (including Amendment)	**X**	
ADM12-0065	Pharmacokinetic Study of CH5424802* after Single Oral and Intravenous Administration of CH5424802 to Rats	**X**	

ADM09-0147	Absorption, Metabolism and Excretion in Rats after Single Oral Administration of [14C]CH5424802*	X	
ADM10-0002S	Pharmacokinetics of CH5424802*-000 in cynomolgus monkeys	X	
ADM10-0001S	Pharmacokinetics of CH5424802*-000 in rats		X
ADM13-0017	Caco-2 permeability of R05428402		X
ADM14-0050	Exploratory pharmacokinetic study of CH5468924*-000 and its metabolite CH5507197-000 after single intravenous and oral administrations of CH5468924*-000 in Wister Hannover rats		X
ADM14-0051	Exploratory pharmacokinetic study of CH5468924-000 and its metabolite CH5507197-000 after single intravenous and oral administrations of CH5468924-000 in cynomolgus monkey		X
TOX13-0009	Validation of a Method for the Determination of CH5468924*-000 in Rat Plasma Using LC-MS/MS		X
TOX13-0010	Validation of a Method for the Determination of CH5468924*-000 in Monkey Plasma Using LC-MS/MS		X
TOX09-0129	Validation of a method for the determination of CH5424802-000 in monkey plasma using LC-MS/MS		X
ADM09-0145	Radiochemical Purity of [14C]CH5424802*		X
TOX09-0128	Validation of a method for the determination of CH5424802-000 in rat plasma using LC-MS/MS		X
ADM10-0030S	Measurements of plasma concentration of CH5424802* in mice of xenograft model		X
TOX09-0130	Long-Term Stability of CH5424802*-000 in Rat Plasma		X
TOX09-0131	Long-Term Stability of CH5424802*-000 in Monkey Plasma		X
TOX12-0030	Validation of a Method for the Determination of CH5424802*-000 in Rabbit Plasma Using LC-MS/MS		X
Safety Pharmacology			
TOX09-0165	The Effects of CH5424802*-002 on the Central Nervous System in Rats	X	
TOX09-0167	The Effects of CH5424802*-002 on the Respiratory System in Rats	X	
TOX09-0150	The Effects of CH5424802*-002 on hERG Current	X	
TOX09-0151	The Effects of CH5424802*-002 on the Cardiovascular System in Conscious Monkeys	X	
TOX09-0048S	Effects of CH5424802*-002 on Cardiovascular System in Cynomolgus Monkeys (Preliminary Study)	X	
TOX09-0152S	Effects of CH5424802-002 on gastrointestinal motor function in rats	X	
TOX09-0055S	Effects of CH5424802*-000 on vasomotion in isolated rat aorta (including Amendment)	X	
TOX12-0060	Effects of CH5424802*-002 on Calcium Currents In Human Transfected CHO Cells	X	

Toxicology Studies			
TOX09-0034S	A Preliminary 2-Week Repeated Oral Dose Toxicity Study of CH5424802*-002 in Cynomolgus Monkeys		**X**
TOX09-0141S	A 1-Month Repeated Oral Dose Toxicity Study of CH5424802*- 002 in Cynomolgus Monkeys (Dose Range Finding Study)		**X**
TOX10-0027	A 13-Week Repeated Dose Oral Gavage Toxicity Study of CH5424802-002* in Cynomolgus Monkeys Followed by an 8- Week Recovery Period	**X**	
TOX10-0026	A 13-Week Repeated Dose Oral Gavage Toxicity Study of CH5424802*-002 in Rats Followed by a 8-Week Recovery Period (Including Amendment 1)	**X**	
TOX13-0127	A 28-day oral gavage repeated-dose toxicity study of CH5424802*-002 in rats comparing formulations of the previous 4-week toxicity studies and the current clinical formulation containing SLS	**X**	
TOX09-0127	A 4-week repeated dose oral gavage toxicity study of CH5424802*-002 in cynomolgus monkeys followed by a 4-week recovery period	**X**[1]	
TOX09-0142S	A 1-Month Repeated Oral Dose Toxicity Study of CH5424802*- 002 in in Rats (Dose Range Finding Study) (including Amendment 1)	**X**[1]	
TOX09-0126	A 4-week repeated dose oral gavage toxicity study of CH5424802*-002 in rats followed by a 4-week recovery period	**X**[1]	
Reproductive Toxicology			
TOX12-0024	A Preliminary Study for Effects of CH5424802*-002 on Embryofetal Development by Oral Gavage Dose in Rabbits (including Amendment)	**X**	
TOX12-0023	A 10-day Repeated Dose Oral Gavage Toxicity Study of RO5424802*-002 in Non-Pregnant Rabbits		**X**
TOX12-0019	A Preliminary Study for Effects of CH5424802*-002 on Embryofetal Development by Oral Gavage Dose in Rats	**X**	
Genetic Toxicology			
1064502	Assessment of RO5424802 to induce non-disjunction		**X**
TOX10-0019S	Study to Elucidate the Mechanisms of Micronucleus Induction by CH5424802*-002 in TK6 Cells (including Amendment 1)		
TOX09-0154	Chromosomal Aberration Test of CH5424802*-002 with CHL Cells	**X**[1]	
TOX09-0153	Bacterial Reverse Mutation Test of CH5424802*-002 (including Amendment 1)	**X**[1]	
TOX09-0155	Micronucleus Test of CH5424802*-002 in Rat Bone Marrow - Oral Administration Route –	**X**[1]	
TOX12-0040	Micronucleus test of CH5424802*-002 in rat bone marrow with FISH analysis	**X**[1]	
Other Toxicology studies			
TOX11-0047	In vitro 3T3 NRU Phototoxicity Test of CH5424802*	**X**	

1055385	Toxicological assessment (b) (4) of SLS ((b) (4)% of API) in the clinical tablet formulation that is being used in Roche phase I/II clinical trials with RO5424802 for registration in NSCLC patients who failed for Crizotinib treatment	X	
TOX13-0018	Microsuspension Ames test of CH5468924*		X
TOX13-0019	In vitro micronucleus test of CH5468924*-000		X
1063196	Tox memo (2 page memo)		X
1063197	Tox memo (2 page memo)		X
TOX15-0016	RO/CH542802*: Micro-Ames tests for potential impurities in the manufacturing process of alactinib		X

[1] *Reviewed during the IND phase*

4 Pharmacology

4.1 Primary Pharmacology

Study Title: CH5424802, a Selective ALK Inhibitor Capable of Blocking the Resistant Gatekeeper Mutant	
Study no.:	N/A (referenced article) Sakamoto et al. 2011
Objectives: to evaluate the binding detail of the (b) (4) structure of alectinib and recombinant human ALK	
Methods	
(b) (4)	

Results

(b) (4)

Conclusion

Alectinib binds to the ATP binding site of human recombinant ALK protein.

Study Title: In vitro evaluation of the inhibitory activity of CH5424802 on various protein kinases	
Study no.:	1054067 (PHM13-023)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan
Date of study start:	12/4/2009
Compliance:	N/A
Drug/lot/purity	CH5424802; 004, 99.8%
Vehicle:	DMSO
Reference article:	Staurosporine, lapatinib, sorafenib
Species:	Wistar-Han rat (7 weeks old)
Objectives: To confirm CH5424802 selectively against a panel of kinases	
Methods The inhibitory activity of CH5424802 against various protein kinases including anaplastic lymphoma kinase (ALK) using a cell-free enzyme assay	

Results

Table 2: IC_{50} values for CH5424802 and staurosporine from various kinases assays

Class	Enzyme	IC_{50} (nmol/L)	
		CH5424802-002	Staurosporine
Receptor tyrosine kinase	ALK	1.9	11
	ALK (F1174L)	1.0	14
	ALK (R1275Q)	3.5	9.9
	FGFR2	>5,000	9.0
	IGF1R	>5,000	880
	INSR	550	770
	KDR	1,400	14
	KIT	>5,000	52
	MET	>5,000	400
	PDGFRβ	>5,000	12
Tyrosine kinase	ABL	>5,000	1,200
	JAK1	>5,000	90
	SRC	>5,000	31
Serine/threonine kinase	Akt1	>5,000	39
	Aurora A	>5,000	7.9
	cdk1/cyclin B	>5,000	13
	cdk2/cyclin A	>5,000	<6.9
	MEK1	>5,000	30
	PKA	>5,000	<6.9
	PKCα	>5,000	<6.9
	PKCβ1	>5,000	<6.9
	PKCβ2	>5,000	<6.9

(Excerpted from Applicant's report)

Table 3: IC_{50} values for CH5424802 and lapatinib from EGFR and HER2 kinase assays

Class	Enzyme	IC_{50} (nmol/L)	
		CH5424802-002	Lapatinib
Receptor tyrosine kinase	EGFR	>5,000	15
	HER2	>5,000	22

Table 4: IC_{50}values for CH5424802 and sorafenib from Raf-1 kinase assays

Class	Enzyme	IC_{50} (nmol/L)	
		CH5424802-002	Sorafenib
Serine/threonine kinase	Raf-1	>5,000	16

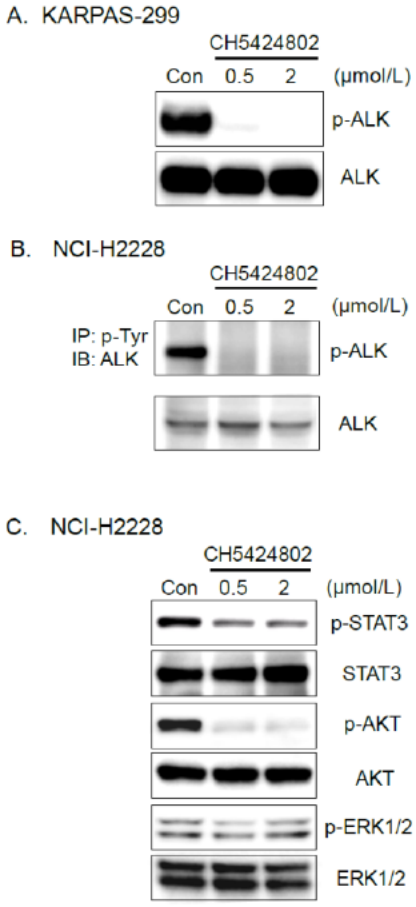
Conclusion

CH5424802 exhibited potent selective inhibitory activity against ALK and ALK mutants (F1174L & R1275Q) with IC_{50} values of 1.0 nmol/L to 3.5 nmol/L in the kinases assayed. These ALK mutants are not crizotinib resistant.

Study Title: Inhibition of cellular ALK activity and ALK signaling pathway by CH5424802	
Study no.:	1054068 (PHM09-0237S)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan
Date of study start:	11/11/2009
Compliance:	N/A
Drug/lot/purity	CH5424802; 004, 99.8%
Vehicle:	DMSO
Species:	Wistar-Han rat (7 weeks old)
Methods The ability of CH5424802 to inhibit ALK activity was examined by measuring phosphorylated ALK using *NPM-ALK*-positive anaplastic large-cell lymphoma cell line, KARPAS-299, and *EML4-ALK*-positive lung cancer cell line, NCIH2228. Further, inhibitory effects on the ALK downstream signaling pathway were evaluated by measuring phosphorylated STAT3, AKT, and ERK1/2 in NCI-H2228 cells. Cells were exposed to 0.5 and 2 μmol/L CH5424802 for 2 hours.	

Results

Figure 2: Effects of Alectinib on ALK phosphorylation and downstream signaling molecules



(Excerpted from Applicant's submission)

Conclusion

CH5424802 can inhibit ALK phosphorylation in cancer cells with ALK gene alterations. The drug also showed more limited effects on downstream ALK-signaling targets, including some inhibition of STAT3 and AKT phosphorylation, in NCI-H2228 cells which express the EML4-ALK fusion protein.

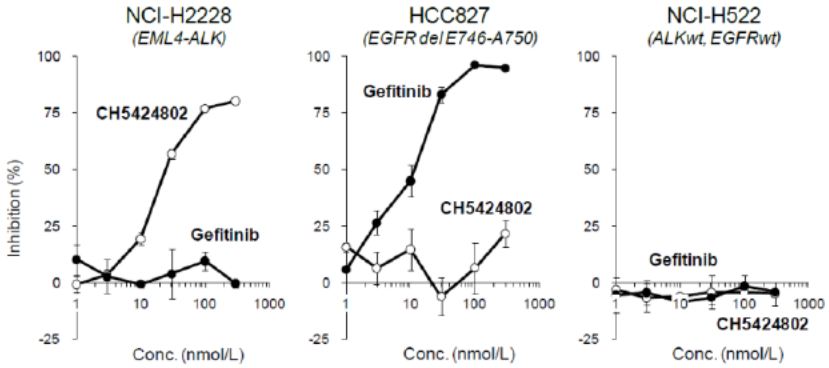
Study Title: Inhibition of spheroid formation by CH5424802 in human lung cancer cell lines	
Study no.:	1054069 (PHM09-0238S)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan
Date of study start:	11/24/2009
Compliance:	N/A

Drug/lot/purity	CH5424802; 004, 99.8%
Vehicle:	DMSO

Methods
Cell growth under spheroid culture was measured using the non-small cell lung cancer cell lines with 3 distinct genotypes. NCI-H2228, HCC827, and NCI-H522 cells were maintained in spheroid culture overnight. Cells were treated with various concentrations of CH5424802 and gefitinib for 5 days. Cell growth inhibition was determined by CellTiter-Glo® Luminescent Cell Viability Assay.

Results

Figure 3: Alectinib Inhibition of ALK-driven tumor cell growth



(Excerpted from Applicant's submission)

Conclusion
CH5424802 had growth-inhibitory activity against tumor cell lines driven by EML4-ALK mutations, but no effect on tumors driven by EGFR or other mutations.

Study Title: In vitro evaluation of the inhibitory activity of CH5424802 onvarious kinases: Part 2	
Study no.:	1054384 (PHM12-0054)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan
Date of study start:	4/23/2012
Compliance:	N/A
Drug/lot/purity	CH5424802; 111392, NA
Vehicle:	DMSO
Reference compound:	Staurosporine

Methods
The inhibitory activity of CH5424802 on ALK C1156Y, ALK L1196M, and RET, was evaluated in a cell-free enzyme assay (Time resolved fluorescence resonance energy transfer assay, TR-FRET assay).

Results

Table 5: Alectinib inhibition of ALK mutants and RET

Enzyme	IC_{50} (nmol/L)	
	CH5424802-002	Staurosporine
ALK C1156Y	0.93	14
ALK L1196M	2.1	4.9

Enzyme	IC_{50} (nmol/L)	
	CH5424802-002	Staurosporine
RET	4.8	5.9

(Excerpted from Applicant's submission)

Conclusion

CH5424802 showed inhibitory activity against two ALK mutants C1156Y and L1196M that have been associated with acquired resistance to crizotinib that was comparable to its activity against wild-type ALK in previous experiments. CH5424802 also exhibited inhibition against RET at clinically achievable concentrations.

Study Title: In vitro evaluation of the inhibitory activity of CH5424802 on various kinases (Part 4)	
Study no.:	1057813 (PHM13-0040)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan
Date of study start:	3/28/2013
Compliance:	N/A
Drug/lot/purity	CH5424802; 111392, NA
Vehicle:	DMSO
Reference compound:	Staurosporine

Methods

The inhibitory potencies of CH5424802 on point mutations of ALK or RET were evaluated in cell-free assay systems. The enzymes were added according to the following table:

Enzyme	Amount of enzyme (ng/assay)
ALK T1151_L1152insT	10
ALK L1152R	10
ALK G1202R	4.0
ALK G1269A	4.0
RET G691S	0.10
RET M918T	0.10
RET S891A	0.10
RET Y791F	0.10
RET V804L	0.20
RET V804M	0.10

Results

Table 6: IC_{50} Values for Alectinib from ALK mutant and RET mutant Kinase Assay

Enzyme	IC_{50} (nmol/L)	
	CH5424802-002	Staurosporine
ALK (T1151_L1152insT)	4.7	41
ALK (L1152R)	4.8	36
ALK (G1202R)	41	37
ALK (G1269A)	17	3.5
RET (G691S)	9.5	4.7
RET (M918T)	5.7	5.7
RET (S891A)	8.3	1.4
RET (Y791F)	14	7.3
RET (V804L)	32	0.81
RET (V804M)	53	3.6

(Excerpted from Applicant's submission)

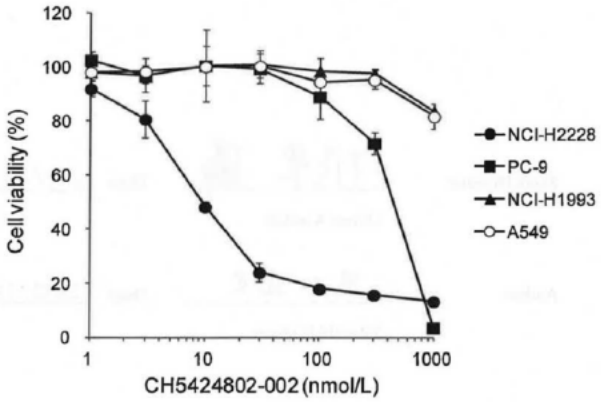
Conclusion

CH5424802 was shown to have inhibitory activity against various ALK point mutations, and RET point mutations. The ALK mutants tested were previously identified in patients who progressed after treatment with crizotinib and showed little response to crizotinib in both in vitro and in vivo assays.

Study Title: Evaluation of cell growth inhibitory activity by CH5424802 in spheroid cultured human non-small cell lung cancer cell lines	
Study no.:	1054385 (PHM12-0105)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan
Date of study start:	7/3/2012
Compliance:	N/A
Drug/lot/purity	CH5424802; 111392, NA
Vehicle:	DMSO
Reference compound:	Staurosporine
Methods Inhibition of cell growth by CH5424802 was investigated using the non-small cell lung cancer cell lines with distinct genotypes. NCI-H2228, PC-9, NCI-HI993 and A549 cells were seeded and cultured overnight, and then treated with CH5424802 for 5 days. Cell viability was analyzed by CellTiter-Glo111 Luminescent Cell Viability Assay (n = 3).	

Results

Figure 4: Alectinib inhibits ALK-driven tumors but not tumors with EGFR, MET, or Ras mutations



(Excerpted from Applicant's submission)

Table 7: Specific alectinib inhibition of an ALK-driven tumor cell line

Cell line	Genetic alteration	Geomean IC_{50} (nmol/L)
NCI-H2228	EML4-ALK	12
PC-9	EGFR deletion of exon 19	400
NCI-H1993	MET amplification	>1,000
A549	KRAS G12S	>1,000

(Excerpted from Applicant's submission)

Conclusion

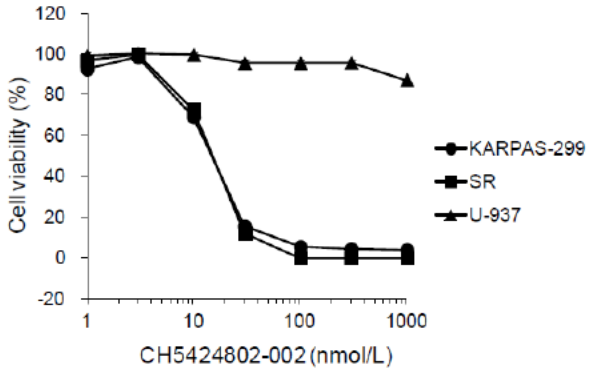
CH5424802 inhibited the growth of NCI-H2228, which harbors EML4-ALK, with an IC_{50} value of approximately 12 nM, but showed little activity against models without ALK alterations.

Study Title: In vitro growth inhibition by CH5424802 in human lymphoma cell lines	
Study no.:	1056351 (PHM12-0067)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan
Date of study start:	6/8/2012
Compliance:	N/A
Drug/lot/purity	CH5424802/111392/NA
Vehicle:	DMSO
Reference compound:	N/A

Methods
Inhibition of cell growth by CH5424802 was investigated using NPM-ALK fusion positive and negative lymphoma cell lines. The inhibitory activity was measured by quantitative analysis of the ATP content in the cells treated with test article (CH5424802), using CellTiter-Glo® Luminescent Cell Viability Assay. CH5424802 was tested at 0, 1, 3, 10, 30, 100, 300, and 1000 nmol/L. Each concentration of the test article was tested in triplicate.

Results

Figure 5: Selective antitumor activity in ALK-positive human lymphoma cell lines



(Excerpted from Applicant's submission)

Table 8: Inhibitory activity of alectinib against human lymphoma cell lines

Cell line	ALK status	Geomean IC_{50} (nmol/L)
KARPAS-299	NPM-ALK	14
SR	NPM-ALK	14
U-937	Negative	>1,000

(Excerpted from Applicant's submission)

Conclusion

CH5424802 inhibited the growth of two NPM-ALK fusion-positive KARPAS-299 and SR cell lines, both with IC_{50} values of 14 nM. On the other hand, it did not affect the growth of ALK-negative U-937 cell line (IC_{50} >1,000 nM). CH5424802 had growth inhibitory activity against human lymphoma cells harboring NPM-ALK.

Study Title: In vitro growth inhibition of CH5424802 in human lymphoma and neuroblastoma cell lines	
Study no.:	1054070 (PHM09-0220S)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan

Date of study start:	10/28/2009
Compliance:	N/A
Drug/lot/purity	CH5424802/004/99.8 %
Vehicle:	DMSO
Reference compound:	N/A

Methods
The inhibitory activities on cell proliferation was measured in human lymphoma and neuroblastoma cell lines, KARPAS-299, SR, HDLM-2, NB-1, KELLY and SK-N-FI, treated with CH5424802.

Results

Table 9: IC_{50} of alectinib against human lymphoma and neuroblastoma cell lines

Tumor type	Cell line	ALK status	IC_{50} (nmol/L) mean ± SD
Lymphoma	KARPAS-299	NPM-ALK	3.0 ± 0.48
Lymphoma	SR	NPM-ALK	6.9 ± 0.89
Lymphoma	HDLM-2	WT	>10,000
Neuroblastoma	NB-1	Gene amplification	4.5 ± 0.28
Neuroblastoma	KELLY	F1174L	62 ± 6.7
Neuroblastoma	SK-N-FI	WT	>10,000

(Excerpted from Applicant's report)

Conclusion

CH5424802 was able to inhibit tumor cells lines with ALK amplifications, and wild-type ALK fusions. CH5424802 also demonstrated activity against a cell line carrying the F1174L ALK mutation at a clinically achievable IC_{50}, though inhibition occurred at a higher concentration (approximately 10X) than that seen for wild type ALK.

Study Title: In vitro evaluation of the inhibitory activity of metabolites of CH5424802 on ALK	
Study no.:	1056380 (PHM12-0143)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan
Date of study start:	12/21/2012
Compliance:	N/A
Drug/lot/purity	CH5468924/002/NA;CH5507197-000/001/NA
Vehicle:	DMSO

Reference compound:	Staurosporine

Methods
The inhibitory activity of CH5468924 (M4) and CH5507197 (M6), the metabolites of CH5424802, on ALK was evaluated using cell-free assay system.

Results

Table 10: Alectinib metabolite inhibition of ALK

	IC_{50} (nmol/L)
CH5468924-000	1.2
CH5507197-000	1.9
Staurosporine	14

(Excerpted from Applicant's report)

Conclusion

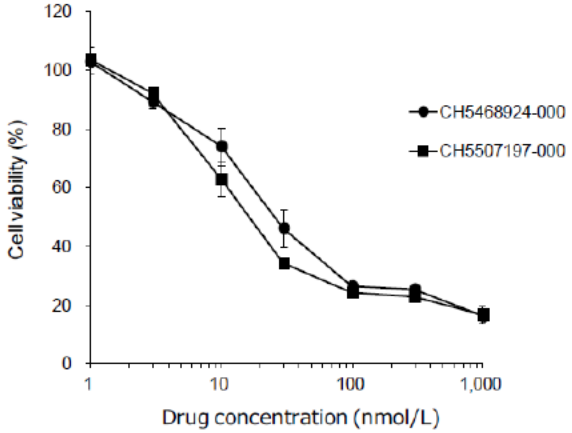
The alectinib metabolites CH5468924-000 and CH5507197-000, exhibited similar activity against ALK as the parent, with IC_{50} values of 1.2 and 1.9 nmol/L.

Study Title: Evaluation of cell growth inhibitory activity by metabolites of CH5424802 in spheroid cultured NCI-H2228 human non-small cell lung cancer cell line	
Study no.:	1056381 (PHM12-0144)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan
Date of study start:	12/26/2012
Compliance:	N/A
Drug/lot/purity	CH5468924/002/NA;CH5507197/001/NA
Vehicle:	DMSO
Reference compound:	N/A

Methods
The inhibitory activity by CH5468924 and CH5507197 on cell growth was measured by quantitative analysis of the ATP content in cells treated with the test articles (CH5468924 and CH5507197), using CellTiter-Glo® Luminescent Cell Viability Assay. They were tested at 1-1,000 nmol/L. Each concentration was tested in triplicate.

Results

Figure 6: Antitumor activity of CH54688294 and CH5507197 in an EML4-ALK+ human NSCLC cell line NCI-H2228



(Excerpted from Applicant's submission)

Table 11: Inhibitory activity by CH54688294 and CH5507197 against NCI-H2228 cells

Test articles	Geomean IC_{50} (nmol/L)
CH5468924-000	37
CH5507197-000	24

(Excerpted from Applicant's submission)

Conclusion

Alectinib metabolites CH5468924 and CH5507197 inhibited the in vitro growth of the NCI-H2228 cell line, which harbors EML4-ALK, with IC_{50} values of 37 and 24 nmol/L, respectively.

Study Title: In vitro evaluation of the inhibitory activity of the metabolite CH5468924 of CH5424802 on various protein kinases and ALK mutations	
Study no.:	1062082 (PHM14-0077)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan
Date of study start:	6/1/2014
Compliance:	N/A
Drug/lot/purity	CH5468924/002/NA

Vehicle:	DMSO
Reference compound:	Staurosporine, lapatinib, sorafenib

Methods

The inhibitory activities of CH5468924 (M4), a metabolite of CH5424802, on various protein kinases including point mutations of ALK were evaluated in a cell-free assay system (time resolved fluorescence resonance energy transfer (TR-FRET) or fluorescence polarization (FP) assay)

Results

Table 12: IC_{50} values for CH5468924 and staurosporine from various kinase assays

Class	Enzyme	IC_{50} (nmol/L)	
		CH5468924-000	Staurosporine
Receptor tyrosine kinase	ALK (1151Tins)	1.1	26
	ALK (L1152R)	0.83	34
	ALK (C1156Y)	0.62	15
	ALK (F1174L)	1.1	15
	ALK (L1196M)	0.87	5.0
	ALK (G1202R)	34	41
	ALK (G1269A)	2.6	2.7
	ALK (R1275Q)	3.4	12
	FGFR2	4100	7.7
	IGF1R	1400	620
	INSR	220	470
	KDR	1000	41
	KIT	>5000	96
	MET	>5000	420
	PDGFRβ	520	7.6
	RET	3.0	4.3
	RET (G691S)	3.3	10
	RET (M918T)	2.3	9.4
	RET (S891A)	2.1	1.1
	RET (Y791F)	3.1	5.4
	RET (V804L)	9.1	<6.9
	RET (V804M)	8.1	0.83
	RON	>5000	140
	ROS1	680	<6.9
Tyrosine kinase	ABL	630	630
	JAK1	>5000	140
	SRC	>5000	250
Serine /threonine kinase	AKT1	>5000	7.7
	AurA	>5000	<6.9
	CDC2/CycB1	>5000	7.5
	CDK2/CycA2	>5000	<6.9
	MEK1	>5000	<6.9
	PKA	>5000	12
	PKCα	>5000	<6.9
	PKCβ1	>5000	<6.9
	PKCβ2	>5000	<6.9

(Excerpted from Applicant's submission)

Table 13: IC_{50} values for CH5468924 and lapatinib from EGFR and HER2 kinase assays

Class	Enzyme	IC_{50} (nmol/L)	
		CH5468924-000	Lapatinib
Receptor tyrosine kinase	EGFR	>5000	10
	HER2	>5000	1.5

(Excerpted from Applicant's submission)

Table 14: IC_{50} values for CH5468924 and sorafenib from RAF1 kinase assay

Class	Enzyme	IC_{50} (nmol/L)	
		CH5468924-000	Sorafenib
Serine/threonine kinase	RAF1	>5000	15

(Excerpted from Applicant's report)

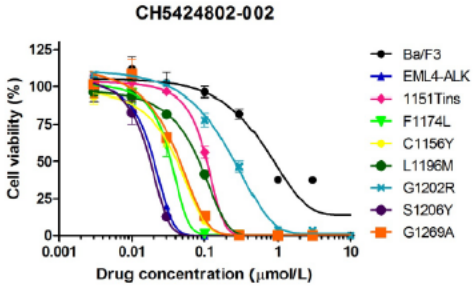
Conclusion

The IC_{50}S of CH5468924 (M4), the metabolite of CH5424802, against ALK mutants, RET, and RET mutants were comparable to those of the parent compound alecitnib demonstrated in previous assays.

Study Title: In vitro growth inhibition by CH5424802 in Ba/F3 cells expressing mutated EML4-ALK	
Study no.:	1061439 (PHM13-0114)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan
Date of study start:	11/1/2012
Compliance:	N/A
Drug/lot/purity	CH5424802/111392/NA Crizotinib/S106810/99.63%
Vehicle:	DMSO
Reference compound:	NA
Methods Inhibition of cell growth by CH5424802 was investigated using Ba/F3 cells expressing native or mutated echinoderm microtubule-associated protein-like 4 (EML4)-ALK (ALK: 1151Tins, C1156Y, F1174L, L1196M, G1202R, S1206Y and G1269A) and in parental Ba/F3 cells. Cell viability was analyzed by CellTiter-Glo® Luminescent Cell Viability Assay. These mutants are associated with crizotinib resistance. SD (n = 3)	

Results

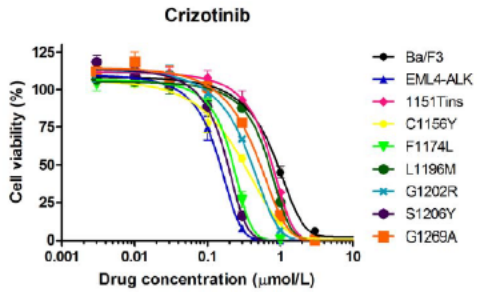
Figure 7: Antitumor activity of alectinib in Ba/F3 cell lines expressing native and mutated EML4-ALK



Cell line	CH5424802-002	
	IC_{50} (μmol/L)[a]	IC_{50} ratio (fold)[b]
Ba/F3	0.91 ± 0.029	1.0
Ba/F3 EML4-ALK	0.019 ± 0.0010	48
Ba/F3 EML4-ALK 1151Tins	0.11 ± 0.0036	8.3
Ba/F3 EML4-ALK C1156Y	0.038 ± 0.0016	24
Ba/F3 EML4-ALK F1174L	0.034 ± 0.0036	27
Ba/F3 EML4-ALK L1196M	0.075 ± 0.00084	12
Ba/F3 EML4-ALK G1202R	0.24 ± 0.0032	3.8
Ba/F3 EML4-ALK S1206Y	0.016 ± 0.0020	57
Ba/F3 EML4-ALK G1269A	0.040 ± 0.00096	23

a: The data was shown as mean ± SD (n=3).
b: The IC_{50} ratio of the native or mutated EML4-ALK-driven cells to the parental cells.

Figure 8: Antitumor activity of crizotinib in Ba/F3 cell lines expressing native and mutated EML4-ALK



Cell line	Crizotinib	
	IC_{50} (μmol/L)[a]	IC_{50} ratio (fold)[b]
Ba/F3	0.87 ± 0.040	1.0
Ba/F3 EML4-ALK	0.14 ± 0.012	6.2
Ba/F3 EML4-ALK 1151Tins	0.69 ± 0.029	1.3
Ba/F3 EML4-ALK C1156Y	0.30 ± 0.015	2.9
Ba/F3 EML4-ALK F1174L	0.20 ± 0.025	4.4
Ba/F3 EML4-ALK L1196M	0.65 ± 0.0070	1.3
Ba/F3 EML4-ALK G1202R	0.37 ± 0.012	2.4
Ba/F3 EML4-ALK S1206Y	0.17 ± 0.010	5.1
Ba/F3 EML4-ALK G1269A	0.47 ± 0.016	1.9

a: The data was shown as mean ± SD (n=3).
b: The IC_{50} ratio of the native or mutated EML4-ALK-driven cells to the parental cells.

(Figures excerpted from Applicant's submission)

Conclusion

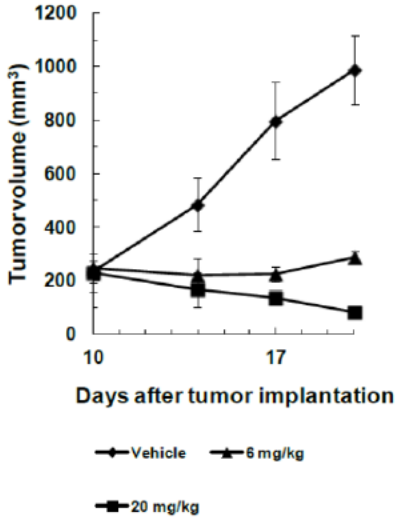
CH5424802 showed more potent inhibitory activity against each cell line expressing mutated EML4-ALK than crizotinib.

Study Title: Anti-tumor activity of CH5424802 in the KARPAS-299 human ALCL xenograft model	
Study no.:	1054075 (PHM09-0242S)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan
Date of study start:	4/4/2008
Compliance:	N/A
Drug/lot/purity	CH5424802/003/98.25%
Vehicle:	DMSO
Doses/Dose vol:	6 and 20 mg/kg/20 mL/kg
Species/Strain:	Mouse, SCID
#/sex/group:	4/males/group

Methods
The antitumor effect of CH5424802-000 was investigated in the KARPAS-299 human anaplastic large-cell lymphoma (ALCL) xenograft model. KARPAS-299 cells were inoculated into the right flank of SCID mice. Tumors were allowed to establish growth after implantation before initiation of treatment. CH5424802 or vehicle was administered orally once daily for 11 days. Tumor size was measured twice per week.

Results

Figure 9: Alectinib Inhibiton of KARPAS-299 xenografts



(Excerpted from Applicant's submission)

Table 15: Antitumor activity of alectinib against KARPAS-299 cells

Cell line	Dose (mg/kg)	TGI (%)	P value
KARPAS-299	6	94	< 0.0001
	20	119	< 0.0001

(Excerpted from Applicant's submission)

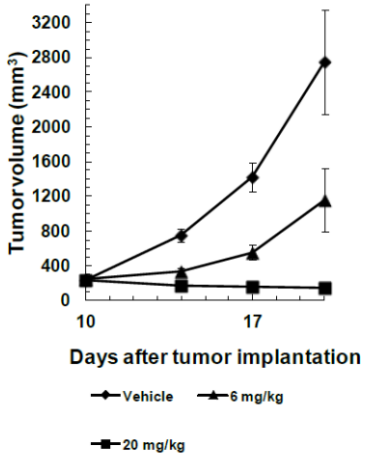
Conclusion
CH5424802 showed statistically significant tumor growth inhibition in the KARPAS-299 human ALCL xenograft model. Body weight changes were unremarkable.

Study Title: Antitumor activity of CH5424802 in the NB-1 human neuroblastoma xenograft model	
Study no.:	1054076 (PHM09-0243S)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan

Date of study start:	5/8/2009
Compliance:	N/A
Drug/lot/purity	CH5424802/014/97.51%
Vehicle:	DMSO
Doses/Dose vol:	6 and 20 mg/kg/20 mL/kg
Species/Strain:	Mouse, SCID
#/sex/group:	5/males/group
Methods The antitumor effect of CH5424802 was investigated in the NB-1 neuroblastoma xenograft model. NB-1 cells were inoculated into the right flank of SCID mice. CH5424802 or vehicle was administered from day 10 to day 20. Tumor size was measured twice per week.	

Results

Figure 10: Alectinib inhibition of NB-1 Xenografts



(Excerpted from Applicant's submission)

Table 16: Alectinib mediated tumor growth inhibition in the NB-1 xenograft model

Cell line	Dose (mg/kg)	TGI (%)	P value
NB-1	6	63	0.0003
	20	104	< 0.0001

(Excerpted from Applicant's submission)

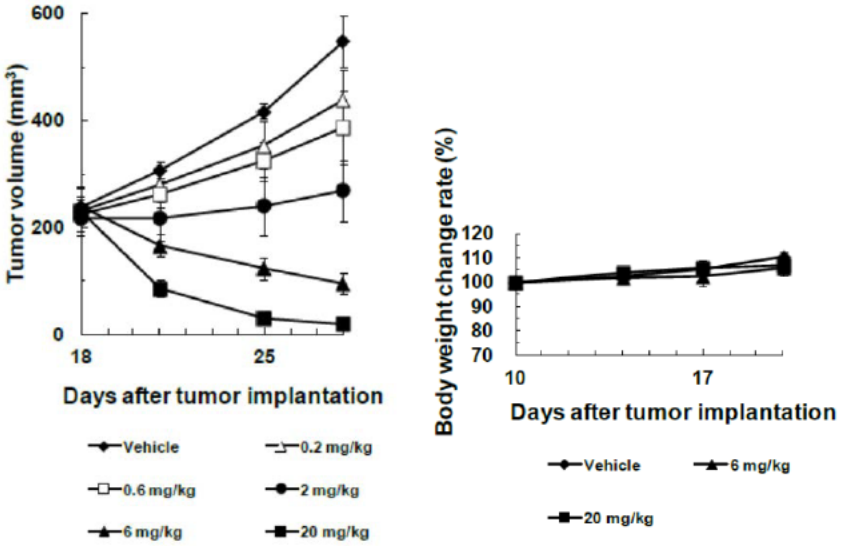
Conclusion

Administration of CH5424802 resulted in statistically significant tumor growth inhibition in mice implanted with NB-1 human neuroblastoma cells. Body weight changes were unremarkable, suggesting that the drug was reasonably tolerated.

Study Title: Antitumor activity of CH5424802 in the NCI-H2228 human lung cancer xenograft model	
Study no.:	1054071 (PHM09-0239S)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan
Date of study start:	11/26/2008
Compliance:	N/A
Drug/lot/purity	CH5424802/003/98.25%
Vehicle:	DMSO
Doses/Dose vol:	0.2, 0.6, 2, 6 and 20 mg/kg (20 mL/kg)
Cell line:	NCI-H2228, human non-small cell lung cancer cells
Species/Strain:	Mouse, SCID
#/sex/group:	4/males/group
Methods The antitumor effect of CH5424802 was investigated in the NCI-H2228 human lung cancer xenograft model. NCI-H2228 cells were inoculated into the right flank of SCID mice. Tumors were allowed to establish growth after implantation before initiation of treatment. CH5424802 or vehicle was administered orally once daily for 11 days. Tumor size was measured twice per week.	

Results

Figure 11: Alectinib effects on NCI-H2228 xenograft tumor growth and mouse body weight



(Excerpted from Applicant's submission)

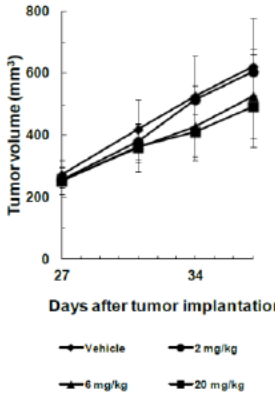
Conclusion

CH5424802 showed statistically significant tumor growth inhibition in the NCI-H2228 xenograft model, with an inhibitory value of 168% at 20 mg/kg/day. Body weight changes were unremarkable.

Study Title: Antitumor activity of CH5424802 in the A549 human lung cancer xenograft model	
Study no.:	1054072 (PHM09-0240S)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan
Date of study start:	2/24/2009
Compliance:	N/A
Drug/lot/purity	CH5424802/014/97.51%
Formulation/Vehicle:	DMSO
Doses (dose volume):	2, 6 and 20 mg/kg (20 mL/kg)
Cell line:	A549, non-small cell lung cancer ALK wild
Species/Strain:	Mouse, BALB-nu/nu
#/sex/group:	5/females/group
Methods The antitumor effect of CH5424802 was investigated in the A549 (ALK wild) human lung cancer xenograft model. A549 cells were inoculated into the right flank of BALB-nu/nu. Tumors were allowed to establish growth after implantation before initiation of treatment. CH5424802 or vehicle was administered orally once daily for 11 days. Tumor size was measured twice per week.	

Results

Figure 12: Antitumor activity of alectinib in the A549 human lung cancer xenograft model



Drug	Dose (mg/kg)	TGI (%)	P value
CH5424802	2	−1	1.0000
	6	22	0.5448
	20	31	0.2874

(Excerpted from Applicant's submission)

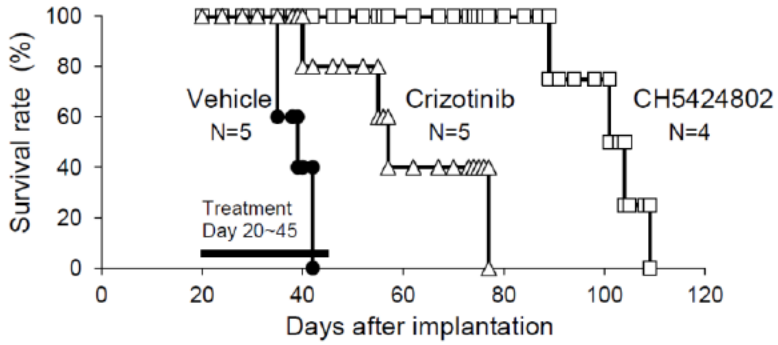
Conclusion
CH5424802 showed little tumor growth inhibition in the A549 xenograft model, with an inhibitory value of 31% at 20 mg/kg/day. Body weight changes were unremarkable. These data suggest that CH5424802 has little effect on tumor cell lines not driven by ALK mutations (or other alectinib targets).

Study Title: Efficacy of CH5424802 in an intracranial NCI-H2228 implantation model	
Study no.:	1056354 (PHM12-0146)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan
Date of study start:	7/13/2011
Compliance:	N/A
Drug/lot/purity	CH5424802; 093, 99.86%
Vehicle:	DMSO
Reference article:	Crizotinib; 006, 95.67%
Cell lines:	NCI-H2228

Methods
To evaluate the feasibility of treating patients with tumors harboring genomic alterations of ALK with metastases to the brain with alectinib, the Applicant investigated the drug's activity in an intracranial implantation model. Mice were implanted intracranially with the NCI-H2228 NSCLC cell line expressing EML4-ALK and randomized into 3 treatment groups with 4 or 5 mice in each group based on body weight 20 days after implantation. Animals were dosed orally once daily for 26 days as follows: vehicle, CH5424802 (60 mg/kg), crizotinib (100 mg/kg). General health status was observed at intervals of 1 or 2 days and body weight and body temperature were measured at least twice a week. Survival time was recorded as the number of days after tumor implantation.

Results

Figure 13: Activity of Alectinib and Crizotinib in an intracranial xenograft EML4-ALK model



(Excerpted from Applicant's submission))

Table 17: Survival time in intracranially implanted mice

Treatment group	Median survival time (day)
Vehicle (control)	39
CH5424802	102.5
Crizotinib	57

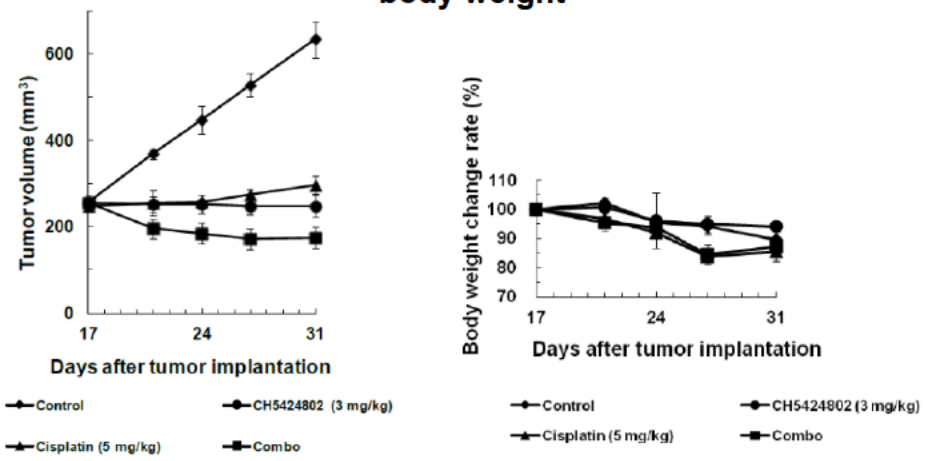
(Excerpted from Applicant's submission)

Conclusion
Treatment with CH5424802 or crizotinib significantly prolonged the survival time of mice implanted intracranially with NCI-H2228 cells that harbor the ALK fusion gene compared to control treatment. The activity of CH5424802 was superior to crizotinib, suggesting better penetration of the blood brain barrier.

Study Title: Antitumor activity of CH5424802 in combination with cisplatin in the NCI-H2228 human lung cancer xenograft model	
Study no.:	1054077 (PHM10-0023S)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan
Date of study start:	10/7/2009
Compliance:	N/A
Drug/lot/purity	CH5424802/031/99.49%
Formulation/Vehicle:	DMSO
Doses (dose volume):	3 mg/kg CH5424802 5 mg/kg cisplatin 3 mg/kg CH5424802 + 5 mg/kg cisplatin (20 mL/kg)
Cell line:	NCI-H2228, non-small cell lung cancer
Species/Strain:	Mouse, SCID
#/sex/group:	5/males/group
Methods The antitumor effect of CH5424802 was investigated in the NCI-H2228, non-small cell lung cancer xenograft model. NCI-H2228 cells were inoculated into the right flank of SCID mice. Tumors were allowed to establish growth after implantation before initiation of treatment. CH5424802, cisplatin, or vehicle was administered orally once daily for 15 days. Body weights and tumor size were measured twice per week.	

Results

Figure 14: Effects of alectinib± cisplatin on NCI-H2228 xenografts and mouse body weight



(Excerpted from Applicant's submission)

Table 18: Tumor growth inhibition by alectinib and/or cisplatin treatment in the NCI-H2228 human lung cancer xenograft model

Group	Tumor growth inhibition(%) on day 31
Vehicle	-
CH5424802 3 mg/kg	102
Cisplatin 5 mg/kg	87
Combo	121

(Excerpted from Applicant's submission)

Conclusion

Treatment with CH5424802 combined with cisplatin resulted in a mild increase in tumor growth inhibition in the NCI-H2228 xenograft model compared to that of either single agent. Body weight changes in treated mice were similar between cisplatin alone or combination treatment, though greater than those observed in mice treated with CH5424802 alone.

Study Title: Antitumor activity of CH5424802 in combination with paclitaxel in the NCI-H2228 human lung cancer xenograft model	
Study no.:	1054078 (PHM10-0024S)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan
Date of study start:	10/7/2009
Compliance:	N/A
Drug/lot/purity	CH5424802/031/99.49%

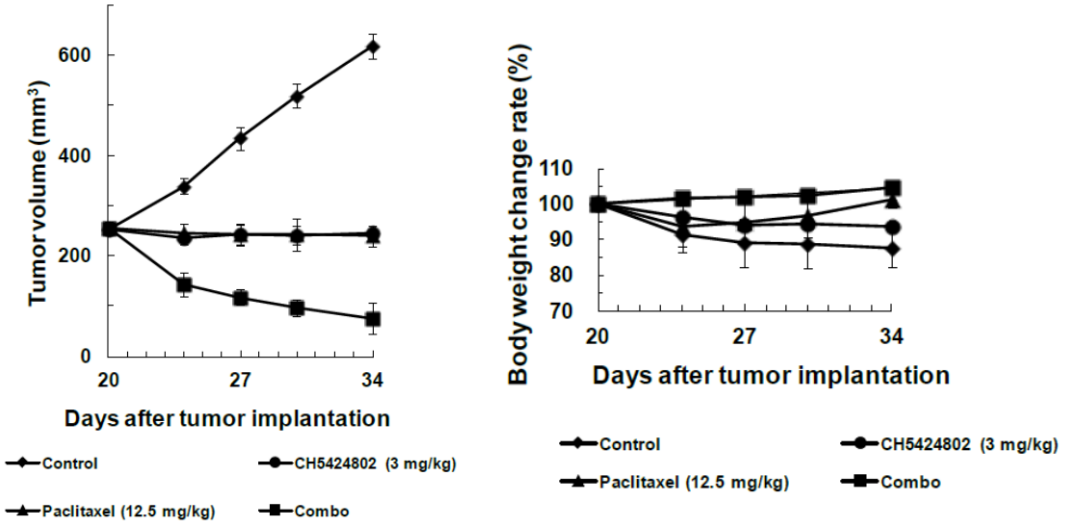
Formulation/Vehicle:	DMSO
Doses (dose volume):	3 mg/kg CH5424802 12.5 mg/kg paclitaxel 3 mg/kg CH5424802 + 12.5 mg/kg paclitaxel (20 mL/kg)
Cell line:	NCI-H2228, non-small cell lung cancer
Species/Strain:	Mouse, SCID
#/sex/group:	5/males/group

Methods

The antitumor effect of CH5424802 was investigated using the NCI-H2228 non-small cell lung cancer cell line harboring the EML4-ALK fusion. NCI-H2228 cells were inoculated into the right flank of SCID mice. Tumors were allowed to establish growth after implantation before initiation of treatment. CH5424802 or vehicle was administered orally once daily for 15 days. Paclitaxel was administered intravenously once daily for 15 days. Body weights and tumor size were measured twice per week.

Results

Figure 15: Effects of alectinib±paclitaxel on NCI-H2228 xenograft growth and body weight



(Excerpted from Applicant's submission)

Table 19: Tumor Growth Inhibition by alectinib±paclitaxel

Group	Tumor growth inhibition(%) on day 34
Vehicle	-
CH5424802 3 mg/kg	102
Paclitaxel 12.5 mg/kg	104
Combo	150

(Excerpted from Applicant's submission)

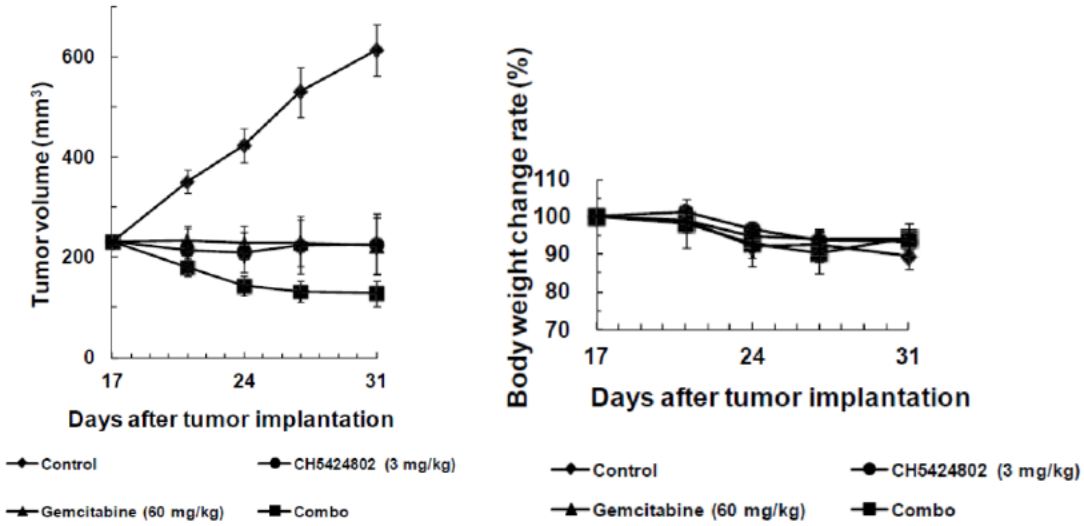
Conclusion

Treatment of mice implanted with the NCI-H2228 human lung cell line with the combination of CH5424802 and paclitaxel combination resulted in increased inhibition of tumor growth compared to either drug alone. Body weight changes were unremarkable with combination treatment.

Study Title: Antitumor activity of CH5424802 in combination with gemcitabine in the NCI-H2228 human lung cancer xenograft model	
Study no.:	1054079 (PHM10-0025S)
Conducting laboratory:	Kamakura Research Laboratories Chugai Pharmaceutical Co., Ltd. Pharmaceutical Research Dept. 2 200 Kajiwara, Kamakura-shi Kanagawa 247-8530, Japan
Date of study start:	10/7/2009
Compliance:	N/A
Drug/lot/purity	CH5424802/031/99.49%
Formulation/Vehicle:	DMSO (CH5424802); saline (gemcitabine)
Doses (dose volume):	3 mg/kg CH5424802 (20 mL/kg) 60 mg/kg gemcitabine 3 mg/kg CH5424802 + 60 mg/kg gemcitabine (10 mL/kg)
Cell line:	NCI-H2228, non-small cell lung cancer
Species/Strain:	Mouse, SCID
#/sex/group:	5/males/group
Methods The antitumor effect of CH5424802 was investigated in the NCI-H2228, non-small cell lung cancer xenograft model. NCI-H2228 cells were inoculated into the right flank of SCID mice. Tumors were allowed to establish growth after implantation before initiation of treatment. CH5424802 or DMSO was administered orally once daily for 15 days. Gemcitabine or saline was administered intraperitoneally twice a week for 2 weeks. Body weights and tumor size were measured twice per week.	

Results

Figure 16: Anti-tumor activity of alectinib±gemcitabine



(Excerpted from Applicant's submission)

Table 20: TGI of alectinib in combination with gemcitabine

Group	Tumor growth inhibition(%) on day 31
Vehicle	-
CH5424802 3 mg/kg	101
Gemcitabine 60 mg/kg	102
Combo	127

(Excerpted from Applicant's submission)

Conclusion

CH5424802 and gemcitabine combination therapy showed greater tumor growth inhibition than administration of CH5424802 or gemcitabine alone in the NCI-H2228 human lung cancer xenograft model. Body weight changes were unremarkable with combination treatment.

4.3 Safety Pharmacology

Study Title: The Effects of CH5424802 on the Central Nervous System in Rats	
Study no.:	1053950 (TOX09-0165)
Conducting laboratory:	(b) (4)

Date of study start:	1/5/2010
Compliance:	GLP
Drug/lot/purity	CH5424802-000; 009, 99.9%

Objectives:

- Determine CNS effects of CH5423802 using Wistar-Han rats (n=6/male) group at dose levels of 3, 30 and 300 mg/kg by observation of general conditions and behaviors using modified Irwin's multiple observation
- Assess systemic exposure

Methods

- Observations were conducted before administration and 1, 4, 8, and 24 hours after administration.
- Control rats (n=3 males/group) were orally administered with dose levels of 3, 30 and 300 mg/kg, and blood samples were collected at 1, 2, 4, 8 and 24 hours after administration to determine the systemic exposure levels of CH5424802-000.

Results

Table 21: Effect of Alectinib on CNS

Test substance	Dose (mg/kg, p.o.)	Signs	Before	Time after administration (hour)			
				1	4	8	24
Vehicle [a]	0	No treatment-related findings	6/6	6/6	6/6	6/6	6/6
CH5424802-002	3	No treatment-related findings	6/6	6/6	6/6	6/6	6/6
	30	No treatment-related findings	6/6	6/6	6/6	6/6	6/6
	300	No treatment-related findings	6/6	6/6	6/6	6/6	6/6

Dose levels are described as CH5424802-000.

Number of animals showing the sign / Number of animals tested

a: 10 w/v% Hydroxypropyl-β-cyclodextrin solution containing 0.01 mol/L methanesulfonic acid and 0.001 mol/L hydrochloric acid

(Excerpted from Applicant's submission)

Table 22: TK parameters of alectinib in CNS safety pharmacology study

Dose (mg/kg)	Dose ratio	C_{max}		AUC_{0-24h}	
		Mean (ng/mL)	Dose proportionality factor *1	Mean (ng·h/mL)	Dose proportionality factor *1
3	1.0	171	1.0	2690	1.0
30	10.0	1250	7.3	22000	8.2
300	100.0	1770	10.4	31500	11.7

*1: Mean of C_{max} or AUC_{0-24h} of on each dosing group/mean of C_{max} or AUC_{0-24h} on the low dosing group

*1: Mean of C_{max} or AUC_{0-24h} of on each dosing group/mean of C_{max} or AUC_{0-24h} on the low dosing group

(*Excerpted from Applicant's submission)*

Conclusion

There were no CH5424802 effects on the CNS in male rats at doses up to 300 mg/kg.

Study Title: The Effects of CH5424802 on the Respiratory System in Rats	
Study no.:	1053951 (TOX09-0167)
Conducting laboratory:	(b) (4)
Date of study start:	1/12/2010
Compliance:	GLP
Drug/lot/purity	CH5424802; 009, 99.9%
Objectives: To evaluate the effects of CH5424802 on the respiratory system in rats	
Methods • The effects of CH5424802 on the respiratory rate (RR), tidal volume (TV) and minute volume (MV) were investigated in conscious rats • The vehicle was 10 w/v% Hydroxypropyl-β-cyclodextrin solution containing 0.01 mol/L methanesulfonic acid and 0.001 mol/L hydrochloric acid • CH5424802 was orally administered at dose levels of 3, 30 and 300 mg/kg • The respiratory parameters were measured before administration and at 1, 4, 8 and 24 hours after administration.	

Results

Table 23: Effect of Alectinib on respiratory rate

Test substance	Dose (mg/kg, p.o.)	Number of animals	Respiratory rate (breaths/min)				
			Before	Time after administration (hour)			
				1	4	8	24
Vehicle [a]	---	8	106 ± 15	92 ± 7	91 ± 7	92 ± 9	94 ± 11 [b]
CH5424802-002	3	8	108 ± 9	97 ± 12	91 ± 6	88 ± 9	97 ± 7 [b]
	30	8	100 ± 10	91 ± 11	92 ± 8	94 ± 7 [b]	93 ± 8
	300	8	101 ± 11	92 ± 12	93 ± 7	96 ± 10	92 ± 12

Dose levels are described as CH5424802-000.

Each value represents the mean ± SD.

[a] 10 w/v% Hydroxypropyl-β-cyclodextrin solution containing 0.01 mol/L methansulfonic acid and 0.001 mol/L hydrochloric acid

[b] n=7 ; Since 1 value in each group was affected by body movement of the animal, the value was excluded from evaluation.

No values were significantly different from the vehicle control group.

Table 24: Effect of Alectinib on tidal volume

Test substance	Dose (mg/kg, p.o.)	Number of animals	Tidal volume (mL/breath)				
			Before	Time after administration (hour)			
				1	4	8	24
Vehicle [a]	---	8	1.00 ± 0.11	0.98 ± 0.10	1.03 ± 0.12	1.01 ± 0.16	0.95 ± 0.15 [b]
CH5424802-002	3	8	0.96 ± 0.13	0.98 ± 0.11	1.02 ± 0.14	1.03 ± 0.11	0.94 ± 0.13 [b]
	30	8	1.02 ± 0.11	0.98 ± 0.11	0.98 ± 0.12	1.02 ± 0.17 [b]	0.91 ± 0.11
	300	8	1.07 ± 0.11	1.04 ± 0.08	1.04 ± 0.10	1.04 ± 0.11	0.98 ± 0.13

Dose levels are described as CH5424802-000.

Each value represents the mean ± SD.

[a] 10 w/v% Hydroxypropyl-β-cyclodextrin solution containing 0.01 mol/L methansulfonic acid and 0.001 mol/L hydrochloric acid

[b] n=7 ; Since 1 value in each group was affected by body movement of the animal, the value was excluded from evaluation.

No values were significantly different from the vehicle control group.

Table 25: Effect of Alectinib on minute volume

Test substance	Dose (mg/kg, p.o.)	Number of animals	Minute volume (mL/min)				
			Before	Time after administration (hour)			
				1	4	8	24
Vehicle [a]	---	8	104.2 ± 8.3	89.8 ± 9.3	93.3 ± 9.8	91.5 ± 8.6	89.4 ± 15.8 [b]
CH5424802-002	3	8	103.3 ± 18.5	94.5 ± 14.6	92.7 ± 13.5	89.9 ± 9.8	91.8 ± 14.4 [b]
	30	8	101.9 ± 10.7	88.6 ± 8.1	89.5 ± 5.4	96.1 ± 17.9 [b]	84.6 ± 11.5
	300	8	108.3 ± 17.1	95.0 ± 10.6	96.2 ± 10.4	98.6 ± 8.2	89.4 ± 12.8

Dose levels are described as CH5424802-000.

Each value represents the mean ± SD.

[a] 10 w/v% Hydroxypropyl-β-cyclodextrin solution containing 0.01 mol/L methansulfonic acid and 0.001 mol/L hydrochloric acid

[b] n=7 ; Since 1 value in each group was affected by body movement of the animal, the value was excluded from evaluation.

No values were significantly different from the vehicle control group.

(Tables excerpted from Applicant's submission)

Conclusion

There were no CH5424802-mediated effects on the respiratory system in male rats at doses up to 300 mg/kg.

Study Title: The Effects of CH5424802 on hERG current	
Study no.:	1053952 (TOX09-0150)
Conducting laboratory:	(b) (4)
Date of study start:	12/14/2009
Compliance:	GLP

Drug/lot/purity	CH5424802; 004, 99.8%
Vehicle:	DMSO
Positive control:	E-4031
Objectives: determine the effects of CH5424802 on current through the hERG channels stably expressed in HEK293 cells	
Methods Human embryonic kidney cells stably expressing the hERG potassium channel were exposed to CH5424802 at concentrations of 0.3, 0.55 and 1 µmol/L. The test substance concentrations after perfusion of application solutions at 0.3, 0.55 and 1 µmol/L were 0.141, 0.246 and 0.470 µmol/L, respectively. Five preparations were made for each concentration. The actual exposure concentrations were used for the evaluation including calculation of IC_{20} and IC_{50} values. The hERG-mediated potassium current was achieved by patch-clamp method.	

Results

Table 26: Effect of Alectinib on hERG current

Test substance	Concentration (µmol/L)	Number of preparations	% before	% inhibition	IC_{20} (µmol/L)	IC_{50} (µmol/L)
Vehicle[a]	---	5	95.1 ± 2.0	0.0 ± 2.1		
CH5424802-002	0.141 (0.3)[b]	5	73.2 ± 5.5	23.1 ± 5.7 *		
	0.246 (0.55)[b]	5	62.5 ± 2.3	34.2 ± 2.4 *	0.120	0.450
	0.470 (1)[b]	5	46.3 ± 6.7	51.3 ± 7.1 *		
E-4031	0.1	5	7.2 ± 2.4	92.5 ± 2.5 #		

% before: percentage of the value just before application
% inhibition : inhibition rate corrected for the mean control value
[a] 0.1 vol% dimethyl sulfoxide
[b] Setting concentration
Each value represents the mean ± SD.
* : Significantly different from the vehicle control group by parametric Dunnett's test, $p<0.05$
# : Significantly different from the control value by Student's *t*-test, $p<0.05$

(Excerpted from Applicant's submission)

Conclusion

CH5424802 inhibited the hERG current with the IC_{20} value of 0.120 µmol/L and IC_{50} value of 0.450 µmol/L, suggesting some potential to effect QTc prolongation.

Study Title: The Effects of CH5424802 on the cardiovascular system in conscious monkeys	
Study no.:	1053953 (TOX09-0151)
Conducting laboratory:	(b) (4)

	(b) (4)
Date of study start:	10/14/2009
Compliance:	Signed GLP and QA statements
Drug/lot/purity	CH5424802-000; 004, 99.8%
Vehicle:	10 w/v% Hydroxypropyl-β-cyclodextrin solution containing 0.01 mol/L methanesulfonic acid and 0.001 mol/L hydrochloric acid
Parameters measured:	blood pressure, heart rate, ECG parameters and body temperature
Objectives: To evaluate the effects of CH5424802 orally administered to monkeys on blood pressure, heart rate, electrocardiographic (ECG) parameters and body temperature, as well as to assess systemic exposure levels of CH5424802	
Methods Four conscious male monkeys per group were administered 0 (vehicle), 1.7, 5, or 15 mg/kg CH5424802. Blood pressure, heart rate, ECG parameters (PR interval, QRS duration, QT interval and QTc) and body temperature were analyzed before administration and at 1, 2, 4, 8, and 24 hours after administration. Simultaneously, blood samples were collected at 4 and 24 hours after administration to determine the systemic exposure level of CH5424802.	
Results • There were no effects on blood pressure, heart rate, ECG or body temperature up to 15 mg/kg in cynomolgus monkeys • No test-article was detected in the vehicle control group • CH5424802-000 plasma concentrations increased in a dose-proportional manner	
Conclusion Under the conditions tested, there were no effects on blood pressure, heart rate, ECG parameters, or body temperature at alectinib doses of up to 15 mg/kg in cynomolgus monkeys.	

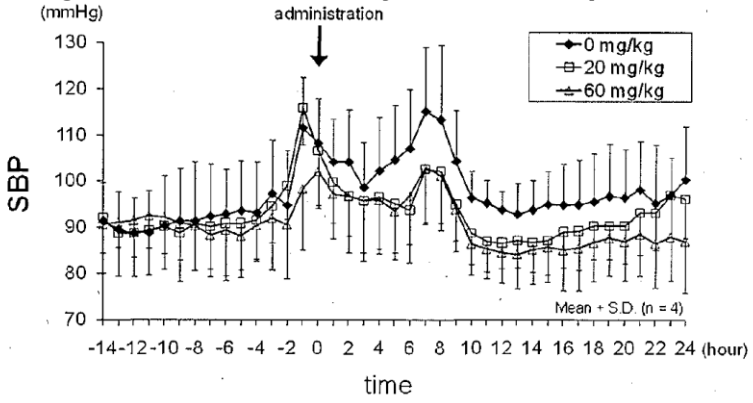
Study Title: The Effects of CH5424802 on the cardiovascular system in cynomolgus monkeys (preliminary study)	
Study no.:	1053954 (TOX09-0048S)
Conducting laboratory:	Fuji Gotemba Research Laboratories Chugai Pharmaceutical Co., Ltd. 1-135 Komakado, Gotemba-shi, Shizuoka 412-8513, Japan
Date of study start:	4/9/2009
Compliance:	Non-GLP
Drug/lot/purity	CH5424802-000; 004, 99.8%
Vehicle:	10 w/v% Hydroxypropyl-β-cyclodextrin solution containing 0.01 mol/L methanesulfonic acid and 0.001 mol/L hydrochloric acid
Parameters measured:	Blood pressure, heart rate, ECG parameters and body temperature
Objectives: To evaluate the effects of CH5424802 on the cardiovascular system in non-restrained conscious cynomolgus monkeys, linked to telemetry system	

Methods
The effects of CH5424802 on blood pressure, heart rate, ECG parameters (PR, QT and RR intervals, QRS duration, and QT) were studied in male and female (n=2/sex/group) non-restrained conscious cynomolgus monkeys using the telemetry system. Monkeys were given single oral doses of 0, 20, and 60 mg/kg CH5424802. The vehicle was 20% SBECD/0.01N HCl).

Results

- There were no effects on heart rate and ECG parameters at 20 and 60 mg/kg

Figure 17: Effects on systolic blood pressure



(Excerpted from Applicant's submission)

Figure 18: Effects on mean blood pressure

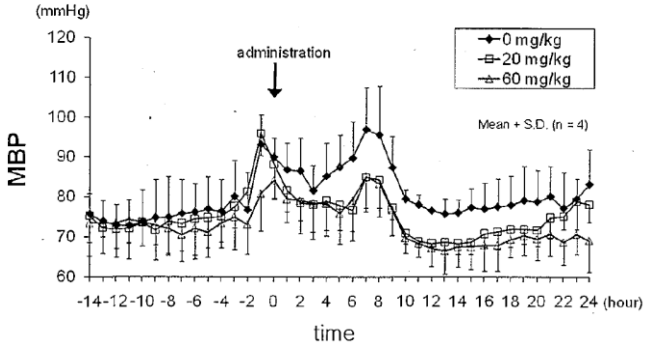
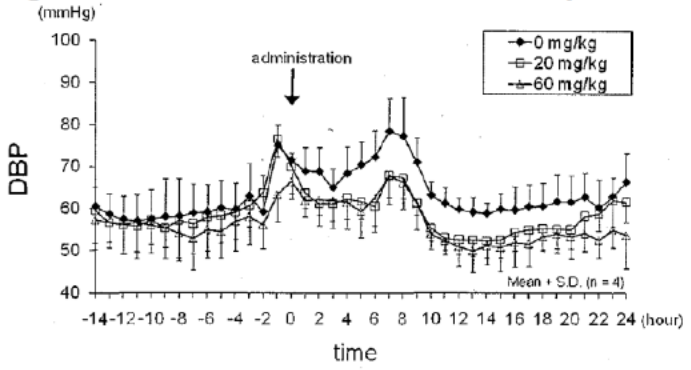


Figure 19: Effects on diastolic blood pressure



(Figures excerpted from Applicant's submission)

Conclusions

Alectinib administration resulted in slight hypotension in monkeys at 20 and 60 mg/kg.

Study Title: The Effects of CH5424802 on gastrointestinal motor function in rats	
Study no.:	1054099 (TOX09-0152S)
Conducting laboratory:	Fuji Gotemba Research Laboratories Chugai Pharmaceutical Co., Ltd. 1-135 Komakado, Gotemba-shi, Shizuoka 412-8513, Japan
Date of study start:	11/24/2009
Compliance:	Non-GLP
Drug/lot/purity	CH5424802; 005, 99.95%
Vehicle:	10% w/v HPCD/0.01 mol/L MsOH/0.001 mol/L HCl aqueous solution
Species:	Wister-Han rat (7 weeks old)
Parameters measured:	Gastric emptying and phenol red small intestinal transit
Objectives: To evaluate the pharmacological effects of orally and singly administered CH5424802 on gastrointestinal motor function	
Methods	

Group	Dose[a] level (mg/kg)	Dose volume (mL/kg)	Dose[a] concentration (mg/mL)	Euthanized time after administration of phenol red[b] (minutes)	Number of animals	Animal Nos.
Non-treatment[c]	0	10	0	0	6	A1-A6
0 mg/kg	0	10	0	20	6	B1-B6
3 mg/kg	3	10	0.3	20	6	C1-C6
30 mg/kg	30	10	3	20	6	D1-D6
300 mg/kg	300	10	30	20	6	E1-E6

Test article was administered singly and orally.

[a] Expressed as CH5424802-000 (the free base of CH5424802-002).

[b] Phenol red (1.5 mL/head) was orally administrated by gavage at 4 hours after vehicle or test compound administration. Animals were euthanized 0 (non-treatment) or 20 minutes (0, 3, 30, 300 mg/kg) after phenol red administration for gastrointestinal motor activity test.

[c] The data of two animals (A1 and A3) were excluded from the calculation of mean absorbance of non-treatment group, because there were leak of phenol red from the stomach at administration in the two animals.

(Excerpted from Applicant's submission)

Results

- There were no effects on there were no dose-dependent effects on gastric transit or gastric emptying

Table 27: Gastric Emptying Time

Test article	Dose[a] (mg/kg)	Number of animals	Gastric emptying[b] (%)	Small intestinal transit (cm)
CH5424802-002	0	6	64.0 ± 19.1	68.7 ± 6.7
	3	6	64.0 ± 21.9	61.2 ± 8.5
	30	6	54.1 ± 18.1	59.2 ± 7.2
	300	6	64.0 ± 12.5	60.6 ± 8.5

Each value represents the mean ± S.D.
a: Test dose levels were described as CH5424802-000
b: Calculated by (1-individual absorbance of the sample/mean absorbance of non-treatment group)*100
No values were significantly different from 0-mg/kg (Dunnett's test)

(Excerpted from Applicant's submission)

Conclusion

Single oral administrations of up to 300 mg/kg CH5424802-000 showed no effects on the gastrointestinal motor function.

Study Title: The Effects of CH5424802 on vasomotion in isolated rat aorta	
Study no.:	1055404 (TOX09-0055S)
Conducting laboratory:	Fuji Gotemba Research Laboratories Chugai Pharmaceutical Co., Ltd. 1-135 Komakado, Gotemba-shi, Shizuoka 412-8513, Japan
Date of study start:	6/1/2009
Compliance:	Non-GLP
Drug/lot/purity	CH5424802; 005, 9%
Vehicle:	DMSO
Reference article:	Nifedipine (positive control)
Species:	Wistar-Han rat (7 weeks old)
Objectives: To evaluate the mechanism causing hypotension seen in study 1053954	

Methods

CH5424802 was applied to the aortic rings following a contractile stimulation with Krebs-Henseleit solution containing 30 mM K+. The study design is below:

Compound	Concentration (μmol/L)	Number of preparations
Vehicle[1]	–	8
CH5424802-000	0.1	4
	0.3	4
	1	4
	3	4
	10	4
Nifedipine	0.0003	4
	0.001	4
	0.003	4
	0.01	4

[1] 0.1% DMSO

(Excerpted from Applicant's submission)

Results

Table 28: Effect of alectinib on 30 mM K+-induced contraction in isolated aortas

Test substance	Concentration[b]	Number of preparations	Corrected % inhibition of 30 mM K^+-induced contraction	IC_{20}[c]	IC_{50}[c]
Vehicle[a]	-	8	0.0 ± 12.6	-	-
CH5424802-000	0.0160 µmol/L (0.1 µmol/L)	4	16.8 ± 10.9	0.0153 µmol/L (7.38 ng/mL)	0.168 µmol/L (81.0 ng/mL)
	0.0723 µmol/L (0.3 µmol/L)	4	40.7 ± 10.1		
	0.247 µmol/L (1 µmol/L)	4	57.2 ± 3.5		
	0.860 µmol/L (3 µmol/L)	4	70.3 ± 8.4		
	3.38 µmol/L (10 µmol/L)	4	83.8 ± 8.9		
Nifedipine	0.0003	4	26.4 ± 6.7	0.000254 µmol/L	0.00139 µmol/L
	0.001	4	39.9 ± 9.8		
	0.003	4	63.7 ± 9.4		
	0.01	4	87.4 ± 4.9		

Corrected % inhibition of 30 mM K^+-induced contraction: % inhibition of 30 mM K^+-induced contraction corrected for the mean vehicle value

[a] 0.1% dimethyl sulfoxide

[b] Concentration of CH5424802-000 represents the actual exposure. Values in parentheses are prepared concentration.

[c] IC_{20} and IC_{50} were calculated from the actual exposure. Values in parentheses are represented as weight concentration converted by the molecular weight of CH5424802-000 (=482.625)

Each value represents mean ± SD.

(Excerpted from Applicant's submission)

Conclusion

The vasodilatory effect of CH5424802 is suggested to be the cause of the hypotension observed in cynomolgus monkeys.

Study Title: The Effects of CH5424802 on calcium currents in human Cav1.2/β2/α2δ Transfected CHO Cells	
Study no.:	1055406 (TOX09-0060)
Conducting laboratory:	Fuji Gotemba Research Laboratories Chugai Pharmaceutical Co., Ltd. 1-135 Komakado, Gotemba-shi, Shizuoka 412-8513, Japan
Date of study start:	9/2/2012

Compliance:	Non-GLP
Drug/lot/purity	CH5424802; 121062, 99.9%
Negative reference article:	DMSO
Positive reference article:	Nifedipine (positive control)
Species:	Wistar-Han rat (7 weeks old)
Parameters measured:	Gastric emptying and phenol red small intestinal transit

Objectives: To clarify the mechanism underlying the vasodilatory effect of CH5424082

Methods

The effect of CH5424802 on Cav1.2 current in CHO cells transfected with human Cav1.2/β2/α2δ was investigated using the patch-clamp technique. The study design is below:

Test and control articles	Concentration of preparation solution	Concentration of pre-application solution [a)]	Number of data (cells)
Negative reference article (0.1% DMSO)	–	–	4
CH5424802-002	0.3 mmol/L	0.3 μmol/L (145 ng/mL [b)])	4
CH5424802-002	0.6 mmol/L	0.6 μmol/L (290 ng/mL [b)])	4
CH5424802-002	1.2 mmol/L	1.2 μmol/L (579 ng/mL [b)])	4
Positive reference article (Nifedipine)	0.1 mmol/L	0.1 μmol/L	4

a) Pre-application solution consists of preparation solution diluted with external solution

b) As CH5424802-000

(Excerpted from Applicant's submission)

Results

Table 29: Effect of Alectinib on Cav1.2 current

Test and reference articles	Concentration (μmol/L)	Number of data (cells)	% inhibition	Corrected % inhibition	IC_{20} (μmol/L)	IC_{50} (μmol/L)
Vehicle (0.1% DMSO)	–	4	10.5 ± 3.1	–	–	–
CH5424802-002	0.189 (0.3) [a)]	4	25.4 ± 5.4*	16.6 ± 6.1		
	0.339 (0.6) [a)]	4	45.3 ± 3.5*	38.9 ± 3.9	0.203	0.461
	0.764 (1.2) [a)]	4	72.6 ± 6.9*	69.4 ± 7.8		
Nifedipine	0.1 [a)]	4	90.5 ± 3.7#	89.3 ± 4.1	–	–

% inhibition: inhibition rate from just before application

Corrected % inhibition: % inhibition corrected for the mean changes in the vehicle group

Each data was expressed as mean ± S.D.

a) Concentration of pre-application solution

–: Not applicable

*: Parametric Dunnet's test, $p<0.05$

#: Student's *t*-test, $p<0.05$

(Excerpted from Applicant's submission)

Conclusion
The inhibitory effect of CH5424802 Cav1.2 current is suggested to be the cause of the vasodilatory effects which caused hypotension observed in cynomolgus monkeys.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

Study Title: Pharmacokinetic study of CH5424802 after single oral and intravenous administration of CH5424802 to rats	
Study no.:	1056248 (ADM12-0065)
Conducting laboratory:	(b) (4)
Date of study start:	8/8/2012
Compliance:	Statement included and signed
QA:	Statement included and signed
Drug/lot/purity	CH5424802; 111392, 99.9%
Species:	Wistar-Han rat (7 weeks old)
Objectives: To evaluate the pharmacokinetics of CH5424802 after a single intravenous and oral administration to fasted male rats (n=3) at 1 mg/kg (as free form) by determining the plasma concentrations of CH5424802-000 (free form of CH5424802) using a high-performance liquid chromatograph-tandem mass spectrometer (LC-MS/MS).	

Methods

Study item	Sex	Route (feeding conditions)	Dose#	Number of dosing	Animal number
Plasma concentration	Male	*p.o.* (fasting)	1 mg/10 mL/kg	3	01101–01103
		i.v. (fasting)	1 mg/5 mL/kg	3	02111–02113

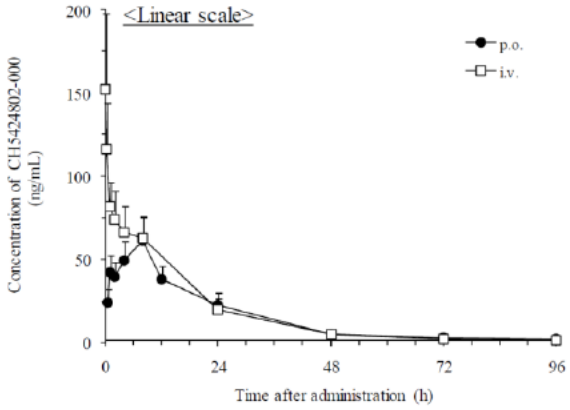
#: As free form

(Excerpted from Applicant's submission)

Plasma was collected from the subclavian vein about 0.1mL without anesthesia at 0.083, 0.25, 1, 2, 4, 8, 24, 48, 72 and 96 h after intravenous administration and a t0.5, 1, 2, 4, 8, 12, 24, 48, 72 and 96 h after oral administration.

Results:

Figure 20: Alectinib single dose plasma concentrations following IV or oral dosing



(Excerpted from Applicant's submission)

Table 30: Alectinib PK in Rats following single dose (IV vs PO)

	Concentration of CH5424802 (ng/mL)	
PK parameter	i.v	p.o
Cmax (ng/mL)	-	60.9 ± 14
Tmax (h)	-	8.0 ± 0
C0(ng/mL)	175 ± 59	-
T1/2 (hr)	24.4 ± 10.9	32.1 ± 4.9
AUC0-t(ng.h/mL)	1530 ± 410	1320 ± 280
AUC inf(ng.h/mL)	1580 ± 450	1400 ± 320
CL (mL/min/kg)	11.0 ± 2.8	-
Vss (L/kg)	13.3 ± 4.3	-
MRT(h)	20.4 ± 5.3	27.6 ± 3.6
F (%)	-	88.6

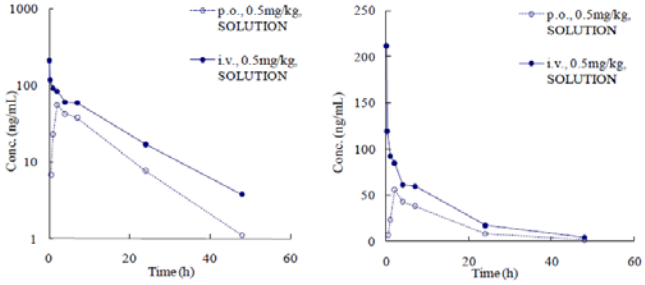
Conclusion: After intravenous administration, alectinib showed a low total body clearance (CL: 11.0 ± 2.8 mL/min/kg) and long elimination half-life (t1/2: 24.4 ± 10.9 h) and a large volume of distribution (Vss : 13.3 ± 4.3 L/kg). After oral administration, alectinib showed good oral bioavailability (F: 88.6%).

Study Title: Pharmacokinetics of CH5424802 in cynomolgus monkeys	
Study no.:	1054083 (ADM10-0002S)
Conducting laboratory:	(b) (4)
Date of study start:	11/25/2008

Compliance:	No
QA:	No
Drug/lot/purity	CH5424802; 111392, 99.9%
Formulation:	DMSO
Species:	Cynomolgus monkey (4-6 years old)
Objectives: To evaluate the pharmacokinetics of CH5424802 after a single intravenous and oral administration to male and female monkeys (n=2/group) at 1 mg/kg (as free form) by determining the plasma concentrations of CH5424802-000 (free form of CH5424802) using a high-performance liquid chromatograph-tandem mass spectrometer (LC-MS/MS).	
Methods CH5424802-000 was administered orally (0.5 mg/kg) and intravenously (0.5mg/kg) to cynomolgus monkeys (n = 2/sex), and pharmacokinetic parameters were calculated. Blood was collected from the forearm cephalic vein using a heparinized syringe with injection needle at the scheduled time points of 5, 15 min, 1, 2, 4, 7, 24 and 48 h after an intravenous administration, or 30 min, 1, 2, 4, 7, 24 and 48 h after an oral administration.	

Results:

Figure 21: Alectinib single dose PK following IV or oral administration in monkeys



Mean plasma concentration of CH5424802-000 after a single intravenous (0.5 mg/kg) or a single oral (0.5 mg/kg) administration of CH5424802-000 to cynomolgus monkeys. Each point represents the mean (male: No.1 and female: No.2).

(Excerpted from Applicant's submission)

Table 31: PK parameters after a single IV alectinib dose (0.5 mg/kg) in monkeys

	C_0 (ng/mL)	AUC_{0-t} (ng•h/mL)	AUC_{inf} (ng•h/mL)	CL (mL/min/kg)	$V_{d,ss}$ (L/kg)	$t_{1/2}$ (h)	MRT (h)
No.1	322	1300	1360	6.13	5.41	10.7	14.7
No.2	244	1350	1400	5.95	5.14	10.1	14.4
Mean	283	1330	1380	6.04	5.28	10.4	14.6

(Excerpted from Applicant's submission)

Table 32: PK parameters of a single oral alectinib dose (0.5 mg/kg) in monkeys

	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng•h/mL)	AUC_{inf} (ng•h/mL)	CL/F (mL/min/kg)	$t_{1/2}$ (h)	MRT (h)	F (%)
No.1	43.3	2.0	437	494	16.8	7.45	11.8	35.8
No.2	68.2	2.0	868	897	9.28	9.31	14.2	65.0
Mean	55.8	2.0	653	696	13.0	8.38	13.0	50.4

(Excerpted from Applicant's submission)

Conclusion: After intravenous administration to monkeys, alectinib showed a low total body clearance (CL: 6.04 mL/min/kg) and long elimination half-life ($t_{1/2}$: 10.4 h) and a large volume of distribution ($Vd_{,ss}$: 5.28 L/kg). After oral administration, alectinib showed a moderate oral bioavailability (F: 50.4%).

Distribution

Study Title: Quantitative Whole-Body Autoradiography in Pigmented Rats after Single Oral Administration of [^{14}C]CH5424802	
Study no.:	1057066 (ADM13-0005)
Conducting laboratory:	(b) (4)
Date of study start:	1/29/2013
Compliance:	Statement included and signed
QA:	Statement included and signed
Drug/lot/purity	CH5424802; 111392, 99.9%
Formulation:	DMSO
Species:	Iar:Long-Evans rat (7 weeks old)
Dose, dose volume, radioactivity:	10 mg, 10 mL/kg, 4.48 MBq/kg

Methods

This study was conducted to examine alectinib distribution in tissues by whole-body autoradiography, following a single oral administration of [^{14}C]CH5424802 to nonfasted male pigmented rats (Iar:Long-Evans) at 10 mg/kg. The radioactivity concentrations in tissues were quantified from the radioluminograms prepared at each time point by whole-body autoradiography 12, 24, 48, 72, 120, 168, 336 and 504 h after administration.

Results

Radiochemical purity of [^{14}C]CH5424802 was 95.2% before administration and 101.3% after administration, which met the acceptance criteria.

Table 33: Radioactivity concentrations in plasma and tissues

Tissue	Radioactivity concentration (ng eq./g) (K_p value; tissue/plasma ratio)			
	01101	02103	03105	04107
	12 h	24 h	48 h	72 h
Plasma*	503 (1.00)	209 (1.00)	52.3 (1.00)	41.6 (1.00)
Blood	1360 (2.70)	601 (2.88)	150 (2.87)	116 (2.79)
Cerebrum	498 (0.99)	268 (1.28)	BLQ (N.C.)	106 (2.55)
Cerebellum	472 (0.94)	242 (1.16)	BLQ (N.C.)	BLQ (N.C.)
Pituitary	15200 (30.22)	6750 (32.30)	1780 (34.03)	1650 (39.66)
Spinal cord	540 (1.07)	245 (1.17)	BLQ (N.C.)	BLQ (N.C.)
Uvea	59100 (117.50)	66900 (320.10)	59000 (1128.11)	61100 (1468.75)
Eyeball	699 (1.39)	458 (2.19)	483 (9.24)	555 (13.34)
Harderian gland	39700 (78.93)	45600 (218.18)	15900 (304.02)	12900 (310.10)
Submaxillary gland	10200 (20.28)	5200 (24.88)	1320 (25.24)	1230 (29.57)
Thyroid	8280 (16.46)	5090 (24.35)	2300 (43.98)	2000 (48.08)
Thymus	5190 (10.32)	2650 (12.68)	944 (18.05)	830 (19.95)
Heart	6330 (12.58)	2970 (14.21)	1110 (21.22)	953 (22.91)
Lung	23600 (46.92)	10500 (50.24)	2680 (51.24)	1970 (47.36)
Liver	15900 (31.61)	7590 (36.32)	2450 (46.85)	1780 (42.79)
Kidney	8170 (16.24)	4580 (21.91)	1340 (25.62)	1080 (25.96)
Adrenal	16300 (32.41)	11500 (55.02)	6040 (115.49)	4700 (112.98)
Pancreas	10600 (21.07)	5170 (24.74)	1410 (26.96)	1080 (25.96)
Spleen	9740 (19.36)	5830 (27.89)	2650 (50.67)	2830 (68.03)
Brown adipose tissue	8100 (16.10)	4450 (21.29)	2430 (46.46)	1550 (37.26)
White adipose tissue	3590 (7.14)	1780 (8.52)	502 (9.60)	251 (6.03)
Bone (femur)	997 (1.98)	578 (2.77)	161 (3.08)	309 (7.43)
Bone marrow (femur)	5810 (11.55)	3170 (15.17)	1250 (23.90)	908 (21.83)
Skin (pigmented)	2870 (5.71)	1360 (6.51)	1260 (24.09)	865 (20.79)
Skin (non-pigmented)	2310 (4.59)	1190 (5.69)	636 (12.16)	701 (16.85)
Skeletal muscle	4380 (8.71)	1680 (8.04)	457 (8.74)	321 (7.72)
Testis	2130 (4.23)	1480 (7.08)	364 (6.96)	267 (6.42)
Epididymis	3100 (6.16)	1520 (7.27)	707 (13.52)	298 (7.16)
Vesicular gland	908 (1.81)	443 (2.12)	121 (2.31)	BLQ (N.C.)
Prostate	2750 (5.47)	1990 (9.52)	490 (9.37)	509 (12.24)
Stomach	4300 (8.55)	1820 (8.71)	802 (15.33)	730 (17.55)
Small intestine	7190 (14.29)	3840 (18.37)	1470 (28.11)	537 (12.91)
Large intestine	6090 (12.11)	2690 (12.87)	1050 (20.08)	1010 (24.28)
Bladder	2600 (5.17)	1420 (6.79)	638 (12.20)	532 (12.79)
Urine in bladder	953 (1.89)	299 (1.43)	121 (2.31)	98.3 (2.36)

* : Values were measured by LSC.

Values in parentheses are expressed as the ratio of tissue concentration to plasma concentration.

BLQ: Below the lower limit of quantification (< 97.9 ng eq./g)

N.C.: Not calculated

(Excerpted from Applicant's submission)

Tissue	Radioactivity concentration (ng eq./g) (K_p value; tissue/plasma ratio)			
	05109	06111	07113	08515
	120 h	168 h	336 h	504 h
Plasma*	N.D. (N.C.)	10.1 (1.00)	N.D. (N.C.)	N.D. (N.C.)
Blood	BLQ (N.C.)	BLQ (N.C.)	BLQ (N.C.)	BLQ (N.C.)
Cerebrum	BLQ (N.C.)	BLQ (N.C.)	BLQ (N.C.)	BLQ (N.C.)
Cerebellum	BLQ (N.C.)	BLQ (N.C.)	BLQ (N.C.)	BLQ (N.C.)
Pituitary	1540 (N.C.)	964 (95.45)	1030 (N.C.)	966 (N.C.)
Spinal cord	BLQ (N.C.)	BLQ (N.C.)	BLQ (N.C.)	BLQ (N.C.)
Uvea	36300 (N.C.)	34000 (3366.34)	34500 (N.C.)	36500 (N.C.)
Eyeball	171 (N.C.)	195 (19.31)	224 (N.C.)	143 (N.C.)
Harderian gland	4160 (N.C.)	1760 (174.26)	749 (N.C.)	341 (N.C.)
Submaxillary gland	506 (N.C.)	402 (39.80)	324 (N.C.)	231 (N.C.)
Thyroid	998 (N.C.)	781 (77.33)	657 (N.C.)	487 (N.C.)
Thymus	463 (N.C.)	339 (33.56)	257 (N.C.)	152 (N.C.)
Heart	477 (N.C.)	380 (37.62)	368 (N.C.)	281 (N.C.)
Lung	1160 (N.C.)	1030 (101.98)	724 (N.C.)	600 (N.C.)
Liver	1070 (N.C.)	717 (70.99)	310 (N.C.)	196 (N.C.)
Kidney	516 (N.C.)	469 (46.44)	214 (N.C.)	134 (N.C.)
Adrenal	1860 (N.C.)	1520 (150.50)	1300 (N.C.)	1950 (N.C.)
Pancreas	531 (N.C.)	420 (41.58)	294 (N.C.)	132 (N.C.)
Spleen	1720 (N.C.)	1930 (191.09)	1940 (N.C.)	1320 (N.C.)
Brown adipose tissue	882 (N.C.)	585 (57.92)	371 (N.C.)	414 (N.C.)
White adipose tissue	165 (N.C.)	BLQ (N.C.)	BLQ (N.C.)	BLQ (N.C.)
Bone (femur)	113 (N.C.)	122 (12.08)	113 (N.C.)	BLQ (N.C.)
Bone marrow (femur)	421 (N.C.)	487 (48.22)	393 (N.C.)	211 (N.C.)
Skin (pigmented)	399 (N.C.)	524 (51.88)	161 (N.C.)	157 (N.C.)
Skin (non-pigmented)	392 (N.C.)	233 (23.07)	117 (N.C.)	BLQ (N.C.)
Skeletal muscle	182 (N.C.)	146 (14.46)	124 (N.C.)	BLQ (N.C.)
Testis	157 (N.C.)	137 (13.56)	127 (N.C.)	105 (N.C.)
Epididymis	214 (N.C.)	221 (21.88)	156 (N.C.)	BLQ (N.C.)
Vesicular gland	BLQ (N.C.)	BLQ (N.C.)	BLQ (N.C.)	BLQ (N.C.)
Prostate	445 (N.C.)	113 (11.19)	110 (N.C.)	125 (N.C.)
Stomach	486 (N.C.)	411 (40.69)	180 (N.C.)	200 (N.C.)
Small intestine	371 (N.C.)	334 (33.07)	230 (N.C.)	243 (N.C.)
Large intestine	427 (N.C.)	133 (13.17)	193 (N.C.)	127 (N.C.)
Bladder	259 (N.C.)	158 (15.64)	220 (N.C.)	BLQ (N.C.)
Urine in bladder	BLQ (N.C.)	BLQ (N.C.)	BLQ (N.C.)	BLQ (N.C.)

* : Values were measured by LSC.
Values in parentheses are expressed as the ratio of tissue concentration to plasma concentration.
BLQ: Below the lower limit of quantification (< 97.9 ng eq./g)
N.C.: Not calculated
N.D.: Not detected (< 10.1 ng eq./g)

(Excerpted from Applicant's submission)

Conclusion: The plasma radioactivity concentration in pigmented rats was highest at 12 hrs postdose and was eliminated with a t1/2 of 49.5 hours. Tissue radioactivity reached Cmax by 12 hrs post dose and was widely distributed into almost the entire body. Radioactivity tissue concentrations exceeded plasma concentrations. The highest radioactivity concentration was observed in the uvea at all time-points, followed by the Harderian gland and lung. The radioactivity concentrations reached the maximum concentration in the uvea and Harderian gland at 24 h after administration. Elimination of radioactivity from 168 hours postdose was slow in many tissues, with 22

of the 34 tissues evaluated still exhibiting radioactivity at 504 hours postdose. Except for the uvea and eyeball, radioactivity detected at 504 hours postdose (105–1950 ng eq/g) had only decreased to 0.7 to 13.6% of their respective maximum concentrations. Concentrations in the cerebrum and cerebellum were approximately 30-40% of the concentration in the blood suggesting reasonable penetration of the blood brain barrier.

Study Title: Quantitative Whole-Body Autoradiography in Pregnant Rats after Single Oral Administration of [^{14}C]CH5424802 – Feto-Placental Transfer of Radioactivity	
Study no.:	1056246 (ADM12-0063)
Conducting laboratory:	(b) (4)
Date of study start:	8/8/2012
Compliance:	Statement included and signed
QA:	Statement included and signed
Drug/lot/purity	[^{14}C]CH5424802; K0135-07, 98.54%
Formulation:	DMSO
Species:	RccHanTM:WIST rat
Dose, dosing volume, radioactivity:	1 mg,10 mL/kg, 4.48 MBq/kg

Methods

This study was conducted to quantitatively examine the feto-placental transfer of radioactivity by whole-body autoradiography following a single oral administration of [^{14}C]CH5424802 to pregnant rats (5/time point) on Day 17 of pregnancy at 1 mg/kg. The radioactivity concentrations in the maternal and fetal tissues were quantified from the radioluminograms prepared at each time point by whole-body autoradiography 4, 8, 24, 48, and 72 h after administration.

Results

Table 34: Maternal-fetal distribution

Tissue	Radioactivity concentration (ng eq./g) (K_p value)				
	01501	02503	03505	04507	05509
	4 h	8 h	24 h	48 h	72 h
Plasma*	107 (1.00)	101 (1.00)	46.4 (1.00)	31.6 (1.00)	14.3 (1.00)
Blood	661 (6.18)	573 (5.67)	137 (2.95)	119 (3.77)	40.5 (2.83)
Brain	52.2 (0.49)	76.5 (0.76)	42.6 (0.92)	19.5 (0.62)	BLQ (N.C.)
Eyeball	14.5 (0.14)	44.8 (0.44)	8.44 (0.18)	20.9 (0.66)	BLQ (N.C.)
Heart	1670 (15.61)	1880 (18.61)	1390 (29.96)	1050 (33.23)	505 (35.31)
Lung	4090 (38.22)	5600 (55.45)	1890 (40.73)	974 (30.82)	601 (42.03)
Liver	2050 (19.16)	2620 (25.94)	1580 (34.05)	994 (31.46)	621 (43.43)
Adrenal	6820 (63.74)	7310 (72.38)	5620 (121.12)	6000 (189.87)	2570 (179.72)
Kidney	1730 (16.17)	1600 (15.84)	1620 (34.91)	1410 (44.62)	566 (39.58)
Mammary gland	750 (7.01)	1030 (10.20)	808 (17.41)	522 (16.52)	306 (21.40)
Ovary	549 (5.13)	1060 (10.50)	537 (11.57)	646 (20.44)	279 (19.51)
Uterus	405 (3.79)	521 (5.16)	359 (7.74)	285 (9.02)	169 (11.82)
Placenta	401 (3.75)	568 (5.62)	291 (6.27)	150 (4.75)	53.5 (3.74)
Amniotic fluid	58.4 (0.55)	38.4 (0.38)	38.3 (0.83)	18.7 (0.59)	BLQ (N.C.)
Fetal membrane	583 (5.45)	555 (5.50)	463 (9.98)	347 (10.98)	175 (12.24)
Fetus (whole-body)	227 (2.12)	295 (2.92)	207 (4.46)	110 (3.48)	41.2 (2.88)
Fetal brain	129 (1.21)	198 (1.96)	64.2 (1.38)	40.9 (1.29)	BLQ (N.C.)
Fetal heart	221 (2.07)	480 (4.75)	88.1 (1.90)	86.4 (2.73)	N.S. (N.C.)
Fetal lung	275 (2.57)	256 (2.53)	104 (2.24)	136 (4.30)	68.3 (4.78)
Fetal liver	398 (3.72)	487 (4.82)	226 (4.87)	183 (5.79)	69.0 (4.83)
Fetal kidney	373 (3.49)	261 (2.58)	145 (3.13)	90.8 (2.87)	15.7 (1.10)
Fetal G.I. tract	316 (2.95)	264 (2.61)	102 (2.20)	163 (5.16)	159 (11.12)

*: Values were measured by LSC.
Values in parentheses are expressed as the ratio of tissue concentration to plasma concentration.
BLQ: Below the lower limit of quantification (< 7.96 ng eq./g)
N.S.: Not specified
N.C.: Not calculated

(Excerpted from Applicant's submission)

Table 35: Tissue half-life

Tissue	$t_{1/2}$ (h)#
Plasma	24.3
Blood	19.0
Brain	N.C.
Eyeball	N.C.
Heart	32.9
Lung	29.0
Liver	35.6
Adrenal	42.5
Kidney	31.6
Mammary gland	34.3
Ovary	50.8
Uterus	44.2
Placenta	19.6
Amniotic fluid	36.6
Fetal membrane	39.4
Fetus (whole-body)	20.6
Fetal brain	N.C.
Fetal heart	N.C.
Fetal lung	42.2
Fetal liver	28.0
Fetal kidney	16.7
Fetal G.I. tract	186.4

#: The $t_{1/2}$ was automatically calculated using the concentrations from 8 h or 24 h to 48 h or 72 h after administration.
The $t_{1/2}$ for the tissues with no three or four consecutive time points in the elimination phase was not calculated.
N.C.: Not calculated

(Excerpted from Applicant's submission)

Conclusion: After oral administration to pregnant rats, radioactivity associated with [^{14}C]CH5424802 was transferred into the fetus and fetal tissues. Levels seen in fetal tissues were similar were similar to those seen in maternal blood. Radioactivity detected in fetal brain tissue, while lower than that recorded in other fetal organs, was higher than that detected in maternal brain tissue.

Study Title: In vitro plasma protein binding and blood cell distribution of [^{14}C]CH5424802	
Study no.:	1054086 (ADM09-0150)
Conducting laboratory:	(b) (4)
Date of study start:	12/11/2009
Compliance:	Statement included and signed
QA:	Statement included and signed
Drug/lot/purity	CH5424802; 3614133, 98.4%
Formulation:	DMSO
Species:	SCDI mice, RccHanTM:WIST, cynomolgus monkey, healthy male volunteers

Methods

The protein binding of [^{14}C]CH5424802 were examined in vitro in the plasma of mice, rats, monkeys, and humans by equilibrium dialysis.

Sample	Sex	Number of samples	[^{14}C]CH5424802 conc. (μg/mL)
Mouse	Male	3 (pool)	1
Rat	Male	3 (pool)	0.1, 1, 10
Monkey	Male	3 (pool)	0.1, 1, 10
Human	Male	3 (pool)	0.1, 1, 10

(Excerpted from Applicant's submission)

Results

Table 36: Plasma protein binding ratio of radiolabelled alectinib

Animal strain or species	[^{14}C]CH5424802 (μg/mL)	Protein binding ratio (%)
Mouse (C.B-17/Icr-*scid*/*scid* Jcl)	1	99.4 ± 0.1
Rat (RccHan™:WIST)	0.1	99.5 ± 0.1
	1	99.5 ± 0.1
	10	99.6 ± 0.0
Monkey (Cynomolgus)	0.1	99.6 ± 0.0
	1	99.6 ± 0.0
	10	99.6 ± 0.0
Human	0.1	99.6 ± 0.1
	1	99.7 ± 0.0
	10	99.7 ± 0.0

Data are expressed as mean±S.D. of 3 samples.

Plasma containing [^{14}C]CH5424802 was incubated at 37°C for 5 min.

(Excerpted from Applicant's submission)

Table 37: Blood cell distribution ratio and blood/plasma (R_B) concentration of radiolabelled alecitinb

Animal strain or species	[^{14}C]CH5424802 (μg/mL)	Distribution ratio (%)	R_B
Mouse (C.B-17/Icr-*scid/scid* Jcl)	1	53.4 ± 1.9	1.11 ± 0.05
Rat (RccHan™:WIST)	0.1	72.8 ± 0.5	2.06 ± 0.04
	1	72.8 ± 0.3	2.06 ± 0.02
	10	60.0 ± 0.1	1.40 ± 0.00
Monkey (Cynomolgus)	0.1	86.6 ± 0.6	3.95 ± 0.17
	1	84.9 ± 0.2	3.52 ± 0.03
	10	68.4 ± 0.2	1.68 ± 0.01
Human	0.1	82.2 ± 0.6	2.92 ± 0.10
	1	80.3 ± 0.2	2.64 ± 0.03
	10	59.8 ± 0.4	1.29 ± 0.01

Data are expressed as mean±S.D. of 3 samples.

Blood containing [^{14}C]CH5424802 was incubated at 37°C for 5 min.

(Excerpted from Applicant's submission)

Conclusions: [^{14}C]CH5424802 exhibits high plasma protein binding, regardless of the concentration or species. The drug blood/plasma distribution is also similar between species. Red blood cell distribution is high and inversely related to dose.

Study Title: In Vitro Plasma Protein Binding and Blood Cell Distribution of CH5468924 (M4)	
Study no.:	1057255 (ADM13-0007)
Conducting laboratory:	(b) (4)
Date of study start:	3/15/2013
Compliance:	Statement included and signed
QA:	Statement included and signed
Drug/lot/purity	CH5468924 (M4); 004, 98.44%
Formulation:	DMSO
Species:	RccHanTM:WIST rat, cynomolgus monkey, healthy male volunteers

Methods

The protein binding (%) of CH5468924-000 (the M4 metabolite) was examined *in vitro* in the plasma of rats, monkeys and humans by equilibrium dialysis.

Equilibrium dialysis

Sample	Sex	Number of samples	CH5468924-000 conc. (μg/mL)*
Rat plasma	Male	3 (pool)	0.04, 0.2, 2

(Excerpted from Applicant's submission)

Plasma protein binding and Blood cell distribution (%)

Sample	Sex	Number of samples	CH5468924-000 conc. (µg/mL)
Rat blood	Male	3 (pool)	0.3, 1, 2
Monkey blood	Male	3 (pool)	0.3, 1, 2
Human blood	Male	3 (pool)	0.3, 1, 2

(Excerpted from Applicant's submission)

Results

The plasma protein binding of CH5468924-000 reached an equilibrium state within 24 h, regardless of the concentration.

Table 38: Plasma protein binding of the M4 metabolite

Animal strain or species	CH5468924-000 (µg/mL)	Protein binding (%)
Rat (RccHan™: WIST)	0.3	99.1 ± 0.1
	1	99.5 ± 0.0
	2	99.6 ± 0.0
Monkey (Cynomolgus monkey)	0.3	99.4 ± 0.2
	1	99.6 ± 0.0
	2	99.7 ± 0.1
Human	0.3	99.1 ± 0.1
	1	99.5 ± 0.0
	2	99.5 ± 0.1

Data are expressed as mean ± S.D. of 3 samples

Plasma containing CH5468924-000 was incubated at 37°C for 5 min

(Excerpted from Applicant's submission)

Table 39: RBC distribution of M4

Animal strain or species	CH5468924-000 (µg/mL)	Distribution (%)	R_B
Rat (RccHan™: WIST)	0.3	77.7 ± 1.3	2.52 ± 0.14
	1	79.2 ± 1.5	2.70 ± 0.19
	2	77.2 ± 0.2	2.45 ± 0.02
Monkey	0.3	76.5 ± 1.2	2.26 ± 0.12
(Cynomolgus monkey)	1	75.8 ± 0.7	2.19 ± 0.06
	2	75.6 ± 1.4	2.18 ± 0.13
Human	0.3	79.8 ± 0.6	2.58 ± 0.08
	1	79.2 ± 0.5	2.50 ± 0.07
	2	79.2 ± 0.4	2.50 ± 0.05

Data are expressed as mean ± S.D. of 3 samples.

Blood containing CH5468924-000 was incubated at 37°C for 5 min.

(Excerpted from Applicant's submission)

Conclusion

Like the alectinib parent compound, CH5468924 (M4) exhibits high plasma protein binding regardless of concentration or species and distributed to red blood cells similarly between species.

Study Title: Identification of [^{14}C]CH5424802 Binding Protein in Human Plasma	
Study no.:	1056250 (ADM12-0067)
Conducting laboratory:	(b) (4)
Date of study start:	8/23/2012
Compliance:	Statement included and signed
QA:	Statement included and signed
Drug/lot/purity	CH5424802; 3614133, 98.4%
Formulation:	DMSO
Species:	SCDI mice, RccHanTM:WIST, cynomolgus monkey, healthy male volunteers

Methods

To identify [^{14}C]CH5424802 binding protein in human plasma, the protein binding ratios of [^{14}C]CH5424802 were examined *in vitro* in the human purified protein (Human serum albumin, HSA; Human α1-acid glycoprotein, α1-AGP) by equilibrium dialysis.

Sample	[^{14}C]CH5424802 conc. (μg/mL)
HSA (40 mg/mL)	0.1, 0.3, 1
α_1-AGP (1 mg/mL)	0.1, 0.3, 1

(Excerpted from Applicant's submission)

Results

Protein binding ratio of [^{14}C]CH5424802

Purified protein	[^{14}C]CH5424802 concentration (μg/mL)	Protein binding ratio (%)
HSA	0.1	97.0 ± 0.0
	0.3	97.0 ± 0.0
	1	96.9 ± 0.1
α_1-AGP	0.1	4.9 ± 4.5
	0.3	2.5 ± 1.5
	1	Not estimated*

Data are expressed as mean ± SD of 3 samples.

When the protein binding ratio was below zero, the value was used as is for calculation of mean (0.1 μg/mL [^{14}C]CH5424802 sample in α_1-AGP solution).

Purified protein solution containing [^{14}C]CH5424802 was incubated at 37°C for 5 min.

* Not estimated: The dissolution of [^{14}C]CH5424802 was suggested at 1 μg/mL in α_1-AGP solution

(Excerpted from Applicant's submission)

Conclusion: Albumin is the main component of the plasma protein of alectinib binding in humans.

Metabolism

Study Title: In vitro metabolic profiles of [^{14}C]CH5424802 in mouse, rat, monkey, dog and human cryopreserved hepatocytes	
Study no.:	1054088 (ADM09-0148)
Conducting laboratory:	(b) (4)
Date of study start:	12/14/2009
Compliance:	Statement included and signed
QA:	Statement included and signed
Drug/lot/purity	[^{14}C]CH5424802; 3614133, 98.4%
Formulation:	DMSO
Species:	CD-1 mice, RccHanTM:WIST, Beagle dog, cynomolgus monkey, healthy volunteers (n=5/sex)

Methods

The Applicant investigated the species difference in metabolism of [^{14}C]CH5424802 in mouse rat, dog, monkey and human hepatocytes. [^{14}C]CH5424802 (10 µmol/L) was incubated for 0, 1, and 4 h at 37°C with mouse, rat, dog, monkey and human hepatocytes. After incubation, [^{14}C]CH5424802 and its metabolites were analyzed by HPLC with a radioisotope detector to calculate the concentrations of CH5424802 and its metabolites. The metabolic clearance of [^{14}C]CH5424802 and metabolite formation velocity were also determined. In addition, the chemical structures of metabolites in human hepatocytes were elucidated by LC/MS.

Species	[^{14}C]CH5424802 (µmol/L)	Cell concentration	Number of samples	Reaction time (h)
Mouse	10	1×10^6 cells/mL	2	0, 1, 4
Rat	10	1×10^6 cells/mL	2	0, 1, 4
Dog	10	1×10^6 cells/mL	2	0, 1, 4
Monkey	10	1×10^6 cells/mL	2	0, 1, 4
Human	10	1×10^6 cells/mL	2	0, 1, 4
Blank-1*1	10	None	2	0, 1, 4
Blank-2*2	0	1×10^6 cells/mL	1	4

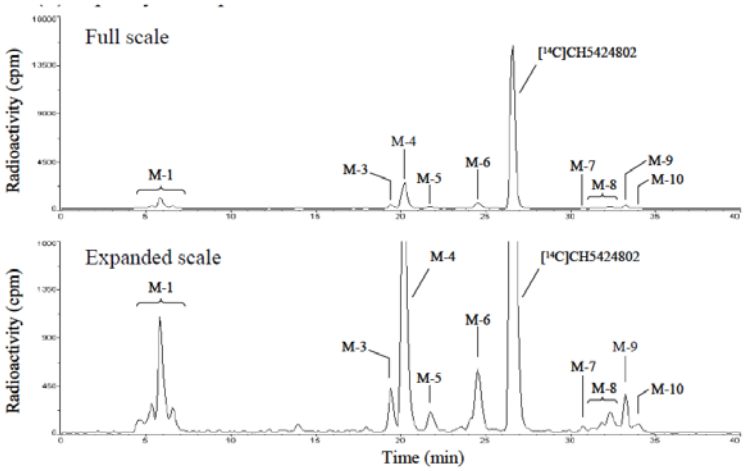
*1: For stability confirmation of [^{14}C]CH5424802 in reaction mixture (HEPES-Krebs-Henseleit buffer [pH 7.4] was added to the reaction mixture instead of hepatocyte suspension)

*2: For stability confirmation of metabolites on the pretreatment for analysis (DMSO was added to the reaction mixture instead of the [^{14}C]CH5424802 solution)

(Excerpted from Applicant's submission)

Results

Figure 22: HPLC radiochromatograms of [^{14}C]-Alectinib and its metabolites



HPLC-radiochromatograms of [^{14}C]-alectinib and its metabolites after 10 μM [^{14}C]-alectinib was incubated with human hepatocytes.

(Excerpted from Applicant's submission)

Table 40: Metabolic clearance of [^{14}C]-Alectinib in mouse, rat, dog, monkey, and human hepatocytes

Species	Incubation time (h)	[^{14}C]CH5424802 (μmol/L)	Metabolic clearance (mL/min/10^6 cells)
Mouse	0	9.80	0.00390
(ICR/CD-1)	1	9.38	
	4	8.81	
Rat	0	9.69	0.0122
(Wistar)	1	8.38	
	4	6.59	
Dog	0	9.78	0.00558
(Beagle)	1	9.30	
	4	8.40	
Monkey	0	9.74	0.0183
(Cynomolgus)	1	6.15	
	4	4.63	
Human	0	9.75	0.0131
	1	8.59	
	4	6.51	

[^{14}C]CH5424802 (10 μmol/L) was incubated with each hepatocytes (1×10^6 cells/mL) at 37°C.

(Excerpted from Applicant's submission)

Table 12: Metabolic formation velocity of [^{14}C]-Alectinib in cryopreserved mouse, rat, dog, monkey, and human hepatocytes

Species	Incubation time	Velocity (pmol/10^6 cells/mL)									
	(h)	M-1	M-2	M-3	M-4*1	M-5	M-6*2	M-7	M-8	M-9	M-10
Mouse	1	3.33	N.D.	0.417	3.50	0.500	0.917	0.917	0.0835	0.167	N.D.
(ICR/CD-1)	4	1.73	N.D.	0.146	1.84	0.0833	0.584	0.146	0.0209	0.146	0.00
Rat	1	3.59	N.D.	0.250	12.1	0.917	0.750	1.92	1.67	2.92	0.333
(Wistar)	4	2.98	N.D.	0.146	8.09	0.292	0.355	0.209	0.480	0.708	0.229
Dog	1	1.75	N.D.	0.334	6.92	1.58	0.167	0.250	0.0835	0.0835	0.0835
(Beagle)	4	1.53	N.D.	N.D.	4.40	0.375	0.0625	0.0417	0.00	0.0209	0.0209
Monkey	1	9.00	N.D.	3.59	14.5	1.59	10.1	2.09	6.25	11.5	1.75
(Cynomolgus)	4	4.40	N.D.	1.10	7.63	0.417	3.46	0.354	1.50	1.94	0.521
Human	1	4.08	N.D.	1.50	7.25	1.42	3.17	0.750	1.00	2.42	0.584
	4	3.48	N.D.	0.771	5.48	0.521	1.75	0.146	0.709	1.06	0.313

[^{14}C]CH5424802 (10 µmol/L) was incubated with each hepatocytes (1×10^6 cells/mL) at 37°C.

*1: M4 = CH5468924

*2: M6 = CH5507197

N.D.: Not detected

(Excerpted from Applicant's submission)

Conclusion

There were no remarkable species differences in the in vitro metabolism of [^{14}C]CH5424802. M-4 was identified as the main metabolite in mouse, rat, dog, monkey, and human hepatocytes. All metabolites confirmed in the human hepatocytes were detected in mouse, rat, dog and/or monkey hepatocytes, suggesting that there are no human-specific metabolites of alectinib.

Study Title: Identification of Cytochrome P450 Isozymes Involved in the Biotransformation of [14C]CH5424802	
Study no.:	1054089 (ADM09-0151)
Conducting laboratory:	(b) (4)
Date of study start:	2/16/2010
Compliance:	Statement included and signed
QA:	Statement included and signed
Drug/lot/purity	[^{14}C]CH5424802; 3614133, 98.4%
Formulation:	DMSO

Methods

The Applicant investigated the CYP isoforms involved in the metabolism of CH5424802. [^{14}C]CH5424802 (10 µmol/L) was incubated for 60 min at 37°C with 13 recombinant CYP isoforms (CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A5 and CYP4A11) in the presence of NADPH. After incubation, [^{14}C]CH5424802 and its metabolites were analyzed using HPLC with radioisotope

detector to calculate the concentration of CH5424802 and the metabolites.

Results

Table 41: Remaining concentration of [^{14}C]CH5424802 in recombinant CYP isoforms

CYP isoform	Remaining conc. of [^{14}C]CH5424802 (μmol/L)	% of control (%)
Control-1	9.61	100.0
Control-2	9.63	100.0
CYP1A1	9.40	97.8[a]
CYP1A2	9.55	99.4[a]
CYP2A6	9.54	99.1[b]
CYP2B6	9.52	98.9[b]
CYP2C8	9.21	95.6[b]
CYP2C9	9.52	98.9[b]
CYP2C18	9.59	99.8[a]
CYP2C19	9.57	99.4[b]
CYP2D6	9.47	98.5[a]
CYP2E1	9.62	99.9[b]
CYP3A4	4.66	48.4[b]
CYP3A5	9.28	96.4[b]
CYP4A11	9.26	96.4[a]

[^{14}C]CH5424802 (10 μmol/L) was incubated with each recombinant human CYP isoform (100 pmol CYP/mL) at 37°C for 60 min.

Data are expressed as the mean values of duplicate determinations.

a: % of control = ([^{14}C]CH5424802 conc. in CYP isoform/[^{14}C]CH5424802 conc. in control-1) × 100

b: % of control = ([^{14}C]CH5424802 conc. in CYP isoform/[^{14}C]CH5424802 conc. in control-2) × 100

(Excerpted from Applicant's submission)

Table 42: Concentration of [^{14}C]CH5424802 metabolites in recombinant human CYP isoforms

CYP isoform	Metabolite concentration (μmol/L)									
	M-1	M-2	M-3	M-4[*1]	M-5	M-6[*2]	M-7	M-8	M-9	M-10
CYP1A1	0.0450	N.D.	0.0850	0.170	0.0400	0.0400	N.D.	N.D.	0.0200	0.00500
CYP1A2	0.0350	N.D.	0.0700	N.D.	N.D.	N.D.	N.D.	N.D.	0.0100	N.D.
CYP2A6	N.D.	N.D.	0.0650	0.155	N.D.	0.0250	N.D.	N.D.	N.D.	N.D.
CYP2B6	0.0300	N.D.	0.0750	0.155	0.0200	N.D.	N.D.	N.D.	N.D.	0.00500
CYP2C8	N.D.	N.D.	0.145	0.265	0.180	0.0350	N.D.	N.D.	0.0150	0.0150
CYP2C9	N.D.	N.D.	0.0800	0.145	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CYP2C18	N.D.	N.D.	0.0650	N.D.	0.0400	N.D.	N.D.	N.D.	0.0100	N.D.
CYP2C19	N.D.	N.D.	N.D.	0.145	0.0150	0.0250	N.D.	N.D.	N.D.	N.D.
CYP2D6	0.0350	N.D.	0.0750	0.165	0.0400	N.D.	N.D.	N.D.	0.0150	0.00
CYP2E1	N.D.	N.D.	N.D.	N.D.	0.0200	N.D.	N.D.	N.D.	N.D.	N.D.
CYP3A4	0.280	0.160	0.355	2.80	0.315	0.515	N.D.	N.D.	0.145	0.180
CYP3A5	0.0300	N.D.	0.125	0.290	0.0550	0.0350	N.D.	N.D.	0.0200	0.0100
CYP4A11	N.D.	N.D.	0.0600	0.170	0.295	N.D.	N.D.	N.D.	N.D.	0.00500

[^{14}C]CH5424802 (10 μmol/L) was incubated with each recombinant human CYP isoform (100 pmol CYP/mL) at 37°C for 60 min.

Data are expressed as the mean values of duplicate determinations.

*1: M4 = CH5468924

*2: M6 = CH5507197

N.D.: Not detected

(Excerpted from Applicant's submission)

Conclusion: The major human CYP isoform involved in the metabolism of [^{14}C]CH5424802 appears to be CYP3A4. Incubation with this enzyme resulted in metabolism of approximately half of the original parent compound during the hour long

incubation, while metabolism by other enzymes was less than 5%. Similar to the data from hepatocytes, M4 was the major metabolite produced by CYP3A4. CYP1A1, 2B6, 2C8/9, 2D6, 3A5, and 4A11 also appear to be involved in CH5424802 metabolism but are expected to be minor contributors.

Excretion

Study Title: Absorption, Metabolism and Excretion in Rats after Single Oral Administration of [14C]CH5424802	
Study no.:	1054090 (ADM09-0147)
Conducting laboratory:	(b) (4)
Date of study start:	12/14/2009
Compliance:	Statement included and signed
QA:	Statement included and signed
Drug/lot/purity/radioactivity	[^{14}C]CH5424802; 3614133, 98.4%, 4.5 MBq/mg
Formulation:	DMSO
Methods The Applicant examined the plasma concentration of radioactivity, urinary and fecal excretion of radioactivity and remaining radioactivity in the body following a single oral administration of [^{14}C]CH5424802 to non-fasting male rats at 1 mg/kg. The Applicant also examined the metabolite profiles in the plasma, urine and feces.	

Results

Table 43: Radioactivity concentration and PK parameters in plasma following a single dose of alectinib

Time (h)	Radioactivity concentration (ng eq./mL)
1	26.0 ± 2.2
2	40.3 ± 2.7
4	59.5 ± 5.8
6	65.0 ± 8.3
8	52.1 ± 12.9
10	58.8 ± 7.3
24	27.5 ± 8.9
48	10.1 ± 3.2
72	4.30 ± 1.51
C_{max} (ng eq./mL)	66.2 ± 6.9
t_{max} (h)	7.3 ± 2.3
AUC_{0-t} (ng eq.·h/mL)	1650 ± 210
AUC_{0-inf} (ng eq.·h/mL)	1760 ± 260
$t_{1/2}$ (h)[a)]	17.9 ± 1.0

Data are expressed as the mean ± S.D. of three animals.

a) The $t_{1/2}$ was automatically calculated by WinNonlin (using the concentrations from 24 h to 72 h).

(Excerpted from Applicant's submission)

Table 44: Cumulative excretion of radioactivity after a single oral dose of alectinib

Time (h)	Cumulative radioactivity excretion (% of dose)			
	Urine	Feces	Cage washings (methanol/water [1:9, v/v]])	Total
0-24	0.2 ± 0.0	51.9 ± 6.0	0.1 ± 0.1	52.2 ± 6.0
-48	0.4 ± 0.1	78.5 ± 2.5	0.2 ± 0.1	79.1 ± 2.6
-72	0.4 ± 0.1	87.2 ± 1.1	0.2 ± 0.1	87.9 ± 1.2
-96	0.5 ± 0.1	91.3 ± 1.0	0.2 ± 0.2	91.9 ± 1.2
-120	0.5 ± 0.1	93.6 ± 0.7	0.2 ± 0.2	94.3 ± 0.6
-144	0.5 ± 0.1	94.8 ± 0.7	0.2 ± 0.2	95.6 ± 0.7
-168	0.5 ± 0.1	95.7 ± 0.7	0.3 ± 0.2	96.4 ± 0.6
Cage washings (methanol) at 168 h				0.1 ± 0.0
Carcass at 168 h				2.9 ± 0.1
Total recovery				99.4 ± 0.8

Data are expressed as the mean ± S.D. of three animals.

(Excerpted from Applicant's submission)

Conclusion: The major excretion route was the feces. Almost the entire amount of the administered radioactivity was excreted in the feces by 168 hours.

Study Title: Excretion and Metabolites Profiling in Bile and Enterohepatic Circulation after Single Intravenous Administration of [14C]CH5424802 to Rats	
Study no.:	1056247 (ADM12-0064)
Conducting laboratory:	(b) (4)
Date of study start:	8/10/2012
Compliance:	Statement included and signed
QA:	Statement included and signed
Drug/lot/purity/radioactivity	[^{14}C]CH5424802; 3614133, 98.4%, 4.5 MBq/mg
Formulation:	DMSO

Methods

The Applicant examined the radioactivity excretion and metabolites profiling in the bile following a single intravenous administration of [^{14}C]CH5424802 to male rats at 1 mg/kg. The Applicant also examined the enterohepatic circulation of radioactivity.

Results

Table 45: Radioactivity in bile, urine, and feces after single dose IV administration of [^{14}C]CH5424802 to bile duct-cannulated rats

Time (h)	Cumulative radioactivity excretion (% of dose)					
	Bile	Urine	Feces	Cage washing (methanol/water [1:9, v/v])	Cage washing (methanol)	Total recovery
0-2	3.8 ± 0.9	--	--	--	--	--
-4	6.6 ± 2.0	--	--	--	--	--
-8	12.8 ± 4.1	--	--	--	--	--
-24	31.5 ± 7.7	2.0 ± 0.2	7.9 ± 3.6	0.1 ± 0.0	--	41.5 ± 10.5
-48	42.5 ± 7.1	2.0 ± 0.1	10.3 ± 4.3	0.1 ± 0.1	0.0 ± 0.0	54.9 ± 8.8

Data are expressed as the mean ± S.D. of four animals.

--: Not applicable

(Excerpted from Applicant's submission)

Table 46: Cumulative radioactivity excretion in bile, urine, and feces after single intraduodenal administration of bile sample (collected 0-24 h after single IV administration of [^{14}C]CH5424802 at 1 mg/kg) to bile duct-cannulated male rats

Time (h)	Cumulative radioactivity excretion (% of dose)					
	Bile	Urine	Feces	Cage washing (methanol/water [1:9, v/v])	Cage washing (methanol)	Total recovery
0-2	0.2 ± 0.1	--	--	--	--	--
-4	0.4 ± 0.1	--	--	--	--	--
-8	1.1 ± 0.2	--	--	--	--	--
-24	2.4 ± 0.4	N.D.	38.4 ± 24.9	N.D.	--	40.8 ± 25.2
-48	3.0 ± 0.6	N.D.	76.3 ± 13.8	N.D.	N.D.	79.3 ± 14.3

Data are expressed as the mean ± S.D. of three animals.
--: Not applicable
N.D.: Not detected

(Excerpted from Applicant's submission)

Table 47: Composition of radioactive metabolites in bile after single IV administration of [^{14}C]CH5424802 to bile duct-cannulated rats

Metabolite No.	Component (Standard)	Retention time (min)	% in analytical sample (*% of dose*)
			0-24 h
BM-1	Unknown	6.7	5.7 (*1.8*)
BM-2	Unknown	7.6	19.2 (*6.0*)
BM-3	Unknown	8.3	21.4 (*6.7*)
BM-4	Unknown	9.4	2.0 (*0.6*)
BM-5	CH5468924	19.7	40.6 (*12.8*)
BM-6	CH5507197	22.8	2.4 (*0.8*)
BM-7	CH5424802	26.4	4.0 (*1.3*)
	Others		4.7 (*1.5*)
	Total radioactivity		(*31.5*)
	Radioactivity recovery		--

--: Not determined (due to directly injection)

(Excerpted from Applicant's submission)

Conclusion: Bile was a major excretion route for alectinib and some of its metabolites. Radioactivity in the bile increased time-proportionally within 24 hours of administration. Reabsorption of radioactivity after biliary excretion was low. M4 was also observed in the bile and accounted for approximately 10% of the total alectinib dose.

6 General Toxicology

6.1 Repeat-Dose Toxicity

Study title: A 13-Week Repeated Dose Oral Gavage Toxicity Study of CH5424802*-002 in Rats Followed by a 8-Week Recovery Period	
Study no.:	TOX10-0026
Sponsor and Conducting laboratory:	Fuji Gotemba Research Laboratories Chugai Pharmaceutical Co., Ltd. 1-135 Komakado, Gotemba-shi, Shizuoka, Japan
Date of study initiation:	27 May, 2010
GLP compliance/QA statement:	Yes (Ministry of Health, Labor and Welfare, Japan). *Reviewer note* Pathology report was not signed by the primary pathologist.
Drug, lot #:	CH5424802-002; G0E01, G0G01

Key Study Findings

- At 27 mg/kg/day, clinical observations (discoloration of eyeball, blackish feces, discoloration of teeth, enlarged, short and crushed teeth) were reported and were accompanied by decreases in mean body weights (up to 22%) and food consumption (up to 13%) at doses ≥9mg/kg/day.

- Test article-related changes in hematology, clinical chemistry, coagulation and urinalysis parameters were reported at doses ≥ 3 mg/kg/day. Effects on reticulocytes, monocytes, total bilirubin, glucose, inorganic phosphorus and Alpha2 Globulin were evident at the end of the recovery period at 27 mg/kg/day.

- Major target systems/organs were: hematopoietic system, gastrointestinal system, respiratory system, urinary system, reproductive system, endocrine system, incisor tooth, mandibular glands and bone. Changes in all organs showed recovery or a trend towards recovery.

- The NOAEL was < 3 mg/kg/day.

Methods:

Doses/ Number/Sex/Group:

Group	Dose[a] level (mg/kg)	Dosing[a] suspension concentration (mg/mL)	Dosing volume (mL/kg)	Number of animals		Animal Nos[b]./Cage Nos.	
				Male	Female	Male	Female
Main group							
0 mg/kg[c]	0	0	10	15	15	1101–1115	5101–5115
3 mg/kg	3	0.3	10	15	15	1201–1215	5201–5215
9 mg/kg	9	0.9	10	15	15	1301–1315	5301–5315
27 mg/kg	27	2.7	10	14[d]	15	1401, 1403–1415[d]	5401–5415
Satellite group							
0 mg/kg TK[c]	0	0	10	4	4	1501–1504	5501–5504
3 mg/kg TK	3	0.3	10	4	4	1601–1604	5601–5604
9 mg/kg TK	9	0.9	10	4	4	1701–1704	5701–5704
27 mg/kg TK	27	2.7	10	4	4	1801–1804	5801–5804

[a] Expressed as CH5424802-000 (free base of CH5424802).

[b] Animals with numbers ending in 11 to 15 were recovery groups.

[c] Groups at 0 mg/kg and 0 mg/kg TK were treated with vehicle.

[d] Animal No. 1402 was excluded from toxicological evaluation in the study.

(Excerpted from Applicant's report)

Route/Frequency of dosing:	Oral gavage/Daily for 92 days.
Formulation/Vehicle:	Vehicle, 0.5% methylcellulose (HPMC)/0.001 mol/L hydrochloric acid (HCL) solution
Sex/Strain/Species:	Male and female RccHan:WIST rats
Age/Weight at start of dosing:	6 weeks / 193 to 170 g for the males 116 to 144 g for the females
Toxicokinetic evaluation:	4/rats/group/sex: blood was collected on Day 1 (1, 2, 4, 8 and 24 hours postdose), prior to Days 28 and 56 of dosing and on Day 91 (prior to dosing, 1, 2, 4, 8, 12 and 24 hours postdose) For control animals, blood was collected at 8 hours postdose.
Endpoints evaluated:	Mortality, clinical observations, ophthalmic examinations, body weight, body weight gain and food consumption. Clinical pathology samples were collected based on the following schedule: • Urinalysis: Days 85 to 86 (treatment period) and 134 to 135 (recovery). • Hematology, coagulation, clinical chemistry and bone marrow test: Days 92 (end of treatment) and Day 148 (end of recovery period).
Termination/ necropsy and histopathology:	Day 92 (end of treatment) and Day 148 (end of recovery): Organ weights, macroscopic and microscopic findings at necropsy. Microscopic examinations were conducted on main study

	animals in the control and 27 mg/kg/day groups. A read down was conducted for tissues which showed pathology.

Results:

Stability and Dosing Analysis

- Samples obtained for concentration verification analysis were within the acceptable limits of ±10% error for all groups (98.1% to 101.7%).
- Suspensions at concentration levels of 0.3 to 2.7 mg/mL were homogeneous.
- The suspensions at concentration levels of 0.05 to 10 mg/mL were stable for up to 6 hours at room temperature and stable for up to 10 days in a refrigerator shielded from light. Stability testing was conducted under study No. TOX10-0027 (13-week monkey toxicology study).

Mortalities

No test article-related mortalities were reported. One male at 27 mg/kg/day was moribund sacrificed, presumably due to a gavage error.

Table 48: Rat – Mortalities

Dose (mg/kg/day)	Day of moribund sacrificed	Sex (animal #)	Prior to Death and Necropsy Findings
27	68	M (1204)	*Clinical observations:* Decreased locomotor activity, deep respiration, decrease of feces.
			Macroscopic findings: Increased dark-red content in the thoracic cavity, lung adhesion, and enlargement and dark-red discoloration of the pulmonary lymph nodes. *Microscopic findings related to the moribund condition:* Diffuse pleurisy in the lung.

Clinical Observations

Test article-related clinical observations were reported at 27 mg/kg/day in male and female rats. Observations persisted throughout the recovery period. The teeth findings correlated to the macro-and microscopic findings. The discoloration of the eyeball and blackish feces were consistent with the reported increase in blood clotting time (APTT and PT).

Table 49: Rat – Clinical Observations

Observations (Duration in Days)	Number of rats affected							
	0		3 mg/kg/day		9 mg/kg/day		27 mg/kg/day	
	M	F	M	F	M	F	M	F
Discoloration of eyeball (56-92 and 93-100)	0	0	0	0	0	0	1	3 (1)
Blackish feces (59-91 and 94-95)	0	0	0	0	0	0	1(1)	2
Discoloration of teeth (52-92 and 93-145)	0	0	0	0	0	0	0	8 (2)
Enlarged teeth (88-92 and 129-135)	0	0	0	0	0	0	0	1 (1)
Crushing of teeth (85-92 and 93-142)	0	0	0	0	0	0	0	1 (1)
Short teeth (88-92 and 129-135)	0	0	0	0	0	0	0	1 (1)

M=male; F=female
( ) = recovery

Body Weight

Table 50 : Rat – Mean Body Weight (% change from control)

Day	3 mg/kg/day		9 mg/kg/day		27 mg/kg/day	
Sex	M	F	M	F	M	F
92	--	--	-6*	-9**	-22**	-16**
148 (recovery)	--	-5*	--	-9**	-10**	-16**

-- = no biologically meaningful findings were reported
shaded numbers = finding was not considered biologically meaningful
** = p<0.05, ** p<0.01 significantly different from control*

Food Consumption

Table 51: Rat – Mean Food Consumption (% change from control)

Days	3 mg/kg/day		9 mg/kg/day		27 mg/kg/day	
Sex	M	F	M	F	M	F
1-92	--	--	--	up to-6*	up to-11**	up to -13**
148 (recovery)	--	--	--	up to-17**	--	--

*-- = no biologically meaningful findings were reported; shaded numbers = finding was not considered biologically meaningful; * = p<0.05, ** p<0.01 significantly different from control*

Ophthalmology

No test article-related ophthalmic findings were reported.

Hematology

Table 52: Rat– Predominant Hematology Changes on Day 92 (% change from control)

Parameters	3 mg/kg/day		9 mg/kg/day		27 mg/kg/day	
Sex	M	F	M	F	M	F
Red blood cells	--	--	--	--	--	8*
Hemoglobin (HGB)	--	--	--	--	--	-8*
Reticulocytes	--	--	22**	26*	224** (30**)	166** (29**)
Hematocrit	--	--	--	--	-4*	--
White Blood Cells	--	--	23*	67**	70**	106**
NEUT Abs.	--	31*	34*	85**	215**	470**
Lymph Abs.	--	--	--	66**	--	28**
Monocytes Abs.	58*	26	42*	53**	216**	282**
Eosinophils	--	--	--	-28*	-53**	-37*
Basophils Abs.	--	--	--	--	--	233**
Large Unstained Cells	--	--	--	--	--	57*
LUC Abs.	86	63	100*	100*	185**	212**
Platelets	--	--	--	--	27**	9
Prothrombin Time (PT)	--	--	--	--	99**	--
Activated Partial Thromboplastin Time (APTT)	10*	--	14**	--	76**	25**
Fragmented red cell (Poikilocytosis)	--	--	↑ (7/10)	↑ (3/10)	↑ (8/9)	↑ (6/10)
Large PLT (morphology)	--	--	--	↑ (2/10)	↑ (6/9)	↑ (5/10)

( ) = recovery on Day 148; -- = no biologically meaningful findings were reported
** = p<0.05, ** p<0.01 significantly different from control*

Clinical Chemistry

Table 53: Rat – Predominant Clinical Chemistry Changes on Day 92 (% change from control)

Parameters	3 mg/kg/day		9 mg/kg/day		27 mg/kg/day	
Sex	M	F	M	F	M	F
AST	--	54**	23*	35**	33**	60**
ALP	--	--	38*	33**	20*	100**
Total Bilirubin	--	--	--	--	20* (37*)	--
Urea Nitrogen	--	--	--	--	24**	15*
Creatinine	--	--	--	-10*	--	-7*

Parameters	3 mg/kg/day		9 mg/kg/day		27 mg/kg/day	
Sex	M	F	M	F	M	F
Glucose	--	--	--	--	-23** (-15*)	--
Total Cholesterol	--	25*	21**	41**	37**	26**
Inorganic Phosphorus	--	--	--	--	33** (28*)	23*
Alkaline Phosphatase Fraction 2 (%) (ALP2-bone)	--	--	55*	--	98**	160**
ALP2 Activity	--	--	116**	53*	133**	423**
Alkaline Phosphatase Fraction 3 activity (ALP3)	--	--	--	--	--	39*
Alkaline Phosphatase Fraction 5 activity (ALP5)	--	26	--	43*	--	114**
Albumin (%)	--	--	--	--	-4*	-5*
Albumin concentration (g/dL)[1]	--	--	--	--	-6**	-10**
Alpha1 Globulin (%)	--	--	--	--	-17**	--
Alpha1 globulin concentration (g/dL)[1]	--	--	--	--	-20**	-10*
Alpha2 Globulin (%)	--	--	7**	--	23**	20** (16*)
Alpha2 G concentration (g/dL)[1]	--	--	14**	13**	18**	23**
Beta Globulin (%) (Beta G)	--	--	9**	14**	34**	33**
Beta G concentration (g/dL)[1]	--	--	9**	19**	29**	23**
Gamma Globulin (%)	--	--	--	--	-27**	-21**
Gamma G concentration (g/dL)[1]	--	--	--	--	-26**	20*

*( ) = recovery; --= no biologically meaningful findings were reported; * = p<0.05, ** p<0.01 significantly different from control;* [1]*= calculated based on* % protein fraction and total protein (g/dL)

Urinalysis

Table 54: Rat – Predominant Urinalysis Changes (% change from control)

Parameter	Day	3 mg/kg/day		9 mg/kg/day		27 mg/kg/day	
		M	F	M	F	M	F
Urine volume (mL)	88	33	--	51*	--	77*	--
	135 (recovery)	--	--	--	--	--	--

*-- = no biologically meaningful findings were reported; * = p<0.05*

Macroscopic Findings

Test article-related macroscopic findings were report at doses≥9 mg/kg/day. Enlarged and discolored (dark red) mesenteric lymph node were evident at the end of the recovery period at doses ≥ 9mg/kg/day. The macroscopic findings correlated with microscopic findings and with the reported increases in adrenal and spleen organ weights.

Table 55: Rat – Macroscopic Findings (incidence)

Dosage Group (mg/kg/day):	**0**		**3**		**9**		**27**	
Number examined	10 (5)	10 (5)	10 (5)	10 (5)	10 (5)	10 (5)	10 (5)	10 (5)
sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Ileum- Dilated	--	--	--	--	--	--	--	7
Cecum – content dark red	--	--	--	--	--	--	1	1
Colon – content dark-red gelatinous	--	--	--	--	--	--	3	1
Rectum	--	--	--	--	--	--	--	1
Prostate Gland – diminished in size	--	--	--	--	--	--	1	--
Seminal vesicles- diminished in size	--	--	--	--	--	--	1	--
Adrenal gland – Enlarged Dark brown discoloration	 -- --	 -- --	 -- --	 -- --	 -- --	 -- --	 -- --	 4 1
Spleen - enlarged	--	--	--	--	--	--	--	6
Mesenteric lymph node – Dark red discoloration Enlarged	 -- --	 -- --	 -- --	 -- --	 3 4(1)	 1 3(1)	 9(3) 9(5)	 10 (2) 10(5)
Teeth- White	--	--	--	--	--	--	--	6

-- = no findings
( ) = recovery

Organ Weights

Test article-related increases in relative organ weights were reported at doses ≥ 9 mg/kg/day. At the end of the recovery period, relative organ weight increases persisted at 27 mg/kg/day (kidneys, spleen, lung and testes) and at doses ≥9 mg/kg/day in females (heart). The increases in brain and heart were not correlated with microscopic findings.

Table 56: Rat –Organ Weight Changes Relative to Body Weight (% change from control)

Organ	3 mg/kg/day		9 mg/kg/day		27 mg/kg/day	
	M	F	M	F	M	F
Kidneys	6*	4	12**	30**	21**	39**(11*)
Spleen	--	--	23**	12*	67**(16*)	57**(18*)
Lung	--	--	5*	7**	23**(11**)	18**(11*)
Heart	--	3*(10*)	9**	14**(12**)	21**	32**(23**)
Adrenal	--	--	--	--	27**	(25**)
Testes	--	--	--	--	25**(12*)	--
Liver	--	--	6*	13**	16**	42**
Mandibular gland	--	--	--	--	--	(18**)

-- = no biologically meaningful findings were reported
shaded numbers = finding was not considered biologically meaningful
( ) = recovery
** = p<0.05, ** p<0.01 significantly different from control*

Histopathology

Adequate Battery – Yes
Signed Pathology Report – No (in compliance with the Japanese GLPs)
Peer Reviewed- No

Test article-related microscopic findings were reported at doses ≥3mg/kg/day in both sexes.
Terminal necropsy (Day 92):

- At doses ≥ 3 mg/kg/day, liver, spleen and adrenal glands.
- At doses ≥ 9 mg/kg/day, stomach, ileum, thymus, axillary lymph nodes, mesenteric and lymph nodes.
- At 27 mg/kg/day, bone marrow (femur and sternum), trachea, lung, jejunum, kidneys, prostate gland, seminal vesicles, mammary glands, pituitary gland, skin/subcutis, bone (sternum and femur), mandibular glands, teeth and adipose tissue.

At the end of the recovery period (Day 148):

- At doses ≥ 3 mg/kg/day, spleen (partial recovery).
- At doses ≥ 9 mg/kg/day, mesenteric and lymph nodes.
- At 27 mg/kg/day, liver and lung. Partial recovery was reported in the adrenals, kidneys, ileum, stomach and trachea.

Table 57: Rat – Histopathology Findings

	No. of animals affected							
Sex	**Males**				**Females**			
Dose (mg/kg/day)	**0**	**3**	**9**	**27**	**0**	**3**	**9**	**27**
No. Examined* (recovery)	10 (5)	10 (5)	10 (5)	10 (5)	10 (5)	10 (5)	10 (5)	10 (5)
Treatment-related Findings:								
TRACHEA								
Macrophage/ multinucleated giant cell in lamina propria,								
– *Minimal*	0	0	0	9	0	0	0	3(3)
– *Slight*	0	0	0	0	0	0	0	4(2)
– *moderate*	0	0	0	0	0	0	0	3
Disarrangement of mucosal epithelium								
– *Minimal*	0	0	0	0	0	0	0	5(2)
– *Slight*	0	0	0	0	0	0	0	1
Inflammatory cell infiltration in lamina propria								
– *Minimal*	0	0	0	0	0	0	0	3
LUNG								
Foamy macrophage infiltration in alveolus								
– *Minimal*	1(2)	5(1)	10(3)	1(5)	3(2)	5(2)	10(4)	4(5)
– *Slight*	0	0	0	8	0	0	0	6
Osseous metaplasia								
– *Minimal*	0	0	0	1(1)	0	0	0	0
STOMACH								
Macrophage infiltration in glandular mucosa								
– *Minimal*	0	0	6	7	0	0	8	6(1)
– *Slight*	0	0	0	0	0	0	0	4
Inflammatory cell infiltration in glandular mucosa								
– *Minimal*	0	0	7	8	0	0	9	10
Hypertrophy with mucin of glandular epithelium								
– *Minimal*	0	0	4	3	0	0	1	4
Extension of proliferating zone in glandular mucosa								
– *Slight*	0	0	0	9	0	0	3	9

	No. of animals affected							
Sex	**Males**				**Females**			
Dose (mg/kg/day)	**0**	**3**	**9**	**27**	**0**	**3**	**9**	**27**
Erosion in glandular stomach								
– *Slight*	0	0	0	0	(1)	0	0	0
– *Moderate*	0	0	0	0	0	0	0	(1)
JEJUNUM								
Macrophage/ multinucleated giant cell in mucosa								
– *Minimal*	0	0	0	4	0	0	0	4
– *Slight*	0	0	0	2	0	0	0	0
– *moderate*	0	0	0	1	0	0	0	0
Disarrangement/ desquamation of mucosal epithelium								
– *Minimal*	0	0	0	1	0	0	0	0
Inflammatory cell infiltration in mucosa								
– *Minimal*	0	0	0	2	0	0	0	0
– *Slight*	0	0	0	1	0	0	0	0
Extension of proliferating zone in mucosa								
– *Minimal*	0	0	0	1	0	0	0	0
ILEUM								
Macrophage/ multinucleated giant cell in mucosa								
– *Minimal*	0	0	4	0(2)	0	0	7	0(2)
– *Slight*	0	0	0	1	0	0	0	1
– *moderate*	0	0	0	8	0	0	0	9
Disarrangement/ desquamation of mucosal epithelium								
– *Minimal*	0	0	0	8	0	0	0	7
Inflammatory cell infiltration in mucosa								
– *Minimal*	0	0	0	2	0	0	0	5
– *Slight*	0	0	0	7	0	0	0	5
Hemorrhage in mucosa								
– *Minimal*	0	0	0	2	0	0	0	3
Extension of proliferating zone in mucosa								
– *Minimal*	0	0	0	8	0	0	0	7
Decreased lymphocyte in Peyer's patch								
– *Minimal*	0	0	0	9	0	0	0	6
– *Slight*	0	0	0	0	0	0	0	4

	No. of animals affected							
Sex	**Males**				**Females**			
Dose (mg/kg/day)	**0**	**3**	**9**	**27**	**0**	**3**	**9**	**27**
LIVER								
Single cell necrosis of hepatocyte								
– *Minimal*	0	0	0	3	0	6	1	6
– *Slight*	0	0	0	0	0	0	1	4
Focal necrosis of hepatocyte								
– *Minimal*	0	0	0	2	0	6	1	4
– *Slight*	0	0	0	0	0	0	1	3
– *moderate*	0	0	0	1	0	0	0	1
Swelling/yellow brown pigmentation of sinusoidal cell								
– *Minimal*	0	0	0	7	0	2	2	1
– *Slight*	0	0	0	0	0	0	0	9
Bile duct proliferation								
Minimal	0	0	0	0	0	0	0	4
Degeneration/necrosis of bile duct epithelium								
– *Minimal*	0	0	0	0	0	0	0	3
Vacuolation of bile ductal epithelium								
– *Minimal*	0	0	0	9	0	0	0	10
Microgranuloma								
– *Minimal*	0	0	0	(2)	0	0	0	(2)
KIDNEYS								
Yellow brown pigmentation in proximal tubule								
– *Minimal*	0	0	0	5(1)	0	0	0	7(3)
– *Slight*	0	0	0	0	0	0	0	2
Pyelonephritis								
– *Slight*	0	0	0	0	0	0	0	1
PROSTATE GLAND								
Glandular atrophy								
– *Minimal*	0	0	0	2	0	0	0	0
– *Slight*	0	0	0	1	0	0	0	0
SEMINAL VESICLES								
Glandular atrophy								
– *Slight*	0	0	0	1	0	0	0	0
PITUITARY GLAND								
Atrophy of anterior cell								
– *Minimal*	0	0	0	1	0	0	0	3

	No. of animals affected							
Sex	**Males**				**Females**			
Dose (mg/kg/day)	**0**	**3**	**9**	**27**	**0**	**3**	**9**	**27**
ADRENAL GLANDS								
Increased large lipoid droplet in fascicular cell								
– *Minimal*	0	1	3	3(1)	0	0	8	7
– *Slight*	0	0	1	2	0	0	0	0
Decreased lipoid droplet in fascicular cell								
– *Minimal*	0	0	0	3	0	0	0	1
– *Slight*	0	0	0	0	0	0	0	5
Yellow brown pigmentation in sinusoidal cell								
– *Minimal*	0	0	0	0	0	0	0	5
SPLEEN								
Increased extramedullary hematopoiesis								
– *Minimal*	0	3(3)	8(2)	5(3)	0	0	1	3(3)
– *Slight*	0	0	0	3(2)	0	0	0	7
Increased mature megakaryocyte								
– *Minimal*	0	0	0	2	0	0	0	8
Decreased lymphocyte in white pulp								
– *Minimal*	0	0	0	5	0	0	0	3
– *Slight*	0	0	0	4	0	0	0	7
BONE MARROW-STERNUM Increased neutrophil								
– *Minimal*	0	0	0	1	0	0	0	0
BONE MARROW-FEMUR *Increased megakaryocyte*								
– *Minimal*	0	0	0	0	0	0	0	1
Increased neutrophil								
– *Minimal*	0	0	0	3	0	0	0	5
– *Slight*	0	0	0	5	0	0	0	3
THYMUS								
Increased tingible body macrophage								
– *Minimal*	0	0	5	8	0	0	6	5

	No. of animals affected							
Sex	**Males**				**Females**			
Dose (mg/kg/day)	**0**	**3**	**9**	**27**	**0**	**3**	**9**	**27**
AXILLARY LYMPH NODES								
Macrophage/ erythrophagy in sinus								
– *Minimal*	0	0	1	6	0	0	7	5
Decreased lymphocyte								
– *Minimal*	0	0	3	9	0	0	0	10
MESENTERIC LYMPH NODE								
Macrophage/erythrophagy in sinus								
– *Minimal*	5(4)	4(1)	0(1)	0	6(5)	5(3)	0(1)	0
– *Slight*	0	0	5(1)	0(5)	0	0	7(3)	0(5)
– *Moderate*	0	0	5	9	0	0	3	10
Blood absorption								
– *Minimal*	1	1	4(3)	0	2	0	2(3)	0
– *Slight*	0	0	2	0(3)	0	0	1	1(3)
– *Moderate*	0	0	0	4(2)	0	0	0	2(2)
– *Marked*	0	0	0	5	0	0	0	7
Hemosiderin deposition								
– *Minimal*	1	0	1	0	2	1	0	1
– *Slight*	0	0	1	8	0	0	0	6
– *Moderate*	0	0	0	1	0	0	0	3
Multinucleated giant cell *in sinus*								
– *Minimal*	0	0	0	1	0	0	0	2
– *Slight*	0	0	0	2	0	0	0	4
– *Moderate*	0	0	0	6	0	0	0	4
Extramedullary hematopoiesis								
– *Slight*	0	0	0	0	0	0	0	3
SKIN/SUBCUTIS								
Thickening of epidermis								
– *Minimal*	0	0	0	2	0	0	0	6
– *Slight*	0	0	0	0	0	0	0	1
BONE, STERNUM								
Decreased trabecular bone								
– *Minimal*	0	0	0	4	0	0	0	6
BONE, FEMUR								
Increased activated osteoclast in metaphysis								
– *Minimal*	0	0	0	8	0	0	0	9
Decreased trabecular bone in metaphysis	0 0	0 0	0 0	1 6	0 0	0 0	0 0	2 3

	No. of animals affected							
Sex	**Males**				**Females**			
Dose (mg/kg/day)	**0**	**3**	**9**	**27**	**0**	**3**	**9**	**27**
– *Minimal*								
– *Slight*								
TEETH								
Remnant of eosinophilic material in enamel space								
– *Minimal*	0	0	0	7	0	0	0	4
– *Slight*	0	0	0	0	0	0	0	6
Degeneration/necrosis of ameloblast in mid region								
– *Minimal*	0	0	0	4	0	0	0	8
– *Slight*	0	0	0	0	0	0	0	2
Dilatation of capillary at papillary layer in mid region								
– *Minimal*	0	0	0	4	0	0	0	8
Disarrangement of ameloblast *in* mid region								
– *Minimal*	0	0	0	1	0	0	0	1
– *Slight*	0	0	0	0	0	0	0	2
Dilatation of capillary at odontoblast layer in mid region								
– *Minimal*	0	0	0	0	0	0	0	3
Disarrangement of odontoblast in mid region								
– *Minimal*	0	0	0	0	0	0	0	4
ADIPOSE TISSUE								
Atrophy of adipose tissue in subcutis								
– *Minimal*	0	0	0	2	0	0	0	2
– *Slight*	0	0	0	0	0	0	0	1
MANDIBULAR GLAND								
Decreased granule in granulated duct								
– *Minimal*	0	0	0	2	0	0	0	2
MAMMARY GLANDS								
Atrophy								
– *Minimal*	0	0	0	0	0	0	0	2

** A read-down was conducted when test article-related histopathology were observed at 27 mg/kg/day.*

Toxicokinetics

- Plasma exposure ($AUC_{0\text{-}24h}$ and C_{max}) increased in a dose-proportional manner between 3 and 9 mg/kg/day, and in a less than dose proportional manner between 9 and 27 mg/kg/day.
- Accumulation was reported after repeat dosing at doses ≤ 9 mg/kg/day.

- Higher systemic exposure was reported in females relative to males.

Table 58: Rat – Toxicokinetics

Dose level (mg/kg/day)	Sex	T_{max} (h)		C_{max} (ng/mL)		AUC_{0-24h} (ng·h/mL)	
		Day 1	Day 91	Day 1	Day 91	Day 1	Day 91
3	Male	3.3	4.0	208	370	3010	5100
9		6.7	5.3	649	1250	9770	18600
27		9.3	9.3	1780	1840	30000	35300
3	Female	4.0	2.7	238	524	4000	8450
9		5.3	3.3	872	1520	15100	25800
27		6.7	8.0	1960	2170	35800	41100

Each value represents the mean of three animals.

(Excerpted from Applicant's submission)

Study title: 13-Week Repeated Dose Oral Gavage Toxicity Study of CH5424802-002* in Cynomolgus Monkeys Followed by an 8-Week Recovery Period	
Study no.:	TOX10-0027
Conducting laboratory:	(b) (4)
Date of study initiation:	14 May, 2010
GLP compliance/QA statement:	Yes (Ministry of Health, Labor and Welfare, Japan) *Reviewer note* Pathology report was not signed by the primary pathologist.
Drug, lot #:	CH5424802-002; G0E01 and G0G01

Key Study Findings

- The NOAEL was 1.3 mg/kg/day.
- Decreases in heart rate occurred in males (-12%) and females (-7%) at 12 mg/kg/day on Day 88.
- Test article-related hematology, clinical chemistry and urinalysis parameters were reported at doses ≥ 4 mg/kg/day; increases in urine volume and urine Na were evident at the end of the recovery period.
- Major histopathological target organs at doses ≥ 4mg/kg/day were the liver, adrenals, cecum, colon and rectum, lung and stomach. Other findings included changes in the epididymis, pancreas, and parathyroid. At the end of the recovery

period, microscopic findings in all organs showed recovery or a trend towards recovery.

Methods:

Study Design

Group (mg/kg/day)	Dose volume	Concentration	Necropsy Day 92 (Number of animals/sex)	Recovery Day 148 (Number of animals/sex)
Control	5 mL/kg	0	3	2
1.3	5 mL/kg	0.26 mg/ml	3	0
4.0	5 mL/kg	0.8 mg/ml	3	2
12	5 mL/kg	2.4 mg/ml	3	2

Route/Frequency of dosing:	Oral gavage/Daily Treatment period: Day 1 to 92 followed by 57 days of recovery (Day 149)
Formulation/Vehicle:	Vehicle, 0.5% methylcellulose (HPMC)/0.001 mol/L hydrochloric acid (HCL) solution
Sex/Strain/Species:	Male and female Cynomolgus monkeys
Age/Weight at start of dosing:	3 years old; 2.8 to 5 kg for males and 2.3 to 3 kg for females
Toxicokinetic evaluation:	Blood was collected from all groups on Day 1 (1, 2, 4, 8, 12 and 24 hours postdose), prior to Days 28 and 56 of dosing and on Day 91 (prior to dosing, 1, 2, 4, 8, 12 and 24 hours postdose)
Diet:	Approximately 100 g/day/animal
Endpoints evaluated:	Mortality, clinical observations, body weight, weight gain and food consumption. ECG: Predose (Day -6), Day 88 (4 hours post dose), recovery (Day 145) Ophthalmic examinations: Predose (Day -9), Day 85 and recovery (Day 145). Clinical pathology: were collected based on the following schedule: • Urinalysis: Predose, Days 85 to 86 and 142 to 143 (recovery). • Hematology, coagulation, clinical chemistry and bone marrow test: Predose, Days 28, 91 and Day 149 (end of recovery).
Termination, necropsy and	Day 92 (end of treatment) and Day 149 (end of recovery): Organ weights, macroscopic and microscopic findings.

histopathology:	Microscopic examinations were conducted on all animals at all groups.

Results:

Stability and Dosing Analysis

- Samples obtained for concentration verification analysis were within the acceptable limits of ±10% error for all groups (97.9% to 102.0%).
- Suspensions at concentration levels of 0.3 to 2.7 mg/mL were homogeneous.
- The suspensions at concentration levels of 0.05 to 10 mg/mL were stable for up to 6 hours at room temperature and stable for up to 10 days in a refrigerator (4.1 to 6.2^{o}C) shielded from light.

Mortalities

No test article-related mortalities were reported. One male at 1.3 mg/kg/day died on Day 81. The Applicant attributed the death to a breathing difficulty followed by acute gastric dilatation.

Table 59: Monkey – Mortalities

Dose (mg/kg/day)	Day of Death	Sex (animal #)	Prior to Death and Necropsy Findings
1.3	81	M (10203)	*Clinical observations and body weight:* No findings
			Macroscopic findings: Dilatation of the stomach, distention of gas in the abdominal cavity and increased pleural/abdominal fluids. *Microscopic findings: Spleen (congestion), trachea (saburra), lung (edema, alveolar wall and saburra bronchus), adrenal (cortex hypotrophy, testis (* seminiferous tubule, focal dilatation*)*

Clinical Observations

There were no test article-related clinical observations reported.

Body Weight and Food Consumption

There were no test article-related effects reported on body weights and food consumption.

Ophthalmology

There were no test article-related ophthalmic findings reported.

Electrocardiography

A decrease in heart rate was reported in males (-12%) and females (-7%) at 12 mg/kg/day on Day 88, relative to the control and pre-dose values.

Urinalysis
Test article-related increases in urine volume and urine Na relative to the control and pre-dose values were reported at doses ≥ 4 mg/kg/day during the treatment and the recovery periods.

Table 60: Monkey – Predominant Urinalysis Changes (% change from control)

Parameter	Day	1.3 mg/kg/day		4 mg/kg/day		12 mg/kg/day	
		M	F	M	F	M	F
Urine volume (mL)	86 (recovery 143)	--	--	21 (27)	139	23# (39)	145 (92)
Urine Na (mmol/15h)		--	--	--	41	15 (21)	84 (21)

-- = no biologically meaningful findings were reported # = animal #10401 was excluded from mean
( ) = recovery

Hematology
Test article-related hematology changes were reported at doses ≥ 4 mg/kg/day.

Table 61: Monkey – Predominant Hematology Changes on Day 92 (% change from control)

Parameters	1.3 mg/kg/day		4 mg/kg/day		12 mg/kg/day	
Sex	M	F	M	F	M	F
Red blood cells (RBC)	--	--	--	-8	-13**	-6
Hemoglobin (HGB)	--	--	--	--	-8	--
Reticulocytes (RETIC)	--	--	23	16	28	68
RETIC Abs.	--	--	18	--	13	58
Hematocrit (HCT)	--	--	--	-8*	-10	-7
Platelets (PLT)	--	--	--	--	75* (78)	19*
Neutrophils (NEUT)	--	--	30	16	12	9
Fragmented red cell (Poikilocytosis)	--	--	--	--	↑	↑

( ) = recovery on Day 149; -- = no biologically meaningful findings were reported
** = p<0.05, ** p<0.01 significantly different from control*

Clinical Chemistry
Test article-related clinical chemistry changes were reported at doses ≥ 4 mg/kg/day.

Table 62: Monkey – Predominant Clinical Chemistry Changes on Day 92 (% change from control)

Parameters	1.3 mg/kg/day		4 mg/kg/day		12 mg/kg/day	
Sex	M	F	M	F	M	F
ALP	--	--	--	--	22 (41)	38
ALT	--	--	--	18	--	36
Gamma-glutamyl transferase	--	--	40*	--	20	13
Creatinine (CRE)	--	--	--	12	16	14
Glucose (GLUC)	--	--	--	-19	-23** (-15*)	-10
Total Cholesterol (TC)	--	--	12	--	33	--
Triglyceride	--	--	52	52	29	86
Alkaline Phosphatase Fraction 2 (%) (ALP2)	--	--	--	--	51	50
Alkaline Phosphatase Fraction 5 (%) (ALP5)	--	--	42*	--	27	--
Alkaline Phosphatase Fraction 3 activity (ALP3)	--	--	--	--	18	21
Globulin α2	--	--	--	--	--	11* (13)

*-- = no biologically meaningful findings were reported; * = p<0.05, ** p<0.01 significantly different from control; ( ) = recovery*

Organ Weights

The increases in liver and lung weights were correlated with macro and/or microscopic findings.

Table 63: Monkey – Relative Organ Weight Changes (% change from control)

Organ	1.3 mg/kg/day		4 mg/kg/day		12 mg/kg/day	
	M	F	M	F	M	F
Brain	--	--	--	--	-15	--
Thyroid	--	--	--	--	44 (22)	--
Thymus	--	--	--	--	--	-29
Submandibular glands	--	--	23	--	63	--
Lung	--	--	45	--	27	--
Heart	--	--	13	--	12	--
Kidneys	--	--	14	--	20	--
Prostate	--	--	-28	--	-17	--
Liver	--	--	27*	--	36* (30)	17

Organ	1.3 mg/kg/day		4 mg/kg/day		12 mg/kg/day	
	M	F	M	F	M	F
Brain	--	--	--	--	-15	--
Ovary	--	--	--	-11 (-38)	--	-24 (-15)

*-- = no biologically meaningful findings were reported; * = p<0.05, significantly different from control*
( ) = recovery

Macroscopic Findings
Test article-related macroscopic findings were report at 12 mg/kg/day. The effects were correlated with microscopic findings.

Table 64: Monkey – Macroscopic Findings (incidence)

Dosage Group (mg/kg/day):	0		1.3		4		12	
sex	M	F	M	F	M	F	M	F
Lung-brownish	--	--	--	--	--	--	1	--
Lung-reddish patch	--	--	--	--	--	--	1	--
Cecum- dilatation	--	--	--	--	--	--	2	1
Colon-dilatation	--	--	--	--	--	--	2	1
Adrenal-dark discoloration	--	--	--	--	--	--	3	1

-- = no biologically meaningful findings were reported

Histopathology

Adequate Battery – Yes
Signed Pathology Report – No (in compliance with the Japanese GLPs)
Peer Reviewed- No

The predominant test article-related microscopic findings were reported at doses ≥4 mg/kg/day in the liver, adrenals, stomach, cecum, colon, rectum and lung. Other findings included epididymis, pancreas and parathyroid.

Terminal necropsy (Day 92):
- At doses ≥4 mg/kg/day, cecum, colon, rectum, liver, epididymis and adrenal.
- At 12 mg/kg/day, stomach, lung, pancreas and parathyroid.

At the end of the recovery period (Day 149):
- At doses≥4 mg/kg/day, liver and adrenal.
- At 12 mg/kg/day, stomach.

Table 65: Monkey – Histopathology Findings

	No. of animals affected							
Gender	**Males**				**Females**			
Dose (mg/kg)	**0**	**1.3**	**4**	**12**	**0**	**1.3**	**4**	**12**
No. Examined	3 (2)	3	3(2)	3(2)	3 (2)	3	3(2)	3(2)
Treatment-related Findings:								
STOMACH								
Extension, proliferative zone, mucosa – *Minimal*	0	0	0	3	0	0	0	3
Erosion – *Minimal*	0	0	0	(1)	0	0	0	0
CECUM								
Extension, proliferative zone, mucosa – *Minimal*	0	0	1	3	0	0	1	3
COLON								
Extension, proliferative zone, mucosa – *Minimal*	0	0	1	3	0	0	1	2
RECTUM								
Extension, proliferative zone, mucosa – *Minimal*	0	0	0	2	0	0	1	1
LUNG								
Accumulation, pigmented macrophage, hemosiderin, perivascular – *Mild*	0	0	0	1	0	0	0	0
Hemorrhage, alveolus, multifocal – *Minimal*	0	0	0	1	0	0	0	0
LIVER								
Cell infiltration, inflammatory, Glisson's sheath – *Minimal*	0	0	0	0	0	0	0	2
Enlargement, hepatocyte – *Minimal*	0	0	0	1	0	0	0	2
Proliferation, bile duct – *Minimal*	0	0	0	1	0	0	2	3
Cell infiltration, lymphocyte, focal – *Minimal*	0	0	(1)	(1)	0	0	(1)	(1)
PANCREAS								
Fibrosis, capsule, focal – *Minimal*	0	0	0	1	0	0	0	0

	No. of animals affected							
Gender	**Males**				**Females**			
Dose (mg/kg)	**0**	**1.3**	**4**	**12**	**0**	**1.3**	**4**	**12**
TESTIS								
Fibrosis, interstitium, unilateral								
– *Minimal*	0	0	0	1	0	0	0	0
Fibrosis, focal								
– *Mild*	(2)	0	0	0	0	0	0	0
Dilatation, seminiferous tubule, focal								
– *Minimal*	0	1	2(1)	(1)	0	0	0	0
– *Mild*	2	0	0	0	0	0	0	0
EPIDIDYMIS								
Cell infiltration, lymphocyte, interstitium, focal, unilateral								
– *Minimal*	0	0	1	1	0	0	0	0
PARATHYROID								
Cyst								
– *Minimal*	0	0	0	2	0	0	0	1
ADRENAL GLANDS								
Decrease, lipid droplet, cortical cell, fascicular zone								
– *Minimal*	0	0	3(1)	0(1)	0	0	0	2(1)
– *Mild*	0	0	0	3	0	0	0	0
Hypertrophy, cortical cell, reticular zone								
– *Minimal*	0	0	0	1	0	0	(1)	0
Accessory adrenocortical tissue								
– *Minimal*	0	0	0	(1)	0	0	0	(1)

( ) = recovery

Toxicokinetics

- Plasma exposure ($AUC_{0\text{-}24}$ and C_{max}) increased in a less than dose-proportional manner between 1.3 and 12 mg/kg/day.
- No accumulation was reported after repeat dosing.
- No gender differences in systemic exposure were reported.

Table 66: Monkey – Toxicokinetics

Dosage level [1] (mg/kg/day)	Sex	T_{max} (h)		C_{max} (ng/mL)		AUC_{0-24h} (ng·h/mL)	
		1st [2]	91st [2]	1st [2]	91st [2]	1st [2]	91st [2]
1.3	Male	5.3	2.0	59.8	83.4	792	894
4		6.8	2.4	170	243	2700	3610
12		8.0	4.4	394	461	6640	7060
1.3	Female	3.3	3.3	87.5	94.3	942	1030
4		4.0	3.2	175	189	2520	2810
12		4.0	4.4	439	463	6410	6920

1) Dosage level as CH5424802-000

2) Dosing time

(Excerpted from Applicant's submission)

7 Genetic Toxicology

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

Study Title: Bacterial Reverse Mutation Test of CH5424802	
Study no.:	1053945
Study report location:	4.2.3.3.1
Conducting laboratory:	(b) (4)
Date of study start:	10/2/2009
Compliance:	Statement included and signed
QA Statement:	Statement included and signed
Drug/lot/purity	CH5424802; 004, 99.8%
Key study findings:	CH5424802 was not mutagenic in bacteria
Methods	
Strains:	*Salmonella typhimurium (*TA100, TA1535, TA98, TA1537) *Escherichia coli (*WP2*uvrA)*
Concentrations in definitive study:	31.3, 62.5, 125, 250, 500, 1000
Basis of concentration selection:	The preliminary test I by the plate method and the preliminary test II by the pre-incubation method were conducted at concentrations of 156, 313, 625, 1250, 2500, and 5000 µg/plate CH5424802. For both the preliminary test I and II, microbial toxicity was not observed in any test strain regardless of the presence or

	absence of S9 mix. Precipitation was observed on the agar plates at doses of 313 µg/plate or more CH5424802 in the presence and absence of S9 mix. The number of revertant colonies in the test substance-treated groups was less than twice that in the corresponding negative (solvent) control in any test strain regardless of the presence or absence of S9 mix. The highest dose in the main test was selected as the dose at which precipitation was clearly observed.
Negative control:	DMSO (solvent)
Positive control:	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2), Sodium azide (NaN3), 2-aminoanthracene (2-AA), 9-aminoacridine hydrochloride (9-AA)
Formulation/vehicle:	DMSO
# of plates:	3 plates/dose
Incubation & sampling times:	48 hours at 37°C
Study Validity The positive controls significantly increased the colonies compared to the solvent controls.	

Results

- For both the plate and incubation methods, there were less than twice the number of revertant colonies in the CH5424802-treated groups compared to the negative control in the presence or absence of S9 mix
- Microbial toxicity was not observed in any test strain regardless of the presence or absence of S9 mix
- Precipitation was observed on the agar plates at doses of 250 µg/plate or more CH5424802 in the presence and absence of S9 mix

Table 67: Ames Results-Plate Method

With (+) or without (-) S9 mix	Dose level (μg / plate)*	Number of revertants (number of colonies / plate)									
		Base-pair change type						Frameshift type			
		TA100		TA1535		WP2*uvrA*		TA98		TA1537	
S9 mix (-)	Negative control	111 113 (112) 111 (1)		12 11 (11) 11 (1)		36 37 (36) 36 (1)		20 21 (21) 22 (1)		14 9 (11) 10 (3)	
	31.3	118 103 (112) 114 (8)		10 10 (11) 13 (2)		35 39 (37) 37 (2)		17 22 (20) 20 (3)		15 12 (13) 11 (2)	
	62.5	112 130 (123) 128 (10)		9 10 (10) 10 (1)		35 34 (34) 33 (1)		19 19 (20) 23 (2)		9 13 (12) 13 (2)	
	125	130 123 (127) 127 (4)		11 11 (11) 11 (0)		34 38 (36) 37 (2)		19 17 (18) 18 (1)		12 15 (13) 11 (2)	
	250 †	119 113 (112) 103 (8)		12 13 (12) 12 (1)		38 34 (36) 36 (2)		16 21 (19) 19 (3)		12 11 (12) 14 (2)	
	500 †	127 123 (125) 126 (2)		10 9 (9) 9 (1)		31 33 (33) 36 (3)		15 19 (19) 22 (4)		10 16 (14) 15 (3)	
	1000 †	111 107 (110) 113 (3)		8 9 (9) 10 (1)		34 35 (35) 37 (2)		17 14 (16) 16 (2)		16 10 (13) 13 (3)	
S9 mix (+)	Negative control	131 116 (119) 111 (10)		15 13 (13) 10 (3)		40 36 (40) 43 (4)		23 29 (26) 26 (3)		19 19 (20) 21 (1)	
	31.3	125 119 (125) 130 (6)		10 14 (12) 11 (2)		41 39 (40) 39 (1)		22 31 (25) 23 (5)		19 22 (19) 17 (3)	
	62.5	118 138 (132) 139 (12)		15 11 (13) 13 (2)		39 44 (41) 40 (3)		25 26 (25) 24 (1)		20 24 (22) 21 (2)	
	125	137 129 (134) 135 (4)		13 13 (12) 11 (1)		41 38 (41) 43 (3)		27 29 (27) 24 (3)		16 20 (18) 18 (2)	
	250 †	139 120 (134) 144 (13)		16 12 (13) 12 (2)		39 40 (40) 40 (1)		25 28 (26) 26 (2)		20 18 (18) 17 (2)	
	500 †	133 113 (129) 142 (15)		9 11 (10) 9 (1)		45 38 (41) 40 (4)		23 27 (25) 26 (2)		15 20 (19) 21 (3)	
	1000 †	122 136 (125) 118 (9)		9 9 (10) 13 (2)		38 43 (42) 44 (3)		20 23 (23) 25 (3)		21 20 (19) 15 (3)	
Positive control S9 mix (-)	Name	AF-2		NaN_3		AF-2		AF-2		9-AA	
	Dose (μg/plate)	0.01		0.5		0.01		0.1		80	
	Number of colonies / plate	888 860 (857) 823 (33)		601 584 (597) 606 (12)		236 219 (237) 256 (19)		1168 1207 (1182) 1171 (22)		372 301 (343) 357 (37)	
Positive control S9 mix (+)	Name	2-AA		2-AA		2-AA		2-AA		2-AA	
	Dose (μg/plate)	1		2		10		0.5		2	
	Number of colonies / plate	1877 1706 (1762) 1703 (100)		291 264 (277) 275 (14)		1315 1372 (1348) 1357 (30)		213 221 (238) 249 (15)		220 197 (215) 229 (17)	

(Mean)

(±S.D.)

(Note): †: Precipitation was observed.

*: As CH5424802-000

Negative control: Dimethyl sulfoxide

Table 68: Ames Results—Pre-Incubation Method

With (+) or without (-) S9 mix	Dose level (μg / plate)*	Number of revertants (number of colonies / plate)									
		Base-pair change type						Frameshift type			
		TA100			TA1535			WP2*uvrA*		TA98		TA1537	
S9 mix (-)	Negative control	101 96 (100) 102 (3)			7 10 (8) 6 (2)			34 22 (28) 28 (6)		22 17 (18) 16 (3)		11 14 (14) 16 (3)	
	31.3	96 100 (98) 97 (2)			13 8 (10) 10 (3)			21 27 (24) 23 (3)		17 20 (18) 18 (2)		14 12 (14) 15 (2)	
	62.5	110 99 (102) 97 (7)			10 9 (9) 7 (2)			23 21 (21) 19 (2)		16 20 (18) 17 (2)		12 15 (14) 14 (2)	
	125	100 102 (99) 96 (3)			14 7 (11) 12 (4)			22 24 (24) 25 (2)		18 16 (18) 20 (2)		15 17 (15) 14 (2)	
	250 †	99 100 (101) 105 (3)			10 9 (9) 9 (1)			23 22 (22) 22 (1)		19 19 (18) 17 (1)		16 14 (14) 12 (2)	
	500 †	92 113 (102) 101 (11)			5 6 (5) 5 (1)			27 27 (26) 25 (1)		15 14 (14) 13 (1)		13 23 (18) 17 (5)	
	1000 †	91 102 (97) 97 (6)			7 6 (6) 6 (1)			25 26 (27) 29 (2)		17 19 (17) 14 (3)		12 14 (15) 19 (4)	
S9 mix (+)	Negative control	104 122 (110) 105 (10)			10 10 (10) 9 (1)			31 35 (33) 34 (2)		28 26 (28) 30 (2)		21 19 (20) 19 (1)	
	31.3	115 110 (109) 101 (7)			11 11 (11) 10 (1)			33 35 (33) 31 (2)		25 26 (25) 25 (1)		15 19 (18) 21 (3)	
	62.5	117 106 (108) 100 (9)			9 10 (10) 10 (1)			33 31 (34) 38 (4)		26 31 (27) 24 (4)		22 21 (21) 19 (2)	
	125	109 109 (109) 109 (0)			9 9 (9) 8 (1)			32 32 (32) 32 (0)		24 23 (23) 21 (2)		18 21 (19) 18 (2)	
	250 †	104 104 (105) 107 (2)			9 11 (9) 8 (2)			32 32 (32) 32 (0)		23 30 (26) 25 (4)		23 17 (20) 20 (3)	
	500 †	106 100 (102) 101 (3)			6 8 (7) 8 (1)			34 31 (33) 33 (2)		21 23 (24) 28 (4)		19 24 (21) 21 (3)	
	1000 †	101 121 (109) 105 (11)			6 7 (7) 7 (1)			36 32 (34) 34 (2)		24 21 (21) 19 (3)		19 25 (19) 14 (6)	
Positive control S9 mix (-)	Name	AF-2			NaN_3			AF-2		AF-2		9-AA	
	Dose (pg/plate)	0.01			0.5			0.01		0.1		80	
	Number of colonies / plate	936 966 (937) 910 (28)			544 573 (566) 581 (19)			181 202 (194) 198 (11)		729 760 (754) 774 (23)		249 232 (235) 225 (12)	
Positive control S9 mix (+)	Name	2-AA			2-AA			2-AA		2-AA		2-AA	
	Dose (pg/plate)	1			2			10		0.5		2	
	Number of colonies / plate	1013 1002 (1029) 1072 (38)			190 202 (200) 208 (9)			1157 1095 (1207) 1370 (144)		256 265 (260) 258 (5)		150 180 (164) 163 (15)	

(Mean)

(±S.D.)

(Note): †: Precipitation was observed.

*: As CH5424802 000

Negative control: Dimethyl sulfoxide

(Excerpted from Applicant's submission)

Conclusions

Under the conditions tested, CH5424802 was not mutagenic in bacteria.

7.2 *In Vitro* Assays in Mammalian Cells

Study Title: Chromosomal Aberration Test of CH5424802 with CHL Cells	
Study no.:	1053946
Study report location:	4.2.3.3.1
Conducting laboratory:	(b) (4)
Date of study start:	10/15/2009
Compliance:	Statement included and signed
QA Statement:	Statement included and signed
Drug/lot/purity	CH5424802; 004, 99.8%
Key study findings:	CH5424802 caused a slight increase in the number of chromosome aberrations (polypoid) in CHL cells

Methods	
Cell line:	CHL/IU (fibroblast derived from lung of female Chinese hamster)
Concentrations in definitive study:	Cell growth inhibition test 0.195, 0.391, 0.781, 1.56, 3.13, 6.25, 12.5, 25, 50 µg/mL Chromosomal aberration test -S9 mix assay: 1, 2, 3, 4, 5, 6, 7 µg/mL +S9 mix assay: 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 µg/mL 24-hour assay: 1, 2, 3, 4, 5, 6, 7 µg/mL
Basis of concentration selection:	For the inhibition assay, the test article produced strong cytotoxicity to CHL cells at 50 µg/mL in the −S9 mix, +S9 mix, and 24-hour assays.
Negative control:	DMSO (solvent)
Positive control:	Mitomycin C(MMC); Benzo [a] pyrene (BP)
Formulation/vehicle:	DMSO
# of plates:	3 plates/dose
Incubation & sampling times:	Short term: 6-hour treatment and 18-hour recovery Long term: 24 hours

Study Validity

The study was valid based on the following criteria:

1. In the negative and positive controls and at three or more concentrations of the test article treatment group, the criterion for acceptance of the data is satisfied.
2. Incidence of structural and numerical aberrant cells in the negative control group is less than 5%.
3. Incidence of structural aberrant cells in the positive control group is 10% or more.

The results of the study were based on the following criteria:

Negative: Both structural and numerical aberrant cells are observed at less than 5% in each test article treatment group.

Inconclusive: The structural or numerical aberrant cells are observed at 5% or more, but less than 10% in the test article treatment group.

Positive: The structural or numerical aberrant cells are observed at 10% or more in the test article treatment group and dose-dependent increase is recognized.

Results

- No precipitation was observed at the beginning or the end of the treatment at any dose under any treatment condition.
- In the presence of S9 activation there was a small increase in polyploidy (up to 4% compared to negative control).

Table 69: Chromosomal Aberration Test (short term +S9)

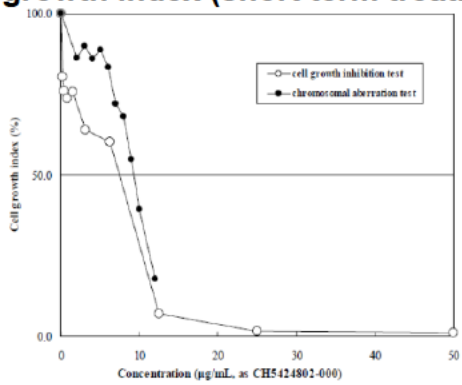
Test substance: CH5424802-002

Exposure-Recovery time (h)	S9 mix	Concentration* (μg/mL)	Number of structural aberrant cells (%)							Number of gap	Cell growth index (%)	Number of numerical aberrant cells (%)			
			Number of cells analyzed	Chromatid breaks	Chromatid exchanges	Chromosome breaks	Chromosome exchanges	Fragments	Total aberrant cells (%)			Number of cells analyzed	polyploids	endoreduplications	Total aberrant cells (%)
6 – 18	+	Negative control (DMSO)	100	0	0	0	0	0	0	0	98.9	100	0	0	0
			100	0	0	0	0	0	0	0	101.1	100	0	0	0
			200	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0	100.0	200	0 (0.0)	0 (0.0)	0 (0.0)
6 – 18	+	2	N.D.								85.9 86.7 86.3	N.D.			
6 – 18	+	3	N.D.								96.9 82.8 89.9	N.D.			
6 – 18	+	4	N.D.								87.2 84.8 86.0	N.D.			
6 – 18	+	5	N.D.								83.1 94.2 88.7	N.D.			
6 – 18	+	6	N.D.								84.9 81.6 83.3	N.D.			
6 – 18	+	7	100	0	0	0	0	0	0	0	73.6	100	1	0	1
			100	0	0	0	0	0	0	0	70.6	100	0	0	0
			200	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0	72.1	200	1 (0.5)	0 (0.0)	1 (0.5)
6 – 18	+	8	100	0	0	0	0	0	0	0	65.8	100	1	0	1
			100	0	0	0	0	0	0	0	70.1	100	1	0	1
			200	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0	68.0	200	2 (1.0)	0 (0.0)	2 (1.0)
6 – 18	+	9	100	0	0	0	0	0	0	0	50.0	100	2	0	2
			100	0	0	0	0	0	0	0	59.7	100	0	0	0
			200	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0	54.9	200	2 (1.0)	0 (0.0)	2 (1.0)
6 – 18	+	10	100	0	0	0	0	0	0	0	44.9	100	5	0	5
			100	0	0	0	0	0	0	0	33.9	100	3	0	3
			200	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0	39.4	200	8 (4.0)	0 (0.0)	8 (4.0)
6 – 18	+	12	N.D.								18.1 17.2 17.7	N.D.			
6 – 18	+	Positive control (BP 10)	100	9	35	0	0	0	39	0		100	0	0	0
			100	12	38	0	0	0	40	0		100	0	0	0
			200	21 (10.5)	73 (36.5)	0 (0.0)	0 (0.0)	0 (0.0)	79 (39.5)	0		200	0 (0.0)	0 (0.0)	0 (0.0)

*:As CH5424802-000
DMSO:Dimethyl sulfoxide BP: Benzo[a]pyrene N.D.: Not determined

(Excerpted from Applicant's submission)

Figure 23: Cell growth index (short term treatment assay +S9)



(Excerpted from Applicant's submission)

Conclusion

Under the conditions tested, CH5424802 caused a 4% increase in number of chromosome aberrations (polyploid) in CHL cells in the presence of metabolic aberration; however, because this increase was within the known variation of the assay and did not meet the criteria for a positive finding, this test was not considered positive.

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

Study Title: Micronucleus Test of CH5424802 in Rat Bone Marrow	
Study no.:	1053947
Study report location:	4.2.3.3.1
Conducting laboratory:	(b) (4)
Date of study start:	12/11/2009
Compliance:	Statement included and signed
QA Statement:	Statement included and signed
Drug/lot/purity	CH5424802; 009, 99.9%
Key study findings:	CH5424802 tested positive for a genotoxic potential to induce chromosome aberration *in vivo*
Methods	
Species:	Wister Han rats (7 weeks at dosing)
#/sex/group:	6/males/group; (n=3 for controls)
Dose:	Original micronucleus test: 500, 1000, or 2000 mg/kg/day Additional micronucleus test: 6, 20, 60, or 200 mg/kg/day
Negative control:	10 w/v% HPCD/0.01 mol/L MsOH/0.001 mol/L HCl

	solution
Positive control:	Cyclophosphamide monohydrate
Treatment:	Oral gavage twice at a 24-hr interval
Study Validity The incidences of MNIMEs in the negative control group were within the range of the historical control data in the test facility, whereas a statistically significant increase in the incidence of MNIMEs was detected in the positive control groups	

Results

- Body weight: statistically significantly lower at ≥ 500 mg/kg/day compared to the negative control group
- Clinical signs: unremarkable
- Food consumption: statistically significantly decreased at doses ≥ 20 mg/kg/day compared to the negative control group
- At doses ≥200 mg/kg PO there were increases in the incidence of micronucleus formation

Table 70: Micronucleus test results

Treatment group	Dose level [1] (mg/kg/day) Frequency Route	Animal number	MNIMEs			IME ratio among total erythrocytes	
			Number of IMEs scored	Number	Incidence (%)	Number of erythrocytes scored	IMEs/ (IMEs+MEs) (%)
Negative control (Vehicle)	0 Twice [2] p.o.	10101	2000	0	0.00	1000	69.2
		10102	2000	1	0.05	1000	69.3
		10103	2000	1	0.05	1000	61.4
		10104	2000	5	0.25	1000	66.4
		10105	2000	3	0.15	1000	61.6
		10106	2000	3	0.15	1000	59.8
	Total / Mean±SD		12000	13	0.11 ± 0.09		64.6 ± 4.2
Test article (CH5424802-002)	500 Twice [2] p.o.	10201	2000	1	0.05	1000	64.5
		10202	2000	10	0.50	1000	63.7
		10203	2000	6	0.30	1000	61.1
		10204	2000	6	0.30	1000	67.1
		10205	2000	2	0.10	1000	62.3
		10206	2000	2	0.10	1000	55.3
	Total / Mean±SD		12000	27 #	0.23 ± 0.17		62.3 ± 4.0
	1000 Twice [2] p.o.	10301	2000	8	0.40	1000	62.8
		10302	2000	6	0.30	1000	57.4
		10303	2000	4	0.20	1000	54.1
		10304	2000	6	0.30	1000	69.0
		10305	2000	6	0.30	1000	56.9
		10306	2000	3	0.15	1000	61.6
	Total / Mean±SD		12000	33 ##	0.28 ± 0.09		60.3 ± 5.3
	2000 Twice [2] p.o.	10401	2000	1	0.05	1000	60.1
		10402	2000	6	0.30	1000	60.1
		10403	2000	6	0.30	1000	58.6
		10404	2000	5	0.25	1000	70.8
		10405	2000	2	0.10	1000	58.6
		10406	2000	6	0.30	1000	55.6
	Total / Mean±SD		12000	26 #	0.22 ± 0.11		60.6 ± 5.2
Positive control (CP)	20 Twice [2] p.o.	10501	2000	79	3.95	1000	32.4
		10502	2000	112	5.60	1000	30.2
		10503	2000	158	7.90	1000	39.2
		10504	2000	146	7.30	1000	45.0
		10505	2000	129	6.45	1000	34.6
		10506	2000	142	7.10	1000	42.0
	Total / Mean±SD		12000	766 ##	6.38 ± 1.43		37.2 ± 5.8 **

IME: Immature erythrocyte, MNIMEs: Micronucleated IMEs, MEs: Mature erythrocytes
Vehicle: 10 w/v% HPCD/0.01 mol/L MsOH/0.001 mol/L HCl solution
CP: Cyclophosphamide monohydrate
[1] As CH5424802-000
[2] In a 24 hr-interval
#, ## Significantly ($p \leq 0.05, 0.01$) different from the negative control by Kastenbaum & Bowman's method
** Significantly ($p \leq 0.01$) different from the negative control by Student's t-test

(Excerpted from Applicant's submission)

Table 71: Micronucleus confirmatory test

Treatment group	Dose level [1] (mg/kg/day) Frequency Route	Animal number	MNIMEs: Number of IMEs scored	MNIMEs: Number	MNIMEs: Incidence (%)	IME ratio among total erythrocytes: Number of erythrocytes scored	IME ratio among total erythrocytes: IMEs/(IMEs+MEs) (%)
Negative control (Vehicle)	0	30101	2000	4	0.20	1000	62.0
		30102	2000	1	0.05	1000	65.2
	Twice [2] p.o.	30103	2000	3	0.15	1000	63.7
		30104	2000	2	0.10	1000	61.5
		30105	2000	2	0.10	1000	59.9
		30106	2000	2	0.10	1000	65.4
	Total / Mean±SD		12000	14	0.12 ± 0.05		63.0 ± 2.2
Test article (CH5424802-002)	6	30201	2000	4	0.20	1000	62.1
		30202	2000	2	0.10	1000	59.5
	Twice [2] p.o.	30203	2000	1	0.05	1000	64.2
		30204	2000	3	0.15	1000	62.9
		30205	2000	1	0.05	1000	61.4
		30206	2000	1	0.05	1000	63.1
	Total / Mean±SD		12000	12	0.10 ± 0.06		62.2 ± 1.6
	20	30301	2000	1	0.05	1000	56.8
		30302	2000	3	0.15	1000	64.4
	Twice [2] p.o.	30303	2000	1	0.05	1000	58.5
		30304	2000	1	0.05	1000	64.9
		30305	2000	4	0.20	1000	56.2
		30306	2000	2	0.10	1000	61.0
	Total / Mean±SD		12000	12	0.10 ± 0.06		60.3 ± 3.8
	60	30401	2000	2	0.10	1000	65.6
		30402	2000	5	0.25	1000	64.8
	Twice [2] p.o.	30403	2000	5	0.25	1000	61.8
		30404	2000	1	0.05	1000	63.5
		30405	2000	2	0.10	1000	64.1
		30406	2000	0	0.00	1000	59.6
	Total / Mean±SD		12000	15	0.13 ± 0.10		63.2 ± 2.2
	200	30501	2000	4	0.20	1000	54.9
		30502	2000	2	0.10	1000	65.2
	Twice [2] p.o.	30503	2000	2	0.10	1000	65.6
		30504	2000	4	0.20	1000	60.5
		30505	2000	5	0.25	1000	67.6
		30506	2000	6	0.30	1000	57.5
	Total / Mean±SD		12000	23	0.19 ± 0.08		61.9 ± 5.0
Positive control (CP)	20	30601	2000	99	4.95	1000	32.5
		30602	2000	60	3.00	1000	32.2
	Twice [2] p.o.	30603	2000	126	6.30	1000	34.0
		30604	2000	85	4.25	1000	40.3
		30605	2000	108	5.40	1000	27.8
		30606	2000	169	8.45	1000	26.3
	Total / Mean±SD		12000	647 **	5.39 ± 1.87		32.2 ± 5.0 **

IME: Immature erythrocyte, MNIMEs: Micronucleated IMEs, MEs: Mature erythrocytes
Vehicle: 10 w/v% HPCD/0.01 mol/L MsOH/0.001 mol/L HCl solution
CP: Cyclophosphamide monohydrate
[1] As CH5424802-000
[2] In a 24 hr-interval
Significantly ($p \leq 0.01$) different from the negative control by Kastenbaum & Bowman's method
** Significantly ($p \leq 0.01$) different from the negative control by Student's t-test

(Excerpted from Applicant's submission)

Toxicokinetics

Dose (mg/kg)	Dose ratio	C_{max}: Mean (ng/mL)	C_{max}: Dose proportionality factor*1	AUC_{0-24h}: Mean (ng·h/mL)	AUC_{0-24h}: Dose proportionality factor*1
200	1.0	1850	1.0	36700	1.0
500	2.5	3030	1.6	55800	1.5
1000	5.0	3020	1.6	59400	1.6
2000	10.0	2810	1.5	53700	1.5

*1: Mean of C_{max} or AUC_{0-24h} of on each dosing group/mean of C_{max} or AUC_{0-24h} on the low dosing group

(Excerpted from Applicant's submission)

Conclusion

Under the conditions tested, CH5424802 was positive in this micronucleus test for the potential to induce chromosome aberrations *in vivo*.

Study Title: Micronucleus test of CH5424802 in rat bone marrow with FISH analysis	
Study no.:	1056185
Study report location:	4.2.3.3.1

Conducting laboratory:	Fuji-Gotemba Research Laboratories Chugai Pharmaceutical Co., Ltd. 1-135 Komakado, Gotemba, Shizuoka 412-8513, Japan
Date of study start:	7/11/2012
Compliance:	Statement included and signed
QA Statement:	Statement included and signed
Drug/lot/purity	CH5424802; 121062; 99.9%

Key Study Findings

- CH5424802-002 was positive in the bone marrow micronucleus test in rats.
- Statistically significant increase in the FISH-Pos micronucleated immature erythrocytes (MNIMEs) in the CH5424802-002 treated groups (500 and 1000 mg/kg/day) suggests aneugenic mode of action.

Methods

Doses in definitive study:	100, 200, 500 and 1000 mg/kg/day
Frequency of dosing:	Once daily for two days
Route of administration:	Oral gavage; Positive control articles were administered by intravenous injection
Dose volume:	20 mL/kg
Formulation/Vehicle:	10% w/v hydroxypropyl-P-cyclodextrin (HPCD)/0.01 mol/L methanesulfonic acid/0.001 mol/L HCl solution
Species/Strain:	RccHan:WIST rats
Number/Sex/Group:	6 male rats/group
Satellite groups:	3 male rats/group for TK analysis
Basis of dose selection:	From a previous study (Study No. TOX09-0J 55)[1]
Negative control:	Vehicle: Sodium citrate dihydrate in water for injection
Positive control:	Clastogen: mitomycin C (MMC) at 2 mg/kg/day Aneugen: vinblastine sulfate (VBS) at 0.5 mg/kg/day

[1] Statistically significant increases in the number of micronucleated immature erythrocytes (MNIMEs) were observed at 500, 1000 and 2000 mg/kg/day and no statistically significant increase in the number of MNIMEs at 6, 20, 60 and 200 mg/kg/day. The dose selection for this study bracketed positive and negative MN dose levels.

Preparation of Bone Marrow Specimens

- All animals were sacrificed 24 hours after the second dosing. A 50 µl of bone marrow suspension from each animal was used to prepare slides for acridine orange staining for standard MN analysis. Rest of the bone marrow cell suspensions were used to prepare fluorescence in situ hybridization (FISH) specimens.

- FISH analysis is performed to distinguish MNIMEs containing centromeres (whole chromosome or aneugenicity) from MNIMEs containing chromosomal fragments (clastogenicity). The centromeres in MN were detected by hybridizing with pan-centromeric DNA probe. FISH specimens were prepared from vehicle, 500, 1000 mg/kg/day, MMC and VBS.
- Erythrocytes were separated by cellulose column[1] and MNIMEs were sorted using fluorescent dye-based method with flow cytometer[2]. Purified MNIMEs were cytospun on to the slides. The DNA on the slides was denatured, followed by hybridization with pan-centromeric DNA probe[3].

Analysis:

- MN analysis: At least 1000 erythrocytes were counted to determine the ratio of IMEs per animal to assess toxicity in the target tissue. Two thousand IMEs per animal were examined for the presence of micronuclei.
- FISH analysis: Fluorescent signals of centromeres on micronuclei out of 100 MNlMEs (where possible) for each animal were observed microscopically to determine the ratio of FISH-Pos MNIMEs and FISH-Neg MNIMEs.

Criteria for Positive Response:

- Test article was considered positive in the micronucleus test, if a statistically significant (Mann-Whitney's U test) increase with dose response (Cochran-Armitage trend test) was observed in the rate of MNIMEs in any treatment group compared to vehicle control group.
- Number of FISH-Pos MNIMEs increased with statistical significance (Fisher exact test) in the test article-treated group compared to the vehicle control group, the test article was considered to induce micronuclei by an aneugenic mode of action.

Study Validity

- The positive control (MMC) induced a significant increase in the frequency of micronucleated PCEs for micronucleus test.
- A statistically significant increase in the number of FISH-Pos MNIMEs was observed in the VBS- but not in the MMC-treated group.

Results

- A statistically significant decrease in the ratio of IMEs to total erythrocytes was observed at 1000 mg/kg/day compared with the vehicle control group with signs of target tissue exposure.

[1] Romagna F. The automated bone marrow micronucleus test. Mutat Res. 1989; 213: 91-104.
[2] Harada A. Fluorescent dye-based simple staining for in vivo micronucleus test with flow cytometer. Mutat Res. 2013; 751: 85-90.
[3] Motoyama S. Establishment of FISH analysis methods for micronucleus test in rats using clastogens and aneugens including CH5424802-002 (Study No. TOX12-0010). Chugai report, 2013.

- Statistically significant increase in the incidence of MNIMEs with dose response was observed at 500 (0.25%) and 1000 mg/kg/day (0.42%) compared with the vehicle control group (0.15%).
- Statistically significant increases in the number of FISH-Pos MNIMEs were observed at 500 (40.7%) and 1000 mg/kg/day (40.9%) in the test article-treated groups compared with the vehicle control group. (15.3%).
- The incidence of FISH-Pos MNIMEs in the MMC-treated group was 11.7% which was comparable to the concurrent vehicle control 15.3%.
  In the VBS group, a statistically significant increase (75.2%) in the incidence of FISH-Pos MNIMEs was observed compared to vehicle control group

Table 72: Micronucleus Test in Rat Bone Marrow

Micronucleus test of CH5424802-002 in rat bone marrow

Test article	Dose (mg/kg/day)	Duration of administration (day)	Administration route	Number of animals	IE/(IE+ME) [1] Mean ± SD (%)	MNIME/IE [2] Mean ± SD (%)		
Vehicle [3]	—	2	p.o.	6	71.6 ± 4.1	0.15 ± 0.06		
CH5424802-002 [4]	100	2	p.o.	6	68.7 ± 6.2	0.18 ± 0.06		##
	200	2		6	72.5 ± 6.6	0.21 ± 0.14		
	500	2		6	66.8 ± 6.3	0.25 ± 0.06	$	
	1000	2		6	66.4 ± 3.7 *	0.42 ± 0.17	$$	
MMC	2	2	i.v.	6	41.6 ± 7.8 **	5.68 ± 1.57	$$	
VBS	0.5	2	i.v.	6	17.5 ± 4.9 **	3.18 ± 1.10	$$	

Each value represents the mean ± S.D.

Abbreviations: IE, immature erythrocytes; ME, mature erythrocytes; MNIME, micronucleated immature erythrocytes; i.v., intravenous administration ; p.o., oral administration; MMC, mitomycin C; VBS, vinblastine sulfate salt.

1) One thousand or more erythrocytes were examined.

2) Two thousand IEs were examined.

3) 10% w/v hydroxypropyl-β-cyclodextrin (HPCD) / 0.01 mol/L methanesulfonic acid / 0.001 mol/L HCl solution used as vehicle control.

4) As CH5424802-000.

* P<0.05; ** P<0.01, Student's t-test (IE(%))

$ P<0.05; $$ P<0.01, Mann-Whitney U-test (MNIME(%))

: P<0.01, Cochran-Armitage trend test

Table 73: Anuegen detection analysis of alectinib using FISH method

Test article	Dose (mg/kg/day)	Number of animals	MN cells [1)a)] (%)	FISH analysis on MN cells Total	FISH-Pos	FISH-Neg	FISH analysis on MN cells FISH-Pos [b)] MNIME (%)	FISH-Neg MNIME (%)	Frequency / 1000 cells [2)] FISH-Pos MNIMEs	FISH-Neg MNIMEs
Vehicle [3]	—	6	0.15	502	(77	425)	15.3	/ 84.7	0.23	1.27
CH5424802-002 [4]	500	6	0.25 *	600	(244 #	356)	40.7	/ 59.3	1.02	1.48
	1000	6	0.42 **	552	(226 #	326)	40.9	/ 59.1	1.72	2.48
MMC	2	6	5.68 **	600	(70	530)	11.7	/ 88.3	6.63	50.17
VBS	0.5	6	3.18 **	600	(451 #	149)	75.2	/ 24.8	23.90	7.90

Abbreviations: MN, micronuclei; MNIME, micronucleated immature erythrocytes; MMC, mitomycin C; VBS, vinblastine sulfate salt.

1) Two thousand immature erythrocytes were examined.

2) Calculated from a) and b) at each treatment; Frequency of FISH-Pos MNIMEs=a×10×b/100, Frequency of FISH-Neg MNIMEs=a×10-Frequency of FISH-Pos MNIMEs.

3) 10% w/v hydroxypropyl-β-cyclodextrin (HPCD) / 0.01 mol/L methanesulfonic acid / 0.001 mol/L HCl solution used as vehicle control.

4) As CH5424802-000.

* P<0.05; ** P<0.01, Mann-Whitney U-test (MN cells(%))

: P<0.01, Fisher's exact test

(Tables excerpted from Applicant's submission)

Conclusion

Under the conditions tested, CH5424802 was positive in this test for micronucleus induction, which may be due to an aneugenic mechanism.

8 Carcinogenicity

No carcinogenicity evaluation was conducted or required to support the approval of a drug intended for the treatment of patients with advanced cancer.

9 Reproductive and Developmental Toxicology

9.1 Embryo-fetal developmental (EFD) toxicity studies

Reviewer comment: The studies that follow were designed as preliminary studies (GLP) and the numbers of litters tested per group were small. As such, statistical power of these studies is low. Per ICH S5, a minimum of 16 litters should be evaluated (more for rare events). Additionally, historical control data for rat and rabbits was not provided by the Applicant. These significant study limitations will impact interpretation of data.

Study title: A Preliminary Study for Effects of CH5424802*-002 on Embryo-Fetal Development by Oral Gavage Dose in Rats	
Study no.:	TOX12-0019
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 8, 2012
GLP compliance:	Yes (Ministry of Health, Labor and Welfare, Japan)
QA statement:	Yes
Drug, lot #, and % purity:	CH5424802-002, G2D01

Key Study Findings

- The maternal and fetal NOAEL was 3 mg/kg/day (0.9 –fold the estimated human $AUC_{ss,24}$ at the recommended human dose of 600 mg BID.
- Maternal toxicity was evident at ≥9 mg/kg/day (≥2.7-fold the estimated human $AUC_{ss,24}$ at the recommended human dose), as was evident by decreased body weights and food consumption.

- At 9 mg/kg/day, there were decreased in fetal weights and increased visceral anomalies, which included the ureter (dilated ureter), thymus (thymic cord) and cardiovascular tissues (small ventricle and thin ventricle wall).

- At 9 mg/kg/day, skeletal anomalies included a decrease in the number of sacral and caudal vertebrae.

- At 27 mg/kg/day (4.5-fold the estimated human $AUC_{ss,24}$ at the recommended dose), total litter loss was reported in all dams (increased in embryofetal lethality).

Objective:
The objective of this preliminary study was to assess the effects on dams and embryo-fetal development when CH5424802-002 was administered orally from implantation to closure of the hard palate in pregnant rats. In addition, the systemic exposure level in pregnant rats was also assessed.

Methods

Species/Strain:	RccHan:WIST rats (SPF).
Supplier:	(b) (4)
Weight:	233.4-274.3 g
Mating:	Male and Female Rats (males used only for breeding). Females with evidence of a sperm positive vaginal smear were randomized and assigned to study groups. The day on which copulation was confirmed visually it was designated as Day 0 of gestation (GD 0).
Study design:	See table below
Vehicle:	0.5 w/v% hydroxypropyl methylcellulose (HPMC)/0.001 mol/L hydrochloric acid (HCl) solution (0.5 w/v% HPMC/0.001 mol/L HCl)
Dosing:	Once daily from Gestation Day (GD) 7 to 17 (Day 0 = Plug date)
Route:	Oral gavage

Group [a]	Dose level [a] (mg/kg/day)	Dosing suspension concentration [a] (mg/mL)	Dosing volume (mL/kg)	Number of dams	Animal Nos./ Cage Nos.
Main group					
0 mg/kg [b]	0	0	10	6	5101–5106
3 mg/kg	3	0.3	10	6	5201–5206
9 mg/kg	9	0.9	10	6	5301–5306
27 mg/kg	27	2.7	10	6	5401–5406
Satellite group					
0 mg/kg TK [b]	0	0	10	2	5501–5502
3 mg/kg TK	3	0.3	10	2	5601–5602
9 mg/kg TK	9	0.9	10	2	5701–5702
27 mg/kg TK	27	2.7	10	2	5801–5802

a) Expressed as CH5424802-000 (free base of CH5424802).
b) Groups at 0 mg/kg and 0 mg/kg TK were treated with vehicle.

(*Excerpted from Applicant's report)*

Analysis:	Concentration, homogeneity and stability were evaluated on study. Stability was tested under study # TOX10-0027
Toxicokinetics (TK):	Blood was collected on GD 7 (1, 2, 4, 8 and 24 hours post dosing) and GD 17 (Predose, 1, 2, 4, 8 and 24 hours post dosing)
In-life end points:	Mortalities and clinical observations. Daily pre and post-dose observations. Body weight – GD 0, 3, 7, 9, 11, 13, 15, 17, 18 and 20. Food consumption – GD 9, 11, 13, 15, 17, 18 and 20.
Cesarean section (C-section):	GD 20
Litter endpoints:	Uterus examination, number of corpora lutea, dead embryos, number of live fetuses, number of dead fetuses, resorptions and placental weight.
Fetal endpoints (control, 3 and 9 mg/kg/day):	Sex, fetal weights External, visceral and skeletal examination anomaly (%): (Number of fetuses in which an anomaly was observed/Number of fetuses on which examination was performed) x 100

Results:

Analysis of Test Formulations

Concentration and homogeneity of the test article in the formulations were within the acceptable range (±10%). The formulation was stable for up to 10 days after preparation in a refrigerator, followed by 6 hours at room temperature.

Maternal data

Toxicokinetics

- On GD 7 and 17, plasma exposure (C_{max} and AUC) increased in a dose-proportional manner between doses of 3 and 9 mg/kg/day and in a less than dose proportional manner between doses 9 and 27 mg/kg/day.
- Accumulation was reported after repeat dosing.

Table 74: EFD Rat – Toxicokinetics

Dose level (mg/kg/day)	Animal No.	C_{max} (ng/mL)		T_{max} (h)		AUC_{0-24h} (ng·h/mL)	
		1st dosing (GD 7)	Last dosing (GD 17)	1st dosing (GD 7)	Last dosing (GD 17)	1st dosing (GD 7)	Last dosing (GD 17)
3	5601	324	842	4.0	4.0	5490	14200
	5602	363	811	4.0	4.0	6620	13500
9	5701	961	2220	4.0	4.0	16200	41400
	5702	1020	2050	2.0	2.0	15700	39800
27	5801	2360	3630	4.0	8.0	47800	64200
	5802	1450	3550	8.0	8.0	29800	68600

(Excerpted from Applicant's report)

Mortality and clinical observations

All rats survived to scheduled termination. There were no treatment-related clinical observations.

Body Weight

Table 75: EFD Rat – Mean Body Weight (% change from control)

GD	3 mg/kg/day	9 mg/kg/day	27 mg/kg/day
	(6)	(6)	(6)
11	--	--	-6*
13	--	--	-7*
15	--	--	-10**
17	--	-5*	-17**
18	--	-6*	-21**
20	--	--	-29**

**p<0.05, **p<0.01, significantly different from control; (N)=number of dams per group; --=no test article-related findings*

Food Consumption

Table 76: EFD Rat – Mean Food Consumption (% change from control)

GD	3 mg/kg/day	9 mg/kg/day	27 mg/kg/day
	(6)	(6)	(6)
9-11	--	--	-32**
11-13	--	--	-29**
13-15	--	--	-28**
15-17	--	--	-33**
17-18	--	--	-34**
18-20	--	--	-36**

*NS=not significant; *p<0.05, **p<0.01, significantly different from control*
(N)=number of dams per group
--=no test article-related findings

Necropsy Data

Table 77: EFD Rat – Number of Dams With Macroscopic Findings (GD 20)

Findings	Control (6)	3 mg/kg/day (6)	9 mg/kg/day (6)	27 mg/kg/day (6)
Red focus in the stomach	--	--	--	4
Dark red mesenteric lymph node	--	--	--	5
Black-brown adrenal gland	--	--	--	1
Diminished in size adrenal gland	--	--	--	1

--=no test article-related findings;
(N)=number of dams per group

Table 78: EFD Rat – F0 Cesarean Section Deliveries

Findings	Control (6)	3 mg/kg/day (6)	9 mg/kg/day (6)	27 mg/kg/day (6)
Number of implantations	12.5±2.6	12.8±1.5	12.5±1.0	12.2±1.3
Mean Number of live fetuses	11.8±2.7	11.5±2.8	11.2±2.0	**0**
Pre-implantation loss (%)	12.9±15.3	11.6±8.7	5.8±9.3	7.6±9.7
Resorptions				
Embryonic death (%)	4.5±5.3	11.5±13.4	8.4±11.1	**69.2** ± 31.7**
Early Fetal Death (%)	1.2±2.9	0.0	2.7±4.1	**30.8** ± 31.7*
Late Fetal Death (%)	0.0	0.0	0.0	0.0
Total fetal death (%) (Post implantation loss)	5.7±6.6	11.5±13.4	11.1±11.6	**100** ± 0.0**

Bold = *test article-related findings (N) = number of dams per group*p<0.05, **p<0.01, significantly different from control*

Table 79: EFD Rat – Percent of Embryonic Death Per Dam

Control (6)	3 mg/kg/day (6)	9 mg/kg/day (6)	27 mg/kg/day (6)
[Dam #]: %	[Dam #]: %	[Dam #]: %	[Dam #]: %
5101:12.5% 5102: 7.1% 5106: 7.1%	5202: 14.3% 5205: 27.3% 5206: 27.3%	5303: 7.7% 5304: 27.3% 5306: 15.4%	5401: 83.3% 5402: 90.0% 5403: 23.1% 5404: 35.7% 5405: 100.0% 5406: 83.3%

(N)=number of dams per group
[Dam #] = number of dam with embryonic death
% =percent of embryonic death per dam

Fetal Examinations

- At 9 mg/kg/day, there was statistically significant 7% decrease in total fetal weights.

- The mean percent of fetuses with any visceral anatomical anomalies per litter was higher at 9 mg/kg/day (44.4%) as compared to the control group (14.5%). The anomalies were reported to affect the ureter (dilated ureter), thymus (thymic cord), and heart (small ventricle and thin ventricular wall). The heart anomalies were found in two fetuses of the same litter (#5306). Small ventricle and thin ventricular wall were found in one fetus and the second fetus had a small ventricle.

Table 80: EFD Rat – Visceral Anomalies (#fetuses/#litters)

Findings (%)*	Control	3 mg/kg/day	9 mg/kg/day	27 mg/kg/day
Number of fetuses evaluated	**38**	**35**	**34**	**0**
Number of Dams	**6**	**6**	**6**	**6**
Incidence of Individual Fetal Visceral Anomaly				
Ureter-dilated ureter (# fetuses / # litters)	1/1	0	**6/2**	--
Thymus-thymic cord (# fetuses / # litters)	2/2	4/2	**10/4**	--
Heart-small ventricle (# fetuses / # litters)	0	0	**2/1**	--
Heart-thin ventricular wall (# fetuses / # litters)	0	0	**1/1**	--

*Total number of fetuses with visceral anomalies /Total number of Litters with visceral anomalies
-- = all dams at 27 mg/kg/day showed total litter loss(N) = number of litters ***Bold=test article-related findings***

Table 81: EFD Rat – Visceral Anomalies (% litter incidence)

Findings		Dose			
		0 mg/kg	3 mg/kg	9 mg/kg	27 mg/kg
	Number of fetuses evaluated	38	35	34	-
Visceral anomaly (%)	Mean	14.5	25.4	44.4	-
	S.D.	13.3	18.6	41.7	-
	N	6	6	6	6
Ureter, Ureter - Dilated (%)	Mean	2.8	0.0	17.8	-
	S.D.	6.8	0.0	28.8	-
	N	6	6	6	6
Thymus, Thymus - Thymic cord (%)	Mean	5.2	9.9	31.4	-
	S.D.	8.0	17.5	30.7	-
	N	6	6	6	6
Umbilical artery, Umbilical artery - Bilateral (%)	Mean	2.1	2.4	0.0	-
	S.D.	5.1	5.8	0.0	-
	N	6	6	6	6
Umbilical artery, Umbilical artery - Transposed (%)	Mean	4.5	8.9	7.0	-
	S.D.	6.9	10.5	11.1	-
	N	6	6	6	6
Heart, Ventricle - Small (%)	Mean	0.0	0.0	6.7	-
	S.D.	0.0	0.0	16.3	-
	N	6	6	6	6
Heart, Ventricular wall - Thin (%)	Mean	0.0	0.0	3.3	-
	S.D.	0.0	0.0	8.2	-
	N	6	6	6	6
Coronary orifice, Coronary orifice - Supernumerary coronary orifice (%)	Mean	0.0	4.2	0.0	-
	S.D.	0.0	10.2	0.0	-
	N	6	6	6	6

(Excerpted from Applicant's report)
Yellow highlight =test article-related anomalies

Skeletal anomalies were reported at 9 mg/kg/day, which included decreased mean number of sacral and caudal vertebrae. The finding of decreased caudal vertebrae was consistent with the growth retardation apparent at 9 mg/kg/day.

Table 82: EFD Rat – Skeletal Anomalies

Findings (%)	Control	3 mg/kg/day	9 mg/kg/day	27 mg/kg/day
Number of fetuses evaluated	**33**	**34**	**33**	**0**
Number of Dams	**6**	**6**	**6**	**6**
Mean number of sacral and caudal vertebrae	8.2±0.2	8±0.5	7.5±0.5*	--

-- = all dams at 27 mg/kg/day showed total litter loss;
(N) = number of litters
**p<0.05, significantly different from control*

Study title: A Preliminary Study for Effects of CH5424802*-002 on Embryo-fetal Development by Oral Gavage Dose in Rabbits	
Study no.:	TOX12-0024 ((b) (4) 036-126)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	14 June 2012
GLP compliance:	Yes (Ministry of Health, Labor and Welfare, Japan)
QA statement:	Yes
Drug, lot #, and % purity:	CH5424802*-002, G2E02

Key Study Findings

- The NOAEL for maternal toxicity and for the embryo-fetal toxicity was 9 mg/kg/day (0.4 -fold the estimated human $AUC_{ss,24}$ at the recommended dose).
- Decreases in body weight (up to -22%) and food consumption (up to -95%) were reported at 27 mg/kg/day.
- Hematological and clinical chemistry effects were reported at 27 mg/kg/day.
- At 27 mg/kg/day (2.9-fold the estimated human $AUC_{ss,24}$ at the recommended dose), two out of 5 rabbits had 100% resorptions and one aborted. Additionally, an increase in embryofetal death, decreases in number of live fetuses, fetal weights and placental weights were reported.
- Retroesophageal subclavian artery was reported at 27 mg/kg/day. Skeletal variations were reported at 27 mg/kg/day, which included an increase in full supernumerary rib and a decrease in short supernumerary rib. Additionally, an increase in supernumerary lumbar vertebra was reported at doses ≥9 mg/kg/day.

Objective: To investigate the effects of CH5424802-002 on pregnant females and embryo-fetal development, and to obtain information to determine dose levels for a subsequent study for effects on embryo-fetal development.	
Methods	
Species/Strain:	*Male and female* Kbl:(NZW) Rabbit (males used only for breeding)
Supplier:	(b) (4)
Age at dosing:	
Weight:	19 weeks
Mating:	2.82-3.16 kg

<table>
<tr><td>Study design:</td><td>The day on which copulation was confirmed visually it was designated as Day 0 of gestation (GD 0).</td></tr>
<tr><td>Vehicle:</td><td>
<table>
<tr><th></th><th>Dose (mg/kg/day)</th><th>Dose Volume (ml/kg)</th><th>Number of Main Study Rabbits</th></tr>
<tr><td>Group 1</td><td>Vehicle</td><td>10</td><td>6</td></tr>
<tr><td>Group 2</td><td>3</td><td>10</td><td>6</td></tr>
<tr><td>Group 3</td><td>9</td><td>10</td><td>6</td></tr>
<tr><td>Group 4</td><td>27</td><td>10</td><td>6</td></tr>
</table>
</td></tr>
<tr><td>Dosing:</td><td>0.5 w/v% hydroxypropyl methylcellulose (HPMC)/0.001 mol/L hydrochloric acid (HCl) solution (0.5 w/v% HPMC/0.001 mol/L HCl).</td></tr>
<tr><td>Route:</td><td>Once daily from GD 6 to 18.</td></tr>
<tr><td>Analysis:</td><td>Oral gavage.</td></tr>
<tr><td>Clinical chemistry:</td><td>Concentration, homogeneity and stability were evaluated on study.</td></tr>
<tr><td>Toxicokinetics (TK):</td><td>GD 18 (prior to dosing) and GD 28</td></tr>
<tr><td>In-life end points:</td><td>Blood was collected on GD 6 (1, 2, 4, 8 and 24 hours post dosing) and GD 18 (Predose, 1, 2, 4, 8 and 24 hours postdosing).</td></tr>
<tr><td>Cesarean section (C-section):</td><td>• Mortalities and clinical observations.
• Body weight and food consumption – every 2 days.</td></tr>
<tr><td>Litter endpoints:</td><td>GD 28.</td></tr>
<tr><td>Fetal endpoints:</td><td>Number of corpora lutea, number of live fetuses, number of dead fetuses, resorptions, and placental weight.</td></tr>
</table>

Results:

Analysis of test formulations

Concentration and homogeneity of the test article in the formulations were within the acceptable range (±10%). The formulation was stable for up to 10 days after preparation in a refrigerator followed by 6 hours at room temperature.

Maternal data:

Toxicokinetics

- On GD 6 and 18, plasma exposure (C_{max} and AUC) increased in approximately dose-proportional manner.
- On GD 18, plasma exposure (C_{max} and AUC) increased in an approximately dose-proportional manner between doses of 3 and 9 mg/kg/day and in a greater than dose proportional manner between doses 9 and 27 mg/kg/day.
- Accumulation was reported after repeat dosing at 27 mg/kg/day.

Table 83: EFD Rabbit – Toxicokinetics

Dosage level [1] (mg/kg/day)	Number of animals	C_{max} (ng/mL)		T_{max} (h)		AUC_{0-24h} (ng·h/mL)	
		Day 6[2]	Day 18[2]	Day 6[2]	Day 18[2]	Day 6[2]	Day 18[2]
3	3	155	163	5.3	4.0	2460	2950
9	3	366	385	8.0	4.7	6130	6650
27	3	914	2090	8.0	9.7	16,600	43,200

1) Dosage level as CH5424802-000
2) Dosing time, Day 6 or Day 18 of gestation

(Excerpted from Applicant's report)

Mortality and clinical observations

All rabbits survived to scheduled termination. There were test article-related clinical observations reported at 27 mg/kg/day, including, one dam that aborted its litter on GD 21.

Table 84: EFD Rabbit – Clinical Observations

Dose (mg/kg)	Control	3	9	27
No. of dams	6	6	6	6
Normal	6	6	6	0
Abortion	0	0	0	1
Decrease in stool volume	0	0	0	6
No stool	0	0	0	4
External genital bleeding	0	0	0	1

Rabbit #10020 showed external genital bleeding on GD 20 and aborted its litter on GD 21.

(Excerpted from Applicant's report)

Body weight

Table 85: EFD Rabbit – Mean Body Weight (% change from control)

GD	3 mg/kg/day	9 mg/kg/day	27 mg/kg/day
12	--	--	-6*
14	--	--	-9**
16	--	--	-11**
18	--	--	-14**
19	--	--	-14**
22	--	--	-19**
26	--	--	-22**
28	--	--	-19**

*-- = no test article-related findings; * = p<0.05, **p<0.01 significantly different from control*

Food consumption

Table 86: EFD Rabbit – Mean Food Consumption (% change from control)

GD	3 mg/kg/day	9 mg/kg/day	27 mg/kg/day
8-9	--	--	-64**
10-11	--	--	-76**
12-13	--	--	-84**
14-15	--	--	-93**
16-17	--	--	-92**
18-19	--	--	-95**
22-23	--	--	-54*

*-- No biologically meaningful change; * = p<0.05, **p<0.01 significantly different from control*

Hematology

Table 87: EFD Rabbit – Hematology (% change from control)

	3 mg/kg/day	9 mg/kg/day	27 mg/kg/day
Erythrocyte count[2]	--	--	-14
Hemoglobin concentration [2]	--	--	-9
Hematocrit[1,2]	--	--	Up to -20
Mean corpuscular hemoglobin concentration[1,2]	--	--	Up to 5
Mean corpuscular hemoglobin volume[1,2]	--	--	Up to -7
Platelet count[1]	--	--	-36
Neutrophil count[1]	--	--	47
Eosinophil count[1]	--	--	-69
Basophil count[1]	--	--	-62
Erythrocyte morphology abnormalities (Poikilocytosis)[2]	0/6 dams	3/6 dams	6/6 dams

-- = not biologically meaningful change = p<0.05, **p<0.01 significantly different from control*
[1] = GD 18; [2]= GD 28

Clinical Chemistry

Table 88: EFD Rabbit – Clinical Chemistry (% change from control)

	3 mg/kg/day	9 mg/kg/day	27 mg/kg/day
Total Protein[1]	--	--	-11**
Total cholesterol[1,2]	--	--	Up to 72**
Triglyceride[1]	--	--	-63*
Creatinine[1]	--	--	71**

	3 mg/kg/day	9 mg/kg/day	27 mg/kg/day
Calcium[1]	--	--	-10**
Chloride	--	--	-4*
Albumin[1]	--	--	-15*
Albumin/Globulin[1]	--	--	-18*
Glucose[2]	--	--	16*
α1 globulin[1,2]	--	--	Up to 18*
α2 globulin[1]	--	--	78**

-- = not biologically meaningful change
** = p<0.05, **p<0.01 significantly different from control*
[1] = GD 18
[2]= GD 28

F_0 Cesarean Section Deliveries

At 27 mg/kg/day, two out of 5 dams had total litter loss, increases in percent of post implantation loss and dead fetuses. Additionally, decreases in number of live fetuses, percent of fetal viability rate and mean placental weight were reported. The increases in the percent post-implantation loss at 3 and 9 mg/kg/day, were not considered test article- related because they were within the range (0 to 11.3%) of the historical control data, as stated by the Applicant.

Table 89: EFD Rabbit – F_0 Cesarean Section Deliveries

Dose (# of dams)	# Resorptions & dead fetuses	% Post implantation loss[1]	% dead fetuses	# of live fetuses	% fetal viability rate[2]	Placental weight (M/ F)	Number of dams aborted
Control (6)	0.2	2.3	0	7.3	97.7	5/ 4.8	0
3 mg/kg (6)	1	9.8	1.5	8.2	90.2	4.6/ 4.1	0
9 mg/kg (6)	0.8	11.3	4.8	5.5	88.7	5.8/ 5.4	0
27 mg/kg (5)[3]	**3.2****	**59.2****	**14.8***	**3.2***	**41****	**3.4*/ 3.7**	**1**

[1] Post implantation loss= (Resorptions & dead fetuses)/implantations
[2] Fetal viability rate=(number of live fetuses)/(number of implantations) X100
[3] =At 27 mg/kg/day, two out of 5 rabbits (40%) had 100% resorptions.
** = p<0.05, **p<0.01 significantly different from control*

Fetal Evaluations

Table 90: EFD Rabbit – Fetal Weight and Anomalies

	Control	3 mg/kg/day	9 mg/kg/day	27 mg/kg/day
Number of dams	**6**	**6**	**6**	**3**
Number of fetuses	**44**	**49**	**33**	**16**
Fetal Weight (M/F)	35.9/ 35.3	34.5/ 32.7	37/ 36.1	**(24.6/ 25.2)****
External	--	--	--	--
Visceral				
Retroesophageal subclavian	--	--	--	**6.7% [1]**
Skeletal				
Mean frequencies of variations (%)	71.6 ±28.5 [31]		75.7 ±39.3 [26]	80.0 ±34.6 [13]
% Thread fusion of sternebra (variation)	0.0 [0]		0.0 [0]	5.6% [1]
% Full supernumerary rib (variation)	53 [22]		66 [23]	80.0 [13]
% Short supernumerary rib (variation)	35.93 [15]		22.22 [6]	0.0 [0]
% Supernumerary lumbar vertebra	1.9 [1]		**16.5 [7]**	**17.7 [3]**

-- No test article-related findings
[ ]= number of fetuses with abnormalities/variation
Shaded area: Skeletal evaluations were not performed for the 3 mg/kg/day group.
*** p<0.01 significantly different from control*

10 Special Toxicology Studies

10.1 Sodium lauryl sulfate (SLS) toxicity

Study title: A 28-Day Oral Gavage Repeated-Dose Toxicity Study of CH5424802*-002 in Rats Comparing Formulations of the Previous 4-Week Toxicity Studies and The Current Clinical Formulation Containing SLS	
Study no.:	TOX13-0127
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1 April 2014

GLP compliance:	Yes (Ministry of Health, Labor and Welfare, Japan)
QA statement:	Yes
Drug, lot # and purity: 1. Clinical formulation: 2. Non-clinical formulation:	1. **CH5424802**; Lot # R2F01; 99.9% (containing SLS) 2. **CH5424802-002**; Lot # 121418; 92.9% (does not contain SLS)
Objectives:	Sodium lauryl sulfate (SLS) is an excipient in the clinical formulation at concentration of (b) (4)% (w/w SLS to API), but was not used in the nonclinical toxicology studies formulations. The objective of the study was to compare the toxicity profiles and systemic exposure levels of two formulations administered orally, at 20 mg/kg/day, in a 28-day repeat dose bridging study in rats: nonclinical formulation CH5424802-002 [does not contain SLS] and clinical formulation of CH5424802 containing SLS

Key Study Findings

- No significant differences in systemic exposure were reported between formulations.
- There were no remarkable differences reported in body weight, food consumption, clinical pathology, organ weights of animals receiving the clinical and nonclinical formulations.
- No significant differences were noted in the histopathology profiles of animals receiving the clinical (SLS) and non-SLS formulations.

Methods:

Study design*:

Group		CH5424802		Dose level of SLS (mg/kg/day)	pH	Male
		Dose level (mg/kg/day) [1]	Concentration (mg/mL) [1]			
1	Clinical formulation of CH5424802 SLS (-) vehicle control	0 [2]	0	0	2.87 to 2.91	10 [5] (10101-10110)[6]
2	Clinical formulation of CH5424802 SLS (+) vehicle control	0 [3]	0	10	2.88 to 2.92	10 (10201-10210)
3	Clinical formulation of CH5424802	20	2	10	2.72 to 2.88	10 (10301-10310)
4	CH5424802-002 Vehicle control	0 [4]	0	0	1.96 to 2.87	10 (10401-10410)
5	CH5424802-002	20	2	0	1.97 to 2.84	10 (10501-10510)

1) Dose and concentration as CH5424802-000, 2) Vehicle 3 (HCl solution, pH 2.9) was dosed to confirm the effect of acid solution. 3) Vehicle 4 (b) (4) SLS solution, pH2.9) was dosed. 4) Vehicle 2 (10 w/v% HPCD/0.01 mol/L MsOH/0.001 mol/L HCl solution) was dosed. 5) Number of animals, 6) Animal numbers

(Excerpted from Applicant's report)

*Toxicokinetics animals (TK): Additional 5 males per group were used for TK evaluation.	
Frequency of dosing:	Once daily for 28 days
Route of administration:	Orally by gavage
Dose Volume:	10 mL/kg
Species/Strain:	Male rats RccHan^{TM}: WIST
Age at start of dosing:	6 weeks
Weight at start of dosing:	147.4g to 175.6g

Observations and Schedules:

Mortality:	Twice daily
Cage side observations:	Twice daily
Body weights and food consumption :	Days 1, 4, 8, 11, 15, 18, 22, 25, and 28
Ophthalmoscopy:	Was not performed
Clinical pathology	Day 29
Bone marrow cytology (femoral bone):	At necropsy for main study animals. Smears were not examined
Toxicokinetics:	Blood was collected on Day 1 and 28 at Predose, 1, 2, 4, 8 and 24 hours post-dosing. In addition, blood was collected on day 14 before dosing.
Terminal procedures: necropsy, organ weights and histopathology	Day 29 from all main study animals
Statistical analysis:	The Applicant stated that “each CH5424802-treated group (the clinical formulation and nonclinical formulation groups) were compared with each vehicle control group separately”.

Results:

Mortality
No mortalities were reported.

Clinical Signs
Findings were unremarkable.

Body Weights
No differences reported between the clinical (group 3 -contains SLS) and nonclinical (group 5 -does not contains SLS) formulations.

Decreases in mean body weight (up to 9%) and body weight gain (up to 23%) were reported between the clinical and nonclinical formulations and their respective control groups.

Table 91: Rat Excipient (SLS) – Mean Body Weight (% change)

Group	Days					
	11	15	18	22	25	28
Group 2 (vehicle control for clinical formulation) vs. **Group 3** (clinical formulation)	--	-5*	-5*	-6*	-7*	-8**
Group 4 (vehicle control for nonclinical formulation) vs. **Group 5** (nonclinical formulation)	-5*	-6**	-6*	-7**	-8**	-9**
Group 3 *(clinical formulation) vs* ***Group 5*** *(nonclinical formulation)*	--	--	--	--	--	--

*-- = no biologically meaningful change; * = p<0.05, ** p<0.01 significantly different*

Table 92: Rat Excipient (SLS) – Body Weight Gain (% change)

Group	Days				
	4-8	8-11	11-15	18-22	25-28
Group 2 (vehicle control for clinical formulation) vs. **Group 3** (clinical formulation)	-14*	--	-20**	-18**	-23**
Group 4 (Vehicle control for nonclinical formulation) vs. **Group 5** (nonclinical formulation)	-23*	-16*	-21*	-17**	-21**
Group 3 *(clinical formulation) vs* ***Group 5*** *(nonclinical formulation)*	--	--	--	--	--

-- = no biologically meaningful change = p<0.05, ** p<0.01 significantly different*

Food Consumption

No differences reported between the clinical (group 3 -contains SLS) and nonclinical (group 5 -does not contains SLS) formulations.

Decreases in food consumption were reported between the clinical (up to 8%) and nonclinical formulations (up to 12%) and their respective control groups.

Table 93: Rat Excipient (SLS) – Food Consumption (% change)

Group	Days				
	1-4	4-8	8-11	11-15	25-28
Group 2 (vehicle control for clinical formulation) vs. **Group 3** (clinical formulation)	--	-5(ns)	-8*	-8*	-7(ns)
Group 4 (vehicle control for nonclinical formulation) vs. **Group 5** (nonclinical formulation)	-7*	-12**	-8*	-7*	-7*
Group 3 *(clinical formulation) vs.* ***Group 5*** *(nonclinical formulation)*	--	--	--	--	--

-- = no biologically meaningful change(ns) = not statistically significant = p<0.05, ** p<0.01 significantly different*

Hematology

Table 94: Rat Excipient (SLS) – Hematology (% change)

Endpoint	**Group 2** (vehicle control for clinical formulation) vs. **Group 3** (clinical formulation)	**Group 4** (vehicle control for nonclinical formulation) vs. **Group 5 (**nonclinical formulation)	**Group 3 vs. Group 5**
Lymphocyte	--	33**	--
White blood cell count	--	32*	--
Reticulocyte (%)	28*	19*	--
Reticulocyte count	28*	27*	--
Eosinophil (%)	-36*	-27*	--
Red blood cell count	--	6*	--
hemoglobin	--	5*	--

-- = no biologically meaningful change
** = p<0.05, **p<0.01 significantly different*

Clinical Chemistry

Table 95: Rat Excipient (SLS) – Clinical Chemistry (% change)

Endpoint	**Group 2** (vehicle control for clinical formulation) vs. **Group 3** (clinical formulation)	**Group 4** (vehicle control for nonclinical formulation) vs. **Group 5 (**nonclinical formulation)	**Group 3 vs. Group 5**
ALP	59*	53*	--
ALP2	76**	62*	--
ALP3	61*	60**	--
ALP5	48**	39 (ns)	--
Alpha 1 Globulin (%)	-9*	-8*	--
Alpha 2 Globulin (%)	10**	--	--
Alpha 2 Globulin count	13*	--	--
Glucose	11**	25*	--
Total Cholesterol	44*	46*	--

-- = no biologically meaningful change
(ns) = not statistically significant
** = p<0.05, **p<0.01 significantly different*

Gross Pathology
Findings were unremarkable.

Organ Weights
The increases in adrenal, lung, heart and liver weights were correlated with microscopic findings in the clinical formulation and nonclinical formulation groups.

Table 96: Rat Excipient (SLS) – Relative Organ Weight (% change)

Dose Group	**Group 2** (vehicle control for clinical formulation) vs. **Group 3** (clinical formulation)	**Group 4** (vehicle control for nonclinical formulation) vs. **Group 5** (nonclinical formulation)	**Group 3 vs. Group 5**
Adrenals	--	18*	--
Brain	11*	10*	--
Lung	8*	--	--
Heart	12*	15*	--
Liver	9*	15*	--
Spleen	16*	16*	--
Kidneys	9*	12*	--
Testes	10**	13*	--
Seminal vesicles	31*	--	--

-- = no biologically meaningful change
** = p<0.05, **p<0.01 significantly different*

Histopathology

Adequate Battery – Yes
Signed Pathology Report – No
All tissues analyzed in all dose groups
Peer Reviewed- No

- Clinical formulation (group 3) and nonclinical formulation (group 5): Lung, stomach and pancreas. Microscopic findings were comparable in incidence and severity.

- Clinical formulation (group 3): heart and prostate; these were the only target organs in the SLS-containing Clinical formulation group (group 3) that were uniquely affected, however, findings in both tissues were minimal cellular infiltrate, in only a single animal.

- Nonclinical formulation (group 5): duodenum, liver and adrenal.

Table 97: Rat Excipient (SLS) – Histopathology (terminal sacrifice)

	No. of animals affected				
Sex	**Males**				
Group (N)	**1 (10)**	**2 (10)**	**3 (10)**	**4 (10)**	**5 (10)**
Treatment	**SLS (-) control**	**Control for Clinical formul-ation**	**Clinical formul-ation**	**Control for CH5424802**	**CH5424802**
Treatment-related Findings:					
LUNG					
Cell infiltration; foamy macrophage, alveolus					
– *minimal*	0	0	**5**	0	**4**
– Osseous metaplasia, Mineralization; arterial wall *minimal*	1	2	1	0	0
STOMACH					
Extension; proliferative zone, mucosa, glandular stomach					
– *minimal*	0	0	**1**	0	**3**
Degeneration; glandular epithelium					
– *minimal*	0	0	**8**	0	**8**
DUODENUM					
Cell infiltration; macrophage, mucosa					
– *minimal*	0	0	0	0	**6**
– *mild*	0	0	0	0	**3**
Cell infiltration; inflammatory, mucosa					
– *minimal*	0	0	0	0	**3**
LIVER					
Enlargement; hepatocyte					
– *minimal*	0	0	0	0	**3**
Necrosis; hepatocyte, focal					
– *minimal*	0	0	0	0	**1**
Vacuolation; bile ductal epithelium					
– *minimal*	0	0	0	0	**3**
Cell infiltration; inflammatory, focal					
– *minimal*	1	2	1	0	1
PANCREAS					
Increase; apoptotic body, acinar cell					
– *minimal*	0	0	**2**	0	**2**
ADRENAL					

	No. of animals affected				
Sex	**Males**				
Group (N)	**1 (10)**	**2 (10)**	**3 (10)**	**4 (10)**	**5 (10)**
Treatment	**SLS (-) control**	**Control for Clinical formul-ation**	**Clinical formul-ation**	**Control for CH5424802**	**CH5424802**
Treatment-related Findings:					
Hypertrophy; cortical cell, reticular zone – *minimal*	0	0	0	0	**2**
HEART					
Cell infiltration; mononuclear cell, focal – *minimal*	0	0	**1**	0	0
KIDNEY					
Basophilic tubule – *minimal*	1	1	2	1	0
Mineralization; medulla – *minimal*	2	1	2	1	0
PROSTATE					
Cell infiltration; lymphocyte – *minimal*	0	0	**1**	0	0

Bold = predominant findings

Toxicokinetics

- Both formulations (clinical and nonclinical) exhibited similar systemic exposure (C_{max} and AUC) levels.
- Accumulation was reported in both formulations following a repeat dose administration.

Table 98: Rat Excipient (SLS) – Toxicokinetics

Group	Day	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-24h} (ng·h/mL)
Clinical formulation of CH5424802*	1	1180	6.4	19600
	28	2000	6.4	34700
CH5424802-002**	1	1380	8.0	24600
	28	2140	4.8	38700

a, analyte: free base of CH5424802-002, *, a formulation with SLS, **, a formulation without SLS

(*Excerpted from Applicant's report)*

Stability and Dosing Formulation Analysis

- Samples obtained for concentration verification analysis for CH5424802-00 and SLS were within the acceptable limits of ±10% error.

- The test article formulation and SLS solution were stable for up to 11 days in refrigerator conditions followed by 6-hour storage at room temperature.

10.2 Phototoxicity Studies

Title: In vitro 3T3 NRU Phototoxicity Test of CH5424802 (Study #: TOX11-0047)	
Objectives: "to evaluate the phototoxic potential of CH5424802 by using an in vitro cytotoxicity assay with Neutral Red uptake in Balb/c 3T3 mouse fibroblast".	
GLP:	Non-GLP
Methods:	
• Species/cell line:Balb/c 3T3 mouse fibroblast cell line. • Cells were incubated with CH5424802 solution (diluted with PBS/1 %DMSO) at concentrations of 0.02, 0.05, 0.16, 0.50, 1.58, 5.01, 15.8 and 50 µg/mL for 60 minutes. • Positive control: Chlorpromazine hydrochloride at concentrations ranging between 0.3211g/mL and 31611g/mL under non-irradiated (dark) conditions and 0.0311g/mL and 31.711g/mL under UVA-irradiated (UVA) conditions. • UV radiation sources: SUNTEST CPS+ (ATLAS) with a filter glass to remove UVB. • The plates for UV A-irradiated conditions were exposed to approximately 5 J/cm^2 UVA light at 1.64 mW/cm^2 for approximately 50 minutes. The other plates were kept in the dark at room temperature and served as the cytotoxicity control. • Cell viability was determined by Neutral Red (NR) staining solution. • Cytotoxicity is expressed as a dose-dependent reduction of the growth rate of cells as determined by uptake of the vital dye Neutral Red one day after treatment. Photo Irritation Factor (PIF), an IC_{50} ratio of dark and UVA conditions, was determined and was used to evaluate the phototoxic potential. Positive threshold of phototoxic activity was PIF >5, according to the OECD guideline.	

Results:

- CH5424802: IC_{50} was calculated as 26.9 µg/mL under dark conditions and 0.30 µg/mL under UVA conditions. PIF was calculated as 94.8, indicating that CH5424802 exhibited a phototoxic response.

Table 99: In vitro 3T3 NRU Phototoxicity Results of CH5424802-000

Table 1 Result of NRU assay of CH5424802-000

CH5424802-000 Concentration (µg/mL)	Dark Mean Values (%)	UVA Mean Values (%)
0.02	91.7	88.4
0.05	76.7	77.7
0.16	86.7	84.2
0.50	97.8	18.7
1.58	101.6	-1.5
5.01	96.7	-1.7
15.8	87.7	-1.6
50.0	36.0	-1.5
IC_{50} value	26.9 µg/mL	0.30 µg/mL

Dark represents the experimental condition under non-irradiation. UVA represents the UVA-irradiation condition. Each value represents the mean cell variability of wells (n=8) with the same dose. Eight wells were used for each concentration in either dark or UVA conditions.

(Excerpted from Applicant's report)

- Positive control: IC_{50} was calculated as 26.0 µg/mL under dark conditions (within the range of the OECD guidelines) and 0.04 µg/mL under UVA conditions (higher than the range of the OECD guideline).

Conclusion:

CH5424802 exhibited a phototoxic response.

11 Integrated Summary and Safety Evaluation

Pharmacology:

The Applicant submitted a series of in vitro and in vivo studies to support the mechanism of action of alectinib as a kinase inhibitor that targets ALK and RET. In Sakamoto et al. 2011, the authors showed that alectinib interacts at the ATP binding site of the ALK enzyme. In in vitro kinase panels, alectinib inhibited ALK, RET, ALK point mutations, and RET mutants, but did not inhibit any other tested kinases at clinically relevant concentrations. Alectinib also showed no activity against tumor cell line growth in cells driven by EGFR, MET, or Ras mutations. In addition, the Applicant investigated the activity of major metabolites of alectinib, including the M4 metabolite (CH5468924) that has been identified at high levels in both humans and animals. M4 showed comparable inhibitory activity to the alectinib parent compound against ALK and RET as well as selected point mutations in each of these proteins. ALK point mutations identified in patients previously treated with crizotinib and associated with crizotinib resistance in vitro include C1156Y, L1196M, 1151Tins, L1152R, and G1269A. Alectinib demonstrated inhibitory activity against each of these mutations both in biochemical kinase panels and in BaF3 cell lines engineered to express these mutant proteins. Alectinib suppressed activation of the ALK kinase, demonstrated by inhibition of ALK phosphorylation, and prevented the phosphorylation of downstream signaling

molecules, such as STAT3 and AKT. Further, alectinib showed inhibitory activity against the growth of human NSCLC, anaplastic large cell lymphoma, and neuroblastoma cell lines harboring ALK fusions and mutations or amplifications

The activity of alectinib was studied in mice implanted with NSCLC (NCI-H2228, harboring the EML4-ALK fusion and A549, ALK wild-type), neuroblastoma (NB-1, ALK gene amplification) and ALCL (KARPAS-299, expressing the nucleophosmin (NPM)-ALK fusion protein) cell lines. Alectinib treatment resulted in tumor growth inhibition and tumor regression in the NSCLC, neuroblastoma, and ALCL models, confirming the anti-tumor activity in cancers with ALK gene amplifications or rearrangements. Little inhibition was observed with alectinib treatment in the ALK wild-type NSCLC model. Alectinib also showed greater inhibition of tumor growth than crizotinib in a mouse model implanted intracranially with the NCI-H2228 cell line, suggesting that alectinib can cross the blood brain barrier and may have activity against brain metastases. The Applicant also conducted studies investigating the combination of alectinib with cisplatin, paclitaxel, gemcitabine, and bevacizumab in mouse xenograft models. In general, the results of these studies showed some improvements in anti-tumor activity of the combinations compared to monotherapy with little effect on body weight, suggesting that these combination treatments did not result in major decrements in tolerability compared to monotherapy.

Safety pharmacology studies included CNS, respiratory, and gastrointestinal motor function studies in rats, two in vivo cardiovascular studies in cynomolgus monkeys, a hERG assay, and studies on the effects of CH5424802 on isolated blood vessels and Cav1.2 currents. Alectinib had no effects on CNS, respiratory, or gastrointestinal motor functions. Alectinib blocked hERG currents with an IC_{20} of 0.12 µM (58 ng/mL) and an IC_{50} of 0.45 µM (217 ng/mL), suggesting some potential for causing QTc prolongation. QTc prolongation has, however, not been reported clinically. At 15 mg/kg, alectinib administration to monkeys had no effects on blood pressure, heart rate, ECG parameters, or body temperature. At 20 and 60 mg/kg, however, slight hypotension was observed as decreases in systolic, mean, and diastolic blood pressures. This hypotension is likely due to the inhibitory effect of alectinib on Cav1.2 currents, which can cause vasodilatory effects. Inhibition of this current occurred at concentrations as low as 0.189 µM. The Applicant also demonstrated an alectinib-mediated effect on vasodilation by inhibition of aortic ring contractility at low concentrations of the drug.

Pharmacokinetic studies:

Intravenous administration of alectinib in rats or monkeys resulted in a low plasma clearance, a large volume of distribution, and a moderately long half-life (t1/2 was 24.4 and 10.4 hr in rats and monkeys, respectively. Alectinib also had high oral bioavailability in rats (F=88.6%) and moderate bioavailability in monkeys (F=50.4%).

Tissue distribution studies in pigmented rats showed high distribution of radiolabeled alectinib. The concentrations of radioactivity in tissues were higher than in the plasma. Alectinib appeared to distribute to the red blood cells, helping to explain the high tissue

distribution. The highest radioactivity was seen in the uvea, likely due to a high degree of protein binding. Alectinib does penetrate the CNS; radioactivity was observed in CNS tissues (cerebrum, cerebellum, and spinal cord) up to 24 hours postdose and was approximately 30-40% of the radioactivity seen in blood at Cmax. In pregnant rats, alectinib was transferred into the fetus and fetal tissues, including the fetal brain. In vitro binding assays showed that alectinib plasma protein binding is high (>99%) in mice, rats, monkeys, and humans.

M4 was the major metabolite formed in mouse, rat, dog, monkey, and human hepatocytes. Further, the major CYP isoform involved in mediating alectinib metabolism of M4 is CYP3A4. Similarly to alectinib, M4 had high plasma protein binding and distribution into blood cells in the same species, and both alectinib and M4 were excreted in the feces at up to 168 hours post administration.

General toxicology:

The toxicity of oral alectinib was evaluated in rats and monkeys in GLP-compliant repeat-dose 13-week toxicology studies. No test article-related mortalities were reported in the rats (3, 9 and 27 mg/kg/day or 18, 54 and 162 mg/m^2) or monkeys (1.3, 4 and 12 mg/kg/day or 15.6, 48 and 144 mg/m^2). Clinical observations were reported in the rat at 162 mg/m^2 (discoloration of eyeball; discoloration of teeth; enlarged, short and crushed teeth; blackish feces) and were accompanied by decreases in mean body weights (up to 22%) and food consumption (up to 13%) at doses ≥ 18mg/m^2. The teeth findings were correlated with macroscopic (white teeth) and microscopic findings in the incisors (eosinophilic material in enamel space, degeneration/necrosis of ameloblast, dilatation of capillary at papillary and odontoblast layers, disarrangement of ameloblast and odontoblast in mid-region), and may have contributed to the reported body weight and food consumption decreases in rats. The macro-and microscopic findings were reversible by the end of the recovery period, while the clinical observation effects persisted throughout recovery. In contrast, there were no body weight or food consumption effects reported in monkeys at doses up to 144 mg/m^2. The dental toxicity in rats was not observed in monkeys, likely due to species differences in tooth growth. In rats, growth of the incisors is continuous throughout the entire lifespan of the animal; in monkeys and humans tooth growth is halted at maturation. Since the dental toxicity appeared to affect only growing teeth (i.e. rats), the effect on incisors is unlikely to be relevant to the targeted patient population (adults) for this indication, but may be relevant for a pediatric population.

Changes in hematology (increases in reticulocytes, platelets, and neutrophils, decreases in hemoglobin and hemocrit as well as changes in red blood cells), increased abnormal red blood cell morphology (poikilocytosis) and urinalysis (increased urine volume) parameters were reported during the dosing phase in the rats and monkeys at doses ≥18 mg/m^2 and at a dose of 48 mg/m^2, respectively. Other changes were reported only in the rat (increased white blood cells, monocytes, and large unstained cells, as well as decreased basophils, eosinophils and lymphocytes). Increases in APTT and PT also occurred in the rat. Effects on reticulocytes and monocytes were evident at the end of the recovery period in rats (162 mg/m^2). The reported increase in

clotting time (APTT and PT) was consistent with the clinical observation of blackish feces in rats and decreased RBC parameters, dark-red gelatinous material in the cecum and colon, and hemorrhage in the mucosa of the ileum.

Changes in clinical chemistry parameters (increased creatinine, total cholesterol, triglyceride, ALP2, ALP3, ALP5, globulin α2 and decreased glucose) were reported in monkeys at doses ≥ 48 mg/m^2, in female rats at 18 mg/m^2, and male rats at 54 mg/m^2. Other changes were reported only in the monkey (increases in ALT and $_{Y}$GT) or only in the rat (increased AST, ALP, urea nitrogen, total bilirubin, inorganic phosphorus, albumin, beta globulin as well as decreased globulin α1 and gamma globulin). In general changes in clinical chemistry were suggestive of liver toxicity and changes in coagulation. At the end of the recovery period, some effects persisted at the high dose level in both species (glucose, globulin α2), but suggested a trend towards reversibility.

The predominant target organs of toxicity in the rat and monkey were the gastrointestinal tract, adrenal gland, liver, reproductive organs, and respiratory system. In the gastrointestinal tract microscopic findings were evident in the rat at doses ≥54 mg/m^2 and in the monkey at doses ≥48 mg/m^2. These findings included degeneration of glandular stomach epithelium and proliferative zone extension in digestive mucosa, mucinous hypertrophy in glandular stomach mucosal epithelium, macrophage/inflammatory cell infiltration in digestive tract mucosa and disarrangement/desquamation of mucosal epithelium in the small intestine. Macroscopically, dilation of cecum, colon and ileum (which contained dark-red gelatinous material- presumably blood) was reported only at the highest doses tested. Presence of blood in large intestine indicates hemorrhage and is consistent with prolonged clotting times noted in the hematology assessments. Consistent with high levels of [^{14}C]-alectinib found in the adrenal glands of male rats in a quantitative whole body autoradiography study, microscopic findings were evident in the adrenal gland in the rat at doses ≥18 mg/m^2 and in monkey at doses ≥48 mg/m^2. These findings included cortical hypertrophy and changes in lipid droplets in fascicular cells of the adrenal gland. Since the zona fasciculata tissue is responsible for the production of glucocorticoids that play a role in regulating the metabolism of glucose, such changes may contribute to the observed glucose effects reported in rats and monkeys as well as decreases in blood pressure and heart rate. Additionally, alteration of cholesterol levels (i.e. reduced lipid droplets) in the adrenal cortex may impact the animal's ability to further synthesize adrenal hormones.

In the liver, microscopic, macroscopic and clinical pathology findings were evident in the rat at doses ≥18 mg/m^2 and in monkey at doses ≥48 mg/m^2. The microscopic findings included degeneration /necrosis /vacuolation of bile duct epithelium, bile duct proliferation, hepatocyte enlargement, focal lymphocyte cell infiltration, swelling/yellow brown pigmentation of sinusoidal cells of the liver, and single cell/focal necrosis of hepatocytes. Additionally, the enlarged liver, and increased liver weight were consistent with the reported clinical chemistry changes.

Respiratory system microscopic and macroscopic findings were evident in the rat at 162 mg/m^2 and in monkey at 144 mg/m^2. In the lung, microscopic findings included alveolus hemorrhage, perivascular hemosiderin pigmented macrophage accumulation and foamy macrophage infiltration in alveoli. In the trachea microscopic findings included multinucleated giant cell in lamina propria, disarrangement of mucosal epithelium, and inflammatory cell infiltration in lamina propria. Macroscopically, brownish and reddish patches on the lung were observed monkeys at 144 mg/m^2, and lung weights were increased.

Additional target organs were identified in the rat (spleen, bone marrow, lymph nodes, thymus, kidneys, pituitary gland, mandibular glands, incisors, and bone) at doses ≥ 18 mg/m^2, and in the monkey (pancreas and parathyroid) at doses ≥48 mg/m^2. In the rat, at the end of the recovery period, effects noted in the lymph nodes (≥54 mg/m^2), the liver, and the lung (162 mg/m^2) had not fully reversed by the end of the recovery period; while effects noted in the spleen (at 18 mg/m^2) and in the adrenals, kidneys, ileum, stomach, and trachea (at 162 mg/m^2) showed partial recovery. In monkeys all organs showed recovery or a trend towards recovery.

The effect on osteoclasts in the metaphysis of the femur in the rat correlated with increases in ALP, ALP2, and IP. Given that such effects might reasonably be expected to affect growth of the long bones, this finding would be a significant safety concern in young/young adult populations, since growth within the metaphysis remains active until the age of ~20 years in the human. Given the average age of the patient population for alectinib, bone toxicities may not be relevant for the current population, but will be added to the pediatric section of the label.

Although ocular toxicity has been observed as a consequence of some tyrosine kinase inhibitors that target ALK (e.g. crizotinib), no test article-related ocular toxicity was reported in rats and monkeys administered alectinib. The Applicant did not submit data from specialized tests, such as ERG or explorations of light/dark adaptation that might further address the type of ocular toxicity observed in patients treated with crizotinib. Exposure data showed that alectinib binds melanin, and a longer residency time might be expected in uveal tissues. Species with pigmented eyes were used for toxicology testing; as such the ocular toxicity of melanin-containing tissues was assessed.

Male reproductive organ effects in the rat were reported at 162 mg/m^2 and included increases in testicular weights (non-recoverable), and prostate and seminal vesicle glandular atrophy. In monkeys, microscopic findings in the epididymis (unilateral interstitium, focal, lymphocyte cell infiltration), testes (unilateral interstitium fibrosis), and decreases in prostate were reported at doses ≥ 48 mg/m^2 which correlated with macroscopic findings of diminished size and weight of the prostate gland, diminished size of seminal vesicles, and increased testes weight. Effects on the male reproductive organs (increases in testes and seminal vesicle weights) were also reported in rats treated with the clinical formulation (120 mg/m^2) which contained the excipient SLS at a concentration of (b)(4)%. Findings in the testis and epididymis were consistent with high levels of [^{14}C]-alectinib found in these organs in rats in a quantitative whole body

autoradiography study. Findings of effects on female reproductive organs were not considered adverse and were limited to decreased ovarian weights at the high dose level in monkeys and mammary gland atrophy at the high dose level in rats.

A standard battery of genotoxicty studies were performed with alectinib. Alectinib was not mutagenic in the in vitro reverse mutation (Ames) assay. Alectinib was positive for clastogenicity, however, in the in vivo micronucleus assay. The chromosomal effects were numerical rather than structural.

Given that SLS is an excipient in the clinical formulation at a concentration of (b)(4)% (w/w SLS to API), but was not tested in the nonclinical repeat-dose toxicity studies, a 28-day bridging repeat-dose study was conducted in rats, based on recommendations of the Agency. Alectinib formulations with and without (b)(4)% SLS were administered at 20 mg/kg/day (120 mg/m^2) by oral gavage. The clinical formulation showed generally comparable systemic exposure and toxicity to the nonclinical formulation, although minimal microscopic findings in the heart (cell infiltration; mononuclear cell, focal), prostate (cell infiltration; lymphocyte), and clinical chemistry (increases in Alpha 2 globulin) occurred in the (b)(4)% SLS-containing group.

Reproductive toxicology:

Male and female fertility and pre-postnatal toxicology developmental studies were not conducted by the Applicant. In the submitted GLP embryofetal dose-range finding studies in rats and rabbits, alectinib administration during organogenesis resulted in abortion, embryo-fetal lethality and structural teratogenicity at maternally toxic doses in both species.

In the dose-range embryofetal developmental study in pregnant rats, alectinib was administered orally throughout organogenesis at doses of 3, 9, and 27 mg/kg/day (18, 54 and 162 mg/m^2). Maternal toxicity (decreases in body weight and food consumption) and total litter loss were reported in all dams (embryo-fetal lethality) at 27 mg/kg/day. At 9 mg/kg/day (approximately 2.7- fold the estimated human $AUC_{ss,24}$ at the recommended dose of 600 mg BID [$AUC_{ss,24}$ 14900 ng.h/ml]), maternal toxicity (decrease in body weight), decreases in fetal weights, increase in visceral (dilated ureter, thymic cord, small ventricle and thin ventricle wall), and skeletal anomalies (decreases in the number of sacral and caudal vertebrae) were reported. The decrease in caudal vertebrae was consistent with the observed growth retardation. The NOAEL for maternal and fetal toxicity was 3 mg/kg/day (approximately 0.9 - fold the estimated human $AUC_{ss,24}$ at the recommended dose).

In the dose range rabbit embryofetal developmental study, pregnant rabbits were dosed orally throughout organogenesis at doses of 3, 9 and 27 mg/kg/day (36, 108 and 324 mg/m^2). Maternal toxicity was observed at an oral dose of 27mg/kg/day (approximately 2.9-fold the estimated human $AUC_{ss,24}$ at the recommended dose). Abortion, increased post-implantation loss, reduced letter size, and decreases in fetal and placental weights were reported at 27 mg/kg/day. Visceral malformation (retroesophageal subclavian)

and skeletal variations (increase in full supernumerary rib and decrease in short supernumerary rib) were reported at 27 mg/kg/day. The NOAEL for maternal and fetal toxicity was 9 mg/kg/day (approximately 0.4-fold the estimated human $AUC_{ss,24}$ at the recommended dose).

Phototoxicity studies:

Alectinib was tested in an *in vitro* cytotoxicity assay with Neutral Red uptake in Balb/c 3T3 mouse fibroblast cell line. A Photo Irritation Factor of 94.8 was calculated, indicating that alectinib has the potential for a phototoxic response.

Table 100: Exposure Margins

Species/sex	Dose (mg/kg/day)	Dose (mg/m^2)	AUC (ng.h/ml)	Human AUC_{ss24} [1] at clinical dose of 600 mg/dose (BID)	Ratio (Animal AUC/Human AUC)
Rat (M)	3	18	5100	14,900 (ng.h/ml)	0.3
	9	54	18600		1.2
	27	162	35300		2.4
Rat (F)	3	18	8450		0.6
	9	54	25800		1.7
	27	162	41100		2.8
Monkey(M)	1.3	15.6	894		0.06
	4	48	3610		0.2
	12	144	7060		0.5
Monkey(F)	1.3	15.6	1030		0.07
	4	48	2810		0.2
	12	144	6920		0.5
Pregnant rat(GD17)	3	18	13850	14,900 (ng.h/ml)	0.9
	9	54	40600		2.7
	27	162	66400		4.5
Pregnant rabbit (GD 18)	3	36	2950	14,900 (ng.h/ml)	0.2
	9	108	6650		0.4
	27	324	43200		2.9

[1]Human $AUC_{ss,24}$ at 600mg BID of 14900 ng.h/mL is based on twice the geometric mean $AUC_{ss,12}$ from the population PK analysis for Phase I/II studies NP28761 and NP28673.

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PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number:	Applicant:	Stamp Date:
208434	Hoffmann-La Roche Inc C/O Genentech Inc	7/06/2015

Drug Name: Alectinib hydrochloride (RO5424802) Or (CH5424802)

NDA Type:

505(b)(1)

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		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
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).	X		Yes, in a type B meeting on July 22, 2013 (Q #9), the division suggested to the Applicant to "consider a 28-day study comparing old toxicology formulation to current clinical formulation". In November 17, 2014, in a type B meeting, the Applicant stated that a 28 day rat toxicology study was conducted (study TOX13). The FDA response was that "based on the data provided in the meeting package, the study appears to be adequate and no additional toxicology studies with SLS are required". The adequacy of the data will be determined during the NDA review.
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		

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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		The following studies followed the Japanese GLPs. Repeat dose Toxicology studies: TOX13-0127, TOX10-0026, TOX10-0027. Reproductive toxicology: TOX12-0024 and TOX12-0019 The impact of non-compliance with the FDA GLP regulations will be assessed as a P/T review issue.
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		Yes, see comment under item # 5.
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		This reviewer is not aware of any pending impurity issues. We defer to CMC for the identification of impurities that may require qualification. Ultimately, this will be determined during the review.
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

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ____yes____

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

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This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

EIAS A ZAHALKA
08/06/2015

EMILY M FOX
08/06/2015