CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

205755Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Zykadia (ceritinib)

Date: April 9, 2014
To: File for NDA 205755
From: John K. Leighton, PhD, DABT
Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting and labeling reviews for Zykadia conducted by Drs. Brower and Fox, and secondary memorandum and labeling provided by Dr. Helms. I concur with Dr. Helms' conclusion that Zykadia may be approved and that no additional nonclinical studies are needed for the proposed indication.

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

/s/

JOHN K LEIGHTON
04/09/2014

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	205,755
Supporting document/s:	1
Applicant's letter date:	December 24, 2013
CDER stamp date:	December 24, 2013
Product:	Zykadia (Ceritinib)
Indication:	ALK-positive Metastatic NSCLC
Applicant:	Novartis, East Hanover, NJ
Review Division:	Division of Hematology Oncology Toxicology
	(Division of Oncology Products 2)
Reviewers:	Margaret Brower, Ph.D., Emily Fox, Ph.D.
Supervisor/Team Leader:	Whitney Helms, Ph.D.
Division Director:	John Leighton, Ph.D.
	(Patricia Keegan, M.D.)
Project Manager:	Karen Boyd

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 205755 are owned by Novartis or are data for which Novartis has obtained a written right of reference.Any information or data necessary for approval of NDA205755 that Novartis 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 205755.

Pharmacology/Toxicology Labeling Review of Zykadia

The following table chronicles the labeling decisions in which the pharmacology/toxicology team had contributions for the initial approval of Zykadia (ceritinib) for the treatment of patients with (b) (4) metastatic non-small cell lung cancer (NSCLC) who have (b) (4) crizotinib.

The Applicant Proposed	FDA Recommends	Reasoning
Highlights **INDICATIONS AND USAGE** (b) (4) is a kinase inhibitor indicated for the treatment of patients with (b) (4) metastatic non-small cell lung cancer (NSCLC) who have (b) (4) (b) (4) Embryofetal Toxicity: (b) (4) (b) (4) cause fetal harm (b) (4) 8.1)	ZYKADIA is a kinase inhibitor indicated for the treatment of patients with anaplastic lymphoma kinase (ALK)-positive metastatic non-small cell lung cancer (NSCLC) who have progressed on or are intolerant to crizotinib. Embryofetal Toxicity: ZYKADIA may cause fetal harm. Advise females of reproductive potential of the potential risk to a fetus. (5.7, 8.1, 8.7)	Established Pharmacologic Class (EPC): The EPC is based on the mechanism of action for ceritinib. The EPC was discussed with the product team. Use in pregnancy: Label was updated to reflect CFR.
5 WARNINGS AND PRECAUTIONS (b) (4) **Embryofetal Toxicity** (b) (4)	**5 WARNINGS AND PRECAUTIONS** **5.7 Embryofetal Toxicity** Based on its mechanism of action, ZYKADIA may cause fetal harm when administered to a pregnant woman. In animal studies, administration of ceritinib to rats and rabbits during organogenesis at maternal plasma exposures below the recommended human dose of 750 mg daily caused increases in skeletal anomalies in rats and rabbits. Apprise women of reproductive potential of the potential hazard to a fetus *[see Use in Specific Populations (8.1)]*. Advise females of	Label was updated to reflect results of reproductive toxicology studies submitted to the NDA. Duration of contraception use in females was based on the half-life of ceritinib and potential risks to a fetus due to the mechanism of action of ceritinib as an inhibitor of ALK signaling on fetal toxicities seen in animal studies. Label was updated to reflect CFR.

(b) (4)	reproductive potential to use effective contraception during treatment with ZYKADIA and for at least 2 weeks following completion of therapy *[see Use in Specific Populations (8.7)]*.	

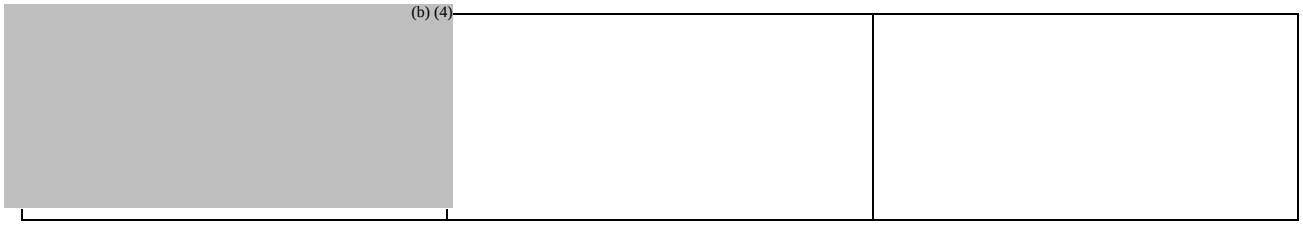
8 USE IN SPECIFIC POPULATIONS **8.1 Pregnancy** **Pregnancy Category D** (b) (4) (b) (4)	**8 USE IN SPECIFIC POPULATIONS** **8.1 Pregnancy** **Pregnancy Category D** *Risk Summary* Based on its mechanism of action, ZYKADIA may cause fetal harm when administered to a pregnant woman. In animal studies, administration of ceritinib to rats and rabbits during organogenesis at maternal plasma exposures below the recommended human dose caused increases in skeletal anomalies in rats and rabbits. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, apprise the patient of the potential hazard to a fetus. *Animal Data* In an embryo-fetal development study in which pregnant rats were administered daily doses of ceritinib during organogenesis, dose-related skeletal anomalies were observed at doses as low as 50 mg/kg (less than 0.5-fold the human exposure by AUC at the recommended dose). Findings included delayed ossifications and skeletal variations. In pregnant rabbits administered ceritinib daily during organogenesis, dose-related skeletal anomalies, including incomplete ossification, were observed at doses equal to or greater than 2 mg/kg/day (approximately 0.015-fold the human exposure by AUC at the recommended dose). A low incidence of visceral anomalies, including absent or malpositioned gallbladder and retroesophageal	Label was updated to reflect results of reproductive toxicology studies submitted to the NDA. Exposure ratios were updated to reflect comparisons to human exposure by AUC. Label was updated using a hybrid approach while PLLR is being finalized. .

	subclavian cardiac artery, was observed at doses equal to or greater than 10 mg/kg/day (approximately 0.13-fold the human exposure by AUC at the recommended dose). Maternal toxicity and abortion occurred in rabbits at doses of 35 mg/kg or greater. In addition, embryolethality was observed in rabbits at a dose of 50 mg/kg.	
8.3 Nursing Mothers It is not known whether (b) (4) (b) (4) in human milk. Because many drugs are (b) (4) in human milk and because of the potential for serious adverse reactions in nursing infants from (b) (4) (b) (4) o discontinue nursing (b) (4)	**8.3 Nursing Mothers** It is not known whether ceritinib or its metabolites are present in human milk. Because many drugs are present in human milk and because of the potential for serious adverse reactions in nursing infants from ceritinib, advise mothers to discontinue nursing.	Label was updated in accordance with CFR.
	8.7 Females and Males of Reproductive Potential *Contraception* Based on its mechanism of action, ZYKADIA may cause fetal harm when administered to a pregnant woman *[see Use in Specific Populations (8.1)]*. Advise females of reproductive potential to use effective contraception during treatment with ZYKADIA and for at least 2 weeks following completion of therapy.	New section of the label was added as proposed by PLLR. Duration of contraception use in females was based on the half-life of ceritinib because of potential risks to a fetus due to the mechanism of ceritinib as an inhibitor of ALK signaling and on fetal toxicities seen in animal studies.
12.1 Mechanism of Action Ceritinib is an (b) (4) kinase inhibitor. Ceritinib inhibits autophosphorylation of ALK, ALK-mediated phosphorylation of downstream signaling protein (b) (4) and proliferation of ALK-dependent cancer cells (b) (4) *in*	**12.1 Mechanism of Action** Ceritinib is a kinase inhibitor. Targets of ceritinib inhibition identified in either biochemical or cellular assays at clinically relevant concentrations include ALK, insulin-like growth factor 1 receptor (IGF-1R), insulin receptor (InsR), and ROS1.	Some language was removed because it was considered to be unnecessary, promotional, or not fully supported by submitted data. Insulin-like growth factor 1 receptor (IGF-1R), insulin

vitro and *in vivo*. (b) (4) **12.2 Pharmacodynamics** (b) (4)	Among these, ceritinib is most active against ALK. Ceritinib inhibited autophosphorylation of ALK, ALK-mediated phosphorylation of the downstream signaling protein STAT3, and proliferation of ALK-dependent cancer cells in in vitro and in vivo assays. Ceritinib inhibited the in vitro proliferation of cell lines expressing EML4-ALK and NPM-ALK fusion proteins and demonstrated dose-dependent inhibition of EML4-ALK-positive NSCLC xenograft growth in mice and rats. Ceritinib exhibited dose-dependent anti-tumor activity in mice bearing EML4-ALK-positive NSCLC xenografts with demonstrated resistance to crizotinib, at concentrations within a clinically relevant range.	receptor (InsR), and ROS1 were added to indicate the kinases inhibited by ceritinib at potentially clinically relevant concentrations. (b) (4) . Pertinent nonclinical pharmacology information was moved to section 12.1 and condensed to reflect the pharmacology data supported by information submitted to the NDA. "downstream signaling proteins" was changed to "downstream signaling protein STAT3" because STAT3 was the only downstream signaling protein that the Applicant demonstrated to be inhibited by ceritinib. "Ceritinib exhibited dose-dependent anti-tumor activity in mice bearing EML4-ALK-positive NSCLC xenografts with demonstrated resistance to crizotinib, at concentrations within a clinically relevant range" was added because it explains the mechanism of ceritinib in the indicated patient population.

(b) (4)		
12.3 Pharmacokinetics Distribution (b) (4)		This information was moved from Section 12.3 to Section 13.2 because it describes nonclinical animal data and not clinical data.
13 NONCLINICAL TOXICOLOGY **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** (b) (4)	**13 NONCLINICAL TOXICOLOGY** **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** Carcinogenicity studies have not been performed with ceritinib. Ceritinib was not mutagenic in vitro in the bacterial reverse mutation (Ames) assay but induced numerical aberrations (aneugenic) in the in vitro cytogenetic assay using human lymphocytes, and micronuclei in the in vitro micronucleus test using TK6 cells. Ceritinib was not clastogenic in the in vivo rat micronucleus assay. There are no data on the effect of ceritinib on human fertility. Fertility/early embryonic development studies were not conducted with ceritinib. There were no adverse effects on male or female reproductive organs in general toxicology studies conducted in monkeys and rats at exposures equal to or greater than 0.5- and 1.5-fold, respectively, of the human exposure by AUC at the recommended dose of 750 mg.	Label was updated in accordance with CFR and edited for brevity. Potential for aneugenic activity was added based on review of data submitted to the NDA. Data on embryofetal development studies was removed as this data is reflected in Section 8.1 Label was updated to reflect reproductive toxicity (fertility/effects on reproductive organs) data submitted to the NDA. Exposure ratios were updated to reflect comparisons to human exposure by AUC.

13.2 Animal Toxicology and/or Pharmacology (b) (4)	**13.2 Animal Toxicology and/or Pharmacology** Target organs in nonclinical animal models included, but were not limited to, the pancreas, biliopancreatic/bile ducts, gastrointestinal tract, and liver. Pancreatic focal acinar cell atrophy was observed in rats at 1.5-fold the human exposure by AUC at the recommended dose. Biliopancreatic duct and bile duct necrosis was observed in rats at exposures equal to or greater than 5% of the human exposure by AUC at the recommended dose. Bile duct inflammation and vacuolation were also noted in monkeys at exposures equal to or greater than 0.5-fold the human exposure by AUC at the recommended dose. Frequent minimal necrosis and hemorrhage of the duodenum was exhibited in monkeys at 0.5-fold the human exposure by AUC, and in rats at an exposure similar to that observed clinically. Ceritinib crossed the blood brain barrier in rats with a brain-to-blood exposure (AUC_{inf}) ratio of approximately 15%.	(b) (4) were removed since clinical data on QT prolongation has been added to the label. Label was updated to reflect the potential for target organ toxicity (pancreas and biliopancreatic/bile duct) exhibited in repeat-dose toxicology studies submitted to the NDA and not clearly reflected in other sections of the label. Exposure ratios were updated to reflect comparisons to human exposure by AUC. Blood-brain barrier exposure data moved from Section 12.3.


(b) (4)

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

/s/

MARGARET E BROWER
04/09/2014

EMILY M FOX
04/09/2014

WHITNEY S HELMS
04/09/2014

MEMORANDUM

Date: March 27, 2014
From: Whitney S. Helms, Ph.D.
Pharmacology Supervisor
Division of Hematology Oncology Toxicology for Division of Oncology Products 2
To: File for NDA # 205755
Zykadia (ceritinib)
Re: Approvability of Pharmacology and Toxicology

On December 24, 2013 Novartis completed the submission of New Drug Application (NDA) 205755 for ceritinib for the treatment of patients with (b) (4) metastatic non-small cell lung cancer (NSCLC) who have (b) (4) with crizotinib. Ceritinib received breakthrough designation on March 6, 2013, for the treatment of patients with metastatic NSCLC that is ALK-positive as detected by an FDA-approved test and which had progressed during treatment with crizotinib or where patients were intolerant to crizotinib. Non-clinical studies examining the pharmacology and toxicology of ceritinib provided to support NDA 205755 were reviewed in detail by Margaret E. Brower, PhD, and Emily M. Fox, PhD. The findings of these studies are summarized in the "Executive Summary" of the NDA review and reflected in the product label.

Ceritinib is a small molecule kinase inhibitor administered orally at the recommended dose of 750 mg daily. In the clinical trials used to support the approval of ceritinib at this dose level, patient exposure was approximately 1010 ng/mL (~1.8 μM) and 22600 ng*hr/mL by Cmax and AUC, respectively. Ceritinib was highly protein bound in all species tested, ≥ 97%; thus, a Cmax of 1.8 μM would result in a free drug concentration of approximately 50 nM in plasma at the recommended dose. In a limited biochemical screening assay of 36 kinases, ceritinib was able to inhibit the anaplastic lymphoma kinase (ALK) as well as two other members of the insulin receptor superfamily, InsR and IGF-1R, with IC_{50}s of less than 10 nM. In cellular assays ROS1 was identified as another potential target of ceritinib inhibition at concentrations that are clinically relevant.

Ceritinib was able to inhibit tumor growth in mouse and rat xenograft models using tumor cell lines with ALK overexpression or harboring ALK fusion proteins (EML4-ALK and NPM-ALK). Ceritinib also showed anti-proliferative activity against Ba/F3 cell lines transduced with EML4-ALK expressing secondary mutations in the ALK kinase domain associated with acquired crizotinib resistance (I1171T, L1196M, C1156Y, S1206A, and G1269S). Additional proof-of-concept studies were conducted using xenograft models of lung cell tumors harboring EML4-ALK fusion proteins with some of the same crizotinib-resistance mutations in the ALK kinase domain. In these models ceritinib showed increased anti-tumor activity compared to crizotinib, supporting the use of ceritinib in patients with ALK positive tumors who have progressed after treatment with crizotinib.

In animal studies ceritinib was absorbed slowly and had moderate bioavailability. While absolute bioavailability of the drug has not been determined in humans, it is predicted to be

low—around 25%. Certinib had a long terminal half-life in all species tested. Elimination was primarily through the fecal route in animals and humans. As predicted by its high volume of distribution, certinib was widely distributed in rat tissues with high levels of the drug observed in the GI tract, liver, and lungs, all target organs identified clinically.

The major target organs identified in general toxicology studies conducted in rats and monkeys included the pancreas, biliopancreatic ducts, bile ducts, gastrointestinal tract, and liver. Gastrointestinal toxicity and hepatotoxicity were observed clinically and are included in the Warnings and Precautions section of the label for ceritinib. The lungs were also identified as a target organ in rats with phospholipidosis observed following 4 weeks of dosing at 1.5-fold clinical exposure, and lung macrophage aggregates following 13 weeks of dosing at similar exposures to that observed clinically. These findings correlate with pneumonitis and interstitial lung disease observed clinically.

Descriptions of pancreatic toxicity were included the Nonclinical Toxicology section of the label. Increases in amylase and lipase were observed in clinical trials and the possibility of an association of ceritinib use with pancreatitis was explored by the clinical review team. In animals, high levels of ceritinib were present in the pancreas in distribution studies and pancreatic atrophy was observed in monkeys and in rats at doses resulting in exposures 0.15 and 1.5-fold the ceritinib exposure at the recommended dose. Effects on the biliopancreatic and bile ducts were also observed in animals at exposures lower than the clinical exposure to ceritinib. Hyperglycemia was also noted clinically and, based on nonclinical findings, may potentially be related to pancreatic effects of the drug or to the pharmacological inhibition of IGF-1R and InsR by ceritinib.

In a CNS safety pharmacology study no significant behavioral or physiological changes were observed following a single dose of certinib. The drug does, however, cross the blood brain barrier. In rats exposure to ceritinib in the brain was approximately 15% that of the exposure in the plasma by AUC. Convulsions were noted in clinical trials using ceritinib, though the large proportion of patients with brain metastases prevents a clear attribution of this finding to the drug. Ceritinib demonstrated potential for causing QTc prolongation in the in vitro hERG assay as well as in vivo in a single dose cardiac safety pharmacology conducted in monkeys. QTc prolonation has been noted clinically and is included in the Warnings and Precautions section of the label.

Carcinogenicity studies were not conducted using ceritinib and are not required for approval for a drug indicated for the treatment of patients with (b) (4) cancer. Ceritinib was not mutagenic in the bacterial reverse mutation assay but was aneugenic in both an in vitro cytogenetic assay using human lymphocytes and an in vitro micronucleus test using TK6 cells.

Reproductive studies investigating the effects of ceritinib on fertility or on post-natal development were not submitted and were not required for approval in this patient population. No adverse effects on male or female reproductive organs were noted in general toxicology studies. In embryofetal development studies conducted in both rats and rabbits, findings were primarily limited to delayed ossifications and skeletal variations. Additional findings in rabbits treated with ceritnib during organogenesis included absent or malpositioned gallbladder and

retroesophageal subclavian cardiac artery. Maximum ceritinib exposures at the highest doses tested in both species were, however, significantly lower than the exposure in humans at the clinically recommended dose and, in rabbits, higher doses of ceritinib resulted in significant maternal toxicity and abortion. In addition, ALK inhibition has been reported to be associated with fetal toxicities including effects on neural development[1-2]. Pregnancy category D is recommended and females of reproductive potential are advised to use contraception during treatment with ceritinib and, based on the half-life in humans of approximately 41 hours, for up to 2 weeks following cessation of treatment.

Recommendations: I concur with the conclusion of Drs. Brower and Fox that the pharmacology and toxicology data support the approval of NDA 205755 for ZYKADIA. There are no outstanding nonclinical issues related to the approval of ZYKADIA for the treatment of patients with (b) (4) metastatic NSCLC who have (b) (4) with crizotinib.

[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

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

/s/

WHITNEY S HELMS
03/27/2014

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	205,755
Supporting document/s:	1
Applicant's letter date:	December 24, 2013
CDER stamp date:	December 24, 2013
Product:	Zykadia (Ceritinib)
Indication:	ALK-positive Metastatic NSCLC
Applicant:	Novartis, East Hanover, NJ
Review Division:	Division of Hematology Oncology Toxicology
	(Division of Oncology Products 2)
Reviewers:	Margaret Brower, Ph.D., Emily Fox, Ph.D.
Supervisor/Team Leader:	Whitney Helms, Ph.D.
Division Director:	John Leighton, Ph.D.
	(Patricia Keegan, M.D.)
Project Manager:	Karen Boyd

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 205755 are owned by Novartis or are data for which Novartis has obtained a written right of reference.
Any information or data necessary for approval of NDA205755 that Novartis 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 205755.

TABLE OF CONTENTS

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

NDA 205,755 has been submitted as a full New Drug Application (NDA) with breakthrough therapy designation for ceritinib, indicated for the treatment of patients with (b) (4) metastatic non-small cell lung cancer (NSCLC) who have (b) (4) with crizotinib. Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase involved in neuronal cell differentiation and regeneration; its expression in normal human tissues is restricted to scattered neural cells, pericytes, and endothelial cells in the brain. Translocations involving the ALK tyrosine kinase domain can result in oncogenic fusion proteins with dysregulated expression and constitutive tyrosine kinase activity, leading to activation of downstream signaling pathways, cell transformation, and increased cell survival. Approximately 2-7% of non-small cell lung cancers (NSCLC) express a novel translocation in which ALK is fused to the echinoderm microtubule-associated protein like protein 4 (EML4). Ceritinib is a small molecule kinase inhibitor with activity at the ALK receptor. The recommended clinical dose of ceritinib is 750 mg administered orally as a capsule once daily; this resulted in a C_{max} and AUC of 1010 ng/mL and 22600 ng·h/mL, respectively, in clinical trials used to support its approval. Nonclinical pharmacology, pharmacokinetic, and toxicology studies have been submitted to support the approval of ceritinib for the proposed indication.

1.2 Brief Discussion of Nonclinical Findings

Against a panel of 36 recombinant human kinases, ceritinib exhibited strong inhibition of ALK (IC_{50} = 0.15 nM); this inhibition was approximately 50-fold higher than its inhibition of other members of the insulin receptor superfamily (InsR; IC_{50} = 7 nM and IGF-1R; IC_{50} = 8 nM) included in the panel and 700-fold more selective for ALK than for the 33 other kinases. In cellular assays ceritinib exhibited anti-proliferative activity against Ba/F3 cells transduced with constitutively active NPM-ALK (IC_{50} = 35 nM), EML4-ALK (IC_{50} = 27 nM), TEL-ALK (IC_{50} = 56 nM), TEL-IGF-1R (IC_{50} = 220 nM), TEL-InsR (IC_{50} = 400 nM), and TEL-ROS1 (IC_{50} = 180 nM), another member of the insulin receptor superfamily. Incubation with ceritinib resulted in a concentration-dependent decrease in the phosphorylation of ALK in vitro and its downstream mediator STAT3 in vitro and in vivo. Treatment with ceritinib inhibited the in vitro proliferation of human NSCLC H2228 (IC_{50} = 11 nM) and Anaplastic Large Cell Lymphoma (ALCL) Karpas299 (IC_{50} = 45 nM) cell lines expressing ALK translocations (EML4-ALK and NPM-ALK, respectively), and human neuroblastoma cells with ALK amplification (IC_{50} = 24 nM). Daily dosing with ceritinib resulted in dose-dependent inhibition of H2228 and Karpas299 xenograft growth in SCID mice and nude rats and induced tumor regression at exposures achievable in the clinic at the recommended dose of ceritinib. Pharmacodynamic studies suggested that a reduction in the ALK signaling pathway of ≥ 70% may be required to achieve tumor regression with ceritinib. Further, ceritinib exhibited inhibitory activity in in vitro and in vivo models of NSCLC with demonstrated resistance to crizotinib, a previously approved inhibitor of ALK, at concentrations within a clinically relevant range.

Nonclinical PK studies indicated that an oral dose of ceritinib exhibited a slow rate of absorption in monkeys, rats, and mice, based on Tmax. Oral bioavailability was moderate in all three species (~43-58%). According to the Applicant, the absolute bioavailability of ceritinib has not been determined in humans. Ceritinib exhibited a relatively long terminal half-life in monkeys and rats following either oral (~12-16 hrs) or IV administration (~10-29 hrs), which was less than that observed in humans (~40 hrs). Following IV administration, ceritinib exhibited low to moderate clearance in rats, monkeys, and mice. Ceritinib distributed slightly more to red blood

cells than plasma in all species tested. The steady-state volume of distribution of ceritinib was high in all three species (6.5-20 L/kg), indicating extensive distribution into tissues. Consistent with this, in vivo distribution studies indicated that ceritinib was widely distributed to most rat tissues, with tissue concentrations generally higher than those observed in blood. Following administration of a single oral dose of radiolabelled ceritinib, concentrations of the drug were the highest in the colon wall and the small intestine wall. Ceritinib concentrations were also high in the GI tract, liver, and lungs, consistent with observations of GI toxicity, increased ALT and AST, and pulmonary toxicity in the clinic. Further, ceritinib exhibited high distribution to the pancreas, potentially associated with symptoms of acute pancreatitis observed in the clinic. In addition, in rats, ceritinib crossed the blood brain barrier with a brain AUC_{inf} of 15% that of the plasma exposure. Convulsions have been noted in patients administered ceritinib, although existing brain metastases in these patients confounds attributing these events to ceritinib. Other ALK inhibitors (e.g. crizotinib) have also been seen to cross the blood brain barrier, without noted adverse effects in the clinic.

Ceritinib exhibited strong plasma protein binding (94.7-98.5%) in all species tested. Ceritinib was 97.2% bound to human plasma protein, resulting in a free drug concentration of approximately 50.7 nM in humans at the recommended dose of ceritinib. Of the 36 recombinant human kinases tested in the biochemical screening panel, ceritinib only inhibited ALK, InsR, and IGF-1R at free drug concentrations achievable in the plasma at the recommended dose of ceritinib, though, based on distribution studies, concentrations in the tissues can be much higher. In cellular assays using cell lines dependent on c-ros1 oncogene (ROS1) activity for growth, incubation with ceritinib resulted in inhibition of the cells at concentrations lower than those necessary to cause InsR or IGF-1R-dependent cell line growth inhibition, suggesting that ROS1 is also a clinically relevant target of ceritinib.

In vivo excretion studies demonstrated that elimination was primarily through the fecal route with both GI and biliary contributions. The nonclinical results were consistent with findings in the clinic, where 91% of the radioactive dose of ceritinib in humans was eliminated in the feces, and only 1.3% was eliminated in the urine. Ceritinib was not extensively metabolized. Following a single IV or oral dose of radiolabelled ceritinib to monkeys or rats, the parent compound was the major component in plasma (~84-100% of the total drug-related AUC) and feces (~60-80% of the administered oral dose). The parent compound was also the main component in human plasma and feces following a single oral 750 mg dose of ceritinib. According to the Applicant, eleven metabolites were found in human plasma, each at levels ≤ 2.3% of the total drug-related AUC; no single metabolite contributed more than 5.8% to the overall plasma radioactivity AUC in the study. Five of the eleven metabolites were not detected in rat or monkey plasma, but two of these five were observed in rat and monkey feces. Given the low levels of the metabolites found only in human plasma and the indicated (b) (4) cancer patient population, no further evaluation of three remaining metabolites is required at this time.

Ceritinib was not mutagenic *in vitro* in the bacterial reverse mutation (Ames) assay but was aneugenic (induced numerical aberrations) in both the *in vitro* cytogenetic assay using human lymphocytes and in micronuclei in the *in vitro* micronucleus test using TK6 cells. Ceritinib was not clastogenic in the *in vivo* mouse micronucleus assay. Seven process impurities were assayed by (b) (4) screening and predicted to be genotoxic, but were controlled to acceptable levels for a drug intended for the treatment of patients with cancer. Ceritinib was found to be phototoxic *in vitro* in Balb/c 3T3 fibroblast cells, and showed association with melanin in distribution studies, but showed no phototoxic potential following administration to mice at dose levels of up to 300 mg/m^2 for 3 days.

In a functional observational battery (FOB) in rats, significant behavioral and physiological changes were not observed following single doses of 600 mg/m^2 ceritinib. In this same study, transient but significantly higher breaths per minute (BPM) (~20%) were observed from 35-60 minutes following single doses of up to 600 mg/m^2 ceritinib.

Ceritinib significantly inhibited hERG channel activity when tested at concentrations of 0.3, 0.4, 1 and 2.4 μM in safety pharmacology studies. The IC_{50} for the inhibitory effect of ceritinib on hERG potassium current was 0.4 μM. In monkeys, administered a single dose of ceritinib at dose levels of 120 or 360 mg/m^2 there was little effect on cardiovascular parameters, although 10-19 hours following administration of 1200 mg/m^2, one of four animals exhibited QT/QTc prolongation at 14-44 ms above predose baseline. QT prolongation has also been noted clinically. No drug-related effects were observed on blood pressure, heart rate or body temperature. In an earlier non-GLP telemetry study in monkeys, no electrocardiography changes were observed following a single dose of 3000 mg/m^2 ceritinib. There were no adverse cardiovascular findings observed in rats or monkeys administered ceritinib for up to 13 weeks in general toxicology studies at exposures equal to or greater than 0.5 or 1.5-fold, respectively, the human exposure by AUC.

Target organs of ceritinib-mediated toxicity were identified in rats and monkeys dosed for up to 13 weeks, and included the pancreas, biliopancreatic ducts, bile ducts, gastrointestinal tract and liver. Marked pancreatic atrophy and inflammation was observed following 4 weeks of ceritinib administration in monkeys and rats at 0.15 and 1.5-fold respectively, the human exposure by AUC at the recommended dose. Correlating with these findings, persistent amylase or lipase elevations were reported in the 13-week toxicology studies in both species. In humans, increased lipase has been reported. Consistent with clinical findings of hyperglycemia, insulin levels were also increased 2-3-fold in monkeys treated for 13 weeks.

Erosion, degeneration/necrosis, inflammation, hyperplasia, dilatation and vacuolation of the biliopancreatic duct and inflammation and dilatation of the bile duct were observed following 4 and 13 weeks of ceritinib administration in rats at exposures ≥ 5% of human exposure by AUC at the recommended dose; the same findings were observed following up to 8 weeks of recovery at a similar incidence. Bile duct hemorrhage and inflammation (cystic bile duct, hepatic bile duct and common bile duct) were also exhibited in monkeys dosed for 13 weeks at exposures equal to or greater than 50% the human exposure by AUC. Necrosis, hyperplasia and hemorrhage of the duodenum were observed at 13 weeks in monkeys at ceritinib exposures 50% of the clinical exposure and in rats at an exposure similar to that observed clinically.

Ceritinib-related hepatotoxic changes were generally reflective of treatment-related systemic toxicity in rats and monkeys and characterized by significant elevations in liver enzymes; similar increases have been noted clinically. In 4 week studies in rats, AST and ALT parameters were elevated 2-4 fold at dose levels resulting in exposures that approximate clinical exposures at the recommended dose of 750 mg. Additional target sites included the lungs with phospholipidosis in rats following 4 weeks of dosing at 1.5-fold clinical exposure, and lung macrophage aggregates in rats following 13 weeks of dosing at similar exposures to that observed clinically. This correlates with pneumonitis and interstitial lung disease observed clinically.

Fertility/early embryonic development studies were not conducted with ceritinib. There were no adverse effects on male or female reproductive organs in general toxicology studies conducted in monkeys and rats at exposures equal to or greater than 0.5 and 1.5-fold, respectively, the human exposure by AUC.

Administration of ceritinib to pregnant rats during the period of organogenesis resulted in dose-related skeletal anomalies at doses resulting in maternal exposures less than 50% the human exposure by AUC at the recommended dose. Findings included delayed ossifications and skeletal variations. ceritinib did not induce embryolethality or fetotoxicity at doses tested in rats. In pregnant rabbits administered ceritinib daily during organogenesis, dose-related skeletal and visceral anomalies, including incomplete ossification, absent or malpositioned gallbladder and retroesophageal subclavian cardiac artery were observed at doses ≥ 118 mg/m^2 (approximately 13% of the human exposure by AUC at the recommended dose). Higher doses resulted in significant maternal toxicity and abortion in rabbits. In addition, ALK inhibition has been reported to be associated with fetal toxicities including effects on neural development[1-2]. Pregnancy category D is recommended. The half-life of ceritinib is ~41 hours at the recommended clinical dose; it is advised that females of reproductive potential use effective contraception during treatment with ceritinib and for up to 2 weeks following cessation of treatment.

1.3 Recommendations

1.3.1 Approvability

Recommended for approval. The non-clinical studies submitted to this NDA provide sufficient information to support the use of ceritinib for the treatment of patients with (b) (4) metastatic NSCLC who have progressed (b) (4) with crizotinib.

1.3.2 Additional Non Clinical Recommendations

1.3.3 Labeling

A separate labeling review will be provided.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number: N/A

2.1.2 Generic Name: None

2.1.3 Code Name: LDK378

[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

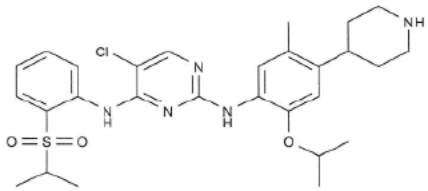
2.1.4 Chemical Name:

5-chloro-N2-[2-isopropoxy-5-methyl-4-(4-piperidinyl)phenyl]-N4-[2-(isopropylsulfonyl)phenyl]-2,4-pyrimidinediamine

2.1.5 Molecular Formula/Molecular Weight:

$C_{28}H_{36}N_5O_3ClS$/ 558.2

2.1.6 Structure



2.1.7 Pharmacologic Class:

Inhibitor of Anaplastic Lymphoma Kinase (ALK)

2.2 Relevant IND/s:

IND 109,272

2.3 Clinical Formulation

2.3.1 Drug Formulation

Ceritinib (b) (4) is manufactured by (b) (4) and packaged by the following manufacturing sites:

Site	Activities performed
Novartis Pharma Stein AG Schaffhauserstrasse 4332 Stein, Switzerland	Manufacture of LDK378 hard gelatin capsules Analytical testing of LDK378 hard gelatin capsules
(b) (4)	(b) (4)
Novartis Pharmaceuticals Corporation 25 Old Mill Road, Suffern, New York 10901, USA	Stability testing of LDK378 hard gelatin capsules

Excerpted from Applicant's submission

During development, (b) (4) were used for manufacture, with (b) (4) identified for commercial use.

Table 1: Composition of ceritinib drug product 150 mg hard gelatin capsule

Ingredient	Function	Amount/150 mg capsule (mg)
LDK378	Active ingredient	150
Microcrystalline cellulose		(b) (4)
(b) (4) Hydroxypropyl cellulose		
Sodium starch glycolate		
Magnesium stearate		
(b) (4) Colloidal (b) (4)		
Empty capsule shell, pre-printed		

LDK378 hard gelatin capsules are packaged in high density polyethylene bottles.

2.3.2 Comments on Novel Excipients:

Excipients meet the US Pharmacopeia/National Formulary standards.

2.3.3 Impurities – Comments and Qualification

Seven process impurities were assayed by (b) (4) screening and predicted to be genotoxic, but were controlled to acceptable levels for a drug intended for the treatment of patients with cancer. Six of these impurities were monitored and controlled in 18 consecutive development batches of drug substance at levels (b) (4) a level which corresponds to a clinical intake of (b) (4) (b) (4) in patients treated at the recommended dose of ceritinib. Based on the consistency of this data the nonclinical and CMC review teams were in agreement that specifications for each of these impurities were not necessary to ensure product quality. Due to its instability, the 7th potential genotoxic impurity, (b) (4) The (b) (4) was not identified as potentially genotoxic according to in-silico screening. This (b) (4) was monitored in development batches of drug substance at (b) (4) which corresponds to a clinical intake of (b) (4) based on the recommended clinical dose of 750 mg/day.

Individual non-genotoxic impurities specified in LDK378, impurities (b) (4) were acceptably qualified at the proposed specification limit of NMT (b) (4) in the 4-week rat toxicology study # 0970416. The daily intake for these impurities resulting from the proposed clinical dose of 750 mg (b) (4) . The nonclinical batch (batch # 0850001) used for Study # 0970416 contained (b) (4) At the highest dose administered to rats at which there was no mortality (75 mg/kg), the animal impurity intake was (b) (4) respectively, which is equal to or above the proposed intake of the impurity in human at the proposed clinical dose. The individual proposed release/stability acceptability criteria for all other specified and unspecified impurities is NMT (b) (4) which is below the ICH Q3B(R2) qualification threshold.

2.3.4 Metabolites

LDK378 was not extensively metabolized. The parent compound was the main component in the plasma and feces of both humans and the nonclinical species used to examine the safety of

the drug. According to the Applicant, eleven metabolites were found in human plasma at levels each at levels ≤ 2.3% of the total drug-related AUC; no single metabolite contributed more than 5.8% to the plasma radioactivity AUC of any individual in the study. Five of the eleven metabolites were not detected in rat or monkey plasma, but two of these five were observed in rat and monkey feces. Given the low levels of the metabolites found only in human plasma and the indicated (b) (4) cancer patient population, no further evaluation of three remaining metabolites is required at this time. No data on metabolite accumulation in plasma following repeat dosing is available.

2.6 Proposed Clinical Population and Dosing Regimen

Patient dose and schedule: The proposed clinical dose of 750 mg is administered orally as five 150 mg gelatin capsules once daily (b) (4) at least 2 hours before and 2 hours after the ingestion of food. Co-administration of ceritinib with strong CYP3A4 inhibitors increases plasma concentrations of the drug, and should be avoided.

2.7 Regulatory Background

IND 109,272 for LDK378 was initially submitted to the Office of Hematology and Oncology Products in October, 2010. The Applicant sought accelerated approval under Subpart H for patients with (b) (4) metastatic non-small cell lung cancer (NSCLC) who have (b) (4). Based on clinical study X2101 in patients with ALK-positive NSCLC and the proposed design for Study A2303, Ceritinib was granted Breakthough therapy designation on March 6, 2013 for the treatment of patients with metastatic NSCLC that is ALK-positive as detected by an FDA-approved test and which had progressed during treatment with crizotinib or where patients were intolerant to crizotinib.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology

Study #	Study title
RD-2010-00649	NVP-LDK378: In vitro effects against a selection of recombinant human protein kinases
RD-2010-00651	NVP-BQK827: In vitro effects against a selection of recombinant human protein kinases
RD-2010-50499	NVP-LDK378: *In vitro* Safety Pharmacology Profile
RD-2008-50904	(b) (4) individual test IC_{50} values for NVP-LDK378-NX-2
RD-2008-50906	Safety Receptor Panel Profiling of LDK378: Individual test EC_{50} and IC_{50} values
RD-2009-50672	In vitro antiproliferative activity of NVP-LDK378 in Ba/F3 cells transduced with ALK fusion kinases
RD-2009-50673	In vitro antiproliferative activity of NVP-LDK378 in Ba/F3 cells transduced with TEL-FES and TEL-FER fusion kinases
RD-2009-50670	*In vitro* antiproliferative activity of NVP-LDK378 in Ba/F3 cells transduced with constitutively activated tyrosine kinases

Study #	Study title
2008-50914	*In vitro* antiproliferative activity of NVP-LDK378 in Karpas299 (ALCL model expressing NPM-ALK) and NCIH2228 (NSCLC model expressing EML4-ALK)
RD-2010-50442	*In vitro* antiproliferative activity of NVP-LDK378 in neuroblastoma cell line NB-1 with ALK amplification
RD-2013-50316	Single agent activity of LDK378 and ALK expression levels in a panel human non-small cell lung cancer cell lines
RD-2008-50926	In vivo evaluation efficacy and exposure of NVP-LDK378-AZ-1 in a subcutaneous tumor model of the human nonsmall cell lung cancer cell line H2228 in SCID beige mice
RD-2008-50933	In vivo evaluation of efficacy and exposure of NVP-LDK378-AZ-1 in a subcutaneous tumor model of the human non-small cell lung cancer cell line H2228 in nude rats
RD-2008-50927	In vivo evaluation efficacy and exposure of NVP-LDK378-AZ-1 in a subcutaneous tumor model of human anaplastic large-cell lymphoma Karpas299 in SCID beige mice
RD-2008-50942	In vivo evaluation of efficacy and exposure of NVP-LDK378-AZ-1 in a subcutaneous tumor model of the human anaplastic large-cell lymphoma Karpas299 in nude rats
RD-2008-50976	In vivo pharmacodynamic and pharmacokinetic studies of NVP-LDK378-AZ-1 in an orthotopic model of human anaplastic large cell lymphoma Karpas299-Luc
RD-2013-00413	Addendum to RD-2008-50976 NVP-LDK378 does not inhibit the IGF-1R pathway *in vivo*
RD-2010-50507	Evaluation of effects of ALK inhibitor LDK378 on glucose tolerance and indicators of insulin resistance in wild-type C57BL/6J mice
RD-2010-50501	Cytochrome p450 3A4 induction potential of the ALK inhibitor NVP-LDK378 by measuring activation of human PXR in a reporter gene assay
RD-2013-50375	*In vitro* antiproliferative activity of NVP-LDK378 in Ba/F3 cells transduced with crizotinib resistant mutant EML4-ALK fusion kinases
RD-2013-50301	Comparison of *in vivo* efficacy of NVP-LDK378-NX-4 with Crizotinib in the H2228 lung cancer xenograft model
RD-2013-50300	In Vivo efficacy of NVP-LDK378-NX-4 in Crizotinib resistant H2228 lung cancer xenograft model carrying the I1171T mutation
RD-2013-50302	In Vivo efficacy of NVP-LDK378-NX-4 in the Crizotinib resistant H2228 xenograft model carrying C1156Y mutation
RD-2013-50303	In vivo efficacy of NVP-LDK378-NX-4 in non-ALK mutation based Crizotinib resistant H2228 xenograft model
RD-2012-50420	Evaluation of NVP-LDK378/NVP-AUY922 combination in the HLUX-1787 human lung primary tumor xenograft model
RD-2012-50517	Evaluation of NVP-LDK378/NVP-AUY922 combination in the LUF-1656 human lung primary tumor xenograft model

Safety Pharmacology

Study #	Study title
0970418	Effects of LDK378 on cloned hERG potassium channels expressed in human embryonic kidney cells
0770889	Telemetry study of cardiovascular effects in male monkeys after a single oral (gavage) administration

Study #	Study title
0970420	LDK378: Telemetry study of cardiovascular effects in male monkeys after a single oral (gavage)administration at multiple dose levels
0970415	LDK378: Single dose oral (gavage) safety pharmacology study in rats (respiratory and nervous systems
RD-2010-50499	NVP-LDK378: In vitro safety pharmacology profile

Pharmacokinetics

Study #	Study title
DMPK R0900773a	Absorption, metabolism and excretion of LDK378 in intact and bile duct-cannulated rats following an intravenous or oral dose of [14C]LDK378
DMPK R1200422	Absorption, metabolism and excretion of LDK378 in the monkey following an intravenous or oral dose of [^{14}C]LDK378
DMPK R0800227-01	Pharmacokinetics of LDK378 following an intravenous or oral dose in the monkey
RD-2008-50924	Pharmacokinetics of NVP-LDK378 after intravenous and oral single dose administration to mice
DMPK R0900773b	Tissue distribution following a single oral or intravenous dose of [^{14}C]LDK378 in the rat
DMPK R0900777	In vitro distribution of ^{14}C-labeled LDK378 to blood cells, serum and plasma proteins in the rat, dog, monkey and human
DMPK R1000033	Preliminary in vitro metabolism of [^{14}C]LDK378 in human, monkey and rat hepatocytes
DMPK R1000487	In vitro assessment of covalent protein binding potential for LDK378 in rat and human liver microsomes and hepatocytes

General Toxicology/Toxicokinetics

Study #	Study title
0970416	4-week oral (gavage)toxicity study in rats with a 4-week recovery period
1270164	13-week oral gavage toxicity and toxicokinetic study with LDK378 in rats with a 8-week recovery phase
0970612	4-week oral (gavage) toxicity study in monkeys with a 4-week recovery period
1270165	13-week oral gavage toxicity and toxicokinetic study with LDK378 in cynomolgus monkeys with a 8-week recovery phase

Genetic toxicology

Study #	Study title
0970421	Mutagenicity test using *S. typhimurium*
0613212	Miniscreen Ames test
0970419	Induction of chromosome aberrations in cultured human peripheral blood lymphocytes
0714901	Micronucleus test in vitro using cultured human peripheral blood lymphocyte
0614110	Micronucleus test in vitro using TK6 cells
0870222	Induction of chromosome aberrations in cultured human peripheral blood lymphocytes
1370166	Induction of micronuclei in bone marrow of treated rats

Reproductive toxicology

Study #	Study title
1370071	Non-pivotal reproductive and developmental toxicity studies
1370073/9000189	LDK378: An oral gavage study of embryo-fetal development in the rat
1370072/9000190	LDK378: An oral gavage study of embryo-fetal development in the rabbit

3.2 Studies Not Reviewed

Pharmacokinetics

Study #	Study title
DMPK R0800367-01	[$^{13}C_6$]LDK378 Synthesis and release analysis
DMPK R0800368a	[$^{14}C_6$]LDK378 Synthesis and release analysis
DMPK R0900827	Quantitative determination of LDK378 and LEH621 in rat plasma by LC-MS/MS, Method validation report
DMPK R0900827-01	Quantitative determination of LDK378 and LEH621 in rat plasma by LC-MS/MS, Amendment 1
DMPK R0900827a	Quantitative determination of LDK378 and LEH621 in monkey plasma by LC-MS/MS, Method validation report
DMPK R0900827a-01	Quantitative determination of LDK378 and LEH621 in monkey plasma by LC-MS/MS, Amendment no. 1
DMPK R1200395	[^{14}C]LDK378 Synthesis and release analysis
DMPK R0700555-01	Oral Bioavailability following a single intravenous (5 mg/kg) or an oral dose (20 mg/kg) of LDK378 in the fed dog (plasma)
DMPK R0700555B	Blood pharmacokinetics following a single intravenous (5 mg/kg) dose of LDK378 in dog

General Toxicology

Study #	Study title
0770888	An oral (gavage) pilot toxicity study in male monkeys
0770561	2-week oral (gavage) exploratory study in male rats
0870025	2-week (4 doses toal) oral (gavage) pilot toxicity study in male rats
0970057	4-week exploratory (gavage) pilot toxicity study in male rats with a 4-week recovery period
0870126	2-week oral (gavage) dose range-finding toxicity study in monkeys

3.3 Previous Reviews Referenced

Review of IND 109272 for LDK378 by G. Sachia Khasar, Ph.D.

Summaries of all following sections are included in the integrated summary.

4 Pharmacology

4.1 Primary Pharmacology

RD-2010-00649: NVP-LDK378: In vitro effects against a selection of recombinant human protein kinases

The Applicant evaluated the selectivity of NVP-LDK378 by testing its in vitro activity against 36 recombinant human protein kinases using the Caliper mobility shift assay, which utilizes fluorescently labeled peptides as kinase substrates and separates phosphorylated from unphosphorylated peptides. The kinases were expressed as histidine- or GST-tagged fusion proteins, except for untagged ERK2, which was produced in E. coli. NVP-LDK378-NX-4 diluted with DMSO to final concentrations of 10, 3.0, 1.0, 0.3, 0.1, 0.03, 0.01, and 0.003 µM was evaluated in the Caliper assay, and the effect of NVP-LDK378 on enzymatic activity was obtained from linear progress curves in the presence and absence of compound. NVP-LDK378 was the most selective for ALK, exhibiting an IC_{50} value of 0.15 nM. NVP-LDK378 also inhibited InsR and IGF-1R with IC_{50} values of 7 nM and 8 nM, respectively. Thus, NVP-LDK378 was approximately 50-fold more specific for ALK than InsR and IGF-1R, other members of the insulin receptor superfamily. All the other kinases tested had IC_{50} values ≥ 110 nM. Since only 36 protein kinases were assessed, this study provides a somewhat limited assessment of NVP-LDK378 specificity.

Table 2: *In vitro* effects of NVP-LDK378 on Human Protein Kinases

No	Kinase	Type	N	Average	SD
1	EPK AURORA_A	S/T	3	0.11	0.015
2	EPK BTKv2	Y	3	3.9	0.82
3	EPK cABLT315	Y	3	0.13	0.012
4	EPK CDK2A	S/T	3	4.2	0.47
5	EPK CDK4D1	S/T	3	5.2	0.59
6	EPK CE ALK (1066-1459)	Y	3	0.00015[1)]	n.d.
7	EPK CE AXL (515-885)	Y	3	0.18	0.026
8	EPK CE EGFR (668-1210)	Y	3	0.9	0.18
9	EPK CE EPHA4 (574-986)	Y	3	9[2)]	n.d.
10	EPK CE EPHB4 (566-987)	Y	3	2.6	0.84
11	EPK CE FGFR3 (411-K650E-806)	Y	3	0.43	0.091
12	EPK CE IGF1R (980-1369)	Y	3	0.008	0.0035
13	EPK CE INSR (871-1343)	Y	3	0.007	0.0013
14	EPK CE KDR (807-1356)	Y	3	0.6	0.21
15	EPK CE KIT (544-976)	Y	3	1.6	0.42
16	EPK CE LCK	Y	3	0.6	0.18
17	EPK CE MET 956-1390	Y	3	3.2	0.64
18	EPK CE PDGFRa (551-V561D-1089)	Y	3	1.0	0.38
19	EPK CE RET (658-1072)	Y	3	0.40	0.031
20	EPK CE SYK (2-635) nonphos	Y	3	3	1.7
21	EPK CE ZAP70	Y	3	9.7[2)]	n.d.
22	EPK COT1	S/T	3	> 10	n.d.
23	EPK ERK2	S/T	3	1.6	0.31
24	EPK GSK3beta	S/T	3	> 10	n.d.
25	EPK JAK1	Y	3	4.5[2)]	0.21
26	EPK JAK2	Y	3	0.6	0.17
27	EPK JAK3v2	Y	3	9.3	0.42
28	EPK p38a	S/T	3	> 10	n.d.
29	EPK PDK1v2	S/T	3	> 10	n.d.
30	EPK PKA	S/T	3	2.9	0.75
31	EPK PKCalpha	S/T	3	4.4	0.55
32	EPK PKCtheta	S/T	3	1.8	0.058
33	EPK PKN1	S/T	3	1.2	0.15
34	EPK PKN2	S/T	3	3.4	0.99
35	EPK ROCK2	S/T	3	0.45	0.062
36	EPK TYK2	Y	3	1.1	0.18

Averages of IC_{50} values in µM are shown. N = Number of individual experiments, Y = Tyrosine-specific protein kinase, S/T = Serine/Threonine-specific protein kinases. [1)] one out of three results were <0.00013µM, [2)] one or two out of three results were >10µM.

(Excerpted from Applicant's submission)

RD-2010-00651: NVP-BQK827: In vitro effects against a selection of recombinant human protein kinases

The Applicant evaluated the selectivity of NVP-BQK827-AA-1 (crizotinib), a reference compound for ALK kinase inhibitors, by testing its in vitro activity against 36 recombinant human protein kinases using the Caliper mobility shift assay as described above in Study RD-2010-00649. NVP-BQK827 was most selective for ALK, exhibiting an IC_{50} value of 3 nM. NVP-BQK827 also inhibited cABL (T315I), MET, AXL, Aurora Kinase A, JAK2, LCK, IGF-1R, and InsR with IC_{50} values of 6 nM, 8 nM, 13 nM, 60 nM, 60 nM, 80 nM, 0.4 µM, and 0.29 µM, respectively.

Table 3: In vitro effects of NVP- BQK827 (Crizotinib) on Human Protein Kinases

No	Kinase	Type	N	Average	SD
1	EPK AURORA_A	S/T	3	0.06	0.052
2	EPK BTKv2	Y	3	> 10	n.d.
3	EPK cABLT315	Y	3	0.006	0.0021
4	EPK CDK2A	S/T	3	8.8[1)]	n.d.
5	EPK CDK4D1	S/T	3	> 10	n.d.
6	EPK CE ALK (1066-1459)	Y	3	0.003	0.0017
7	EPK CE AXL (515-885)	Y	3	0.013	0.0078
8	EPK CE EGFR (668-1210)	Y	3	> 10	n.d.
9	EPK CE EPHA4 (574-986)	Y	3	0.9	0.21
10	EPK CE EPHB4 (566-987)	Y	3	0.15	0.064
11	EPK CE FGFR3 (411-K650E-806)	Y	3	1.7	0.12
12	EPK CE IGF1R (980-1369)	Y	3	0.4	0.24
13	EPK CE INSR (871-1343)	Y	3	0.29	0.071
14	EPK CE KDR (807-1356)	Y	3	2.7	0.29
15	EPK CE KIT (544-976)	Y	3	8.7[1)]	n.d.
16	EPK CE LCK	Y	3	0.08	0.040
17	EPK CE MET 956-1390	Y	3	0.008	0.0036
18	EPK CE PDGFRa (551-V561D-1089)	Y	3	3.3	0.25
19	EPK CE RET (658-1072)	Y	3	2.2	0.35
20	EPK CE SYK (2-635) nonphos	Y	3	4	3.6
21	EPK CE ZAP70	Y	3	5.5	0.72
22	EPK COT1	S/T	3	> 10	n.d.
23	EPK ERK2	S/T	3	> 10	n.d.
24	EPK GSK3beta	S/T	3	> 10	n.d.
25	EPK JAK1	Y	3	1.0	0.29
26	EPK JAK2	Y	3	0.06	0.031
27	EPK JAK3v2	Y	3	5.6	0.56
28	EPK p38a	S/T	3	> 10	n.d.
29	EPK PDK1v2	S/T	3	> 10	n.d.
30	EPK PKA	S/T	3	> 10	n.d.
31	EPK PKCalpha	S/T	3	> 10	n.d.
32	EPK PKCtheta	S/T	3	> 10	n.d.
33	EPK PKN1	S/T	3	8.6[1)]	n.d.
34	EPK PKN2	S/T	3	> 10	n.d.
35	EPK ROCK2	S/T	3	2.5	0.55
36	EPK TYK2	Y	3	1.5	0.36

Averages of IC50 values in µM are shown. N = Number of individual experiments, Y = Tyrosine-specific protein kinase, S/T = Serine/Threonine-specific protein kinases. [1)] one or two out of three results were >10µM.

(Excerpted from Applicant's submission)

RD-2010-50499: NVP-LDK378: *In vitro* Safety Pharmacology Profile
RD-2008-50904: (b) (4) individual test IC_{50} values for NVP-LDK378-NX-2
RD-2008-50906: Safety Receptor Panel Profiling of LDK378: Individual test EC_{50} and IC_{50} values

The off-target activity of NVP-LDK378 was evaluated in study RD-2010-50499 using an in vitro safety pharmacology profiling panel including 139 G protein-coupled receptors (GPCRs), ion channels, nuclear receptors, transporters, and enzymes that have been previously linked to potential side effects. At least eight concentrations of NVP-LDK378 were tested. NVP-LDK378 interacted with 42 of the targets (30%), exhibiting > 50% inhibition at 10 µM. Targets with IC_{50} values less than 10 µM are shown in the table below.

Table 4: NVP-LDK378 targets with IC_{50} values less than 10 µM

Target	NVP-LDK378-AA-2 (IC_{50}, µM)	NVP-LDK378-NX-4 (IC_{50}, µM)
H2	0.17	n.t.
OpK	0.57	4.5
MC4	0.60	5.6
MC3	0.67	3.4
Ad3	0.73 (n=2)	3.1
NK1	0.99	n.t.
D2L	1.2	6.7
NET	1.7	7.3
GHS	2.9	28
V1a	4	20
PDE4d	4.1	1.8
AI2C	4.3	13
Ca2+(L) (rat)	4.3	9.3
AI1 (rat)	4.4	n.t.
AI1A	n.t.	3.9
PXR	n.t.	4.4
5HT2A	4.7	6.4
OpM	4.8	n.t.
D1	5.0	n.t.
Y1	5.4	20
Beta2	5.8	14
Beta1	7.4	7.6
CCKa	8.5	29
hr Mot	8.5	3.5
Ad1	8.5	20
5HT2B	8.5	5.0 (n=2)
5HT2B agonism	n.t.	EC_{50} = 14 (n=3)
5HTT	8.6	7.4
DAT	10	9.4

(Excerpted from Applicant's submission)

Targets with IC_{50} values < 1.8 µM included human recombinant histamine H2 receptor (H_2; 0.17 µM), opiate kappa receptor (0.57 µM), melanocortin 3 receptor (MC_3; 0.67 µM), melanocortin 4 receptor (MC_4; 0.6 µM), adenosine 3 receptor (Ad_3; 0.73 µM), tachykinin NK1 receptor (NK_1; 0.99 µM), dopamine 2 receptor (long form) (D_2;1.2 µM), and norepinephrine transporter (NET; 1.7 µM). Follow-up functional assays were performed on the 42 targets listed in Table 4 by Novartis, (b) (4) (RD-2008-50904), and (b) (4) (RD-2008-50906).

The off-target activity of NVP-LDK378 was evaluated in Study RD-2008-50904 using a safety pharmacology broad spectrum screen with 84 receptors at (b) (4) NVP-LDK378-NX-2 was tested at 10 µM in duplicate. Table 5 shows the receptors inhibited > 50% by 10 µM NVP-LDK378, and Table 5 shows the binding IC_{50} values for receptors that are inhibited >80% by 10 µM NVP-LDK378. NVP-LDK378 had a binding affinity of < 1 µM for rabbit vesicular monoamine transporter (VMAT2; 0.331µM) and rat potassium channel (K_A; 0.682 µM).

Table 5: Percent inhibition elicited at 10 µM NVP-LDK378

Assay	Species	Concentration	% Inhibition
Adregenic α_{2B}	human	10 µM	62
Calcium channel L-type Benzodiazepine	rat	10 µM	99
Calcium channel L-type Phenylalkylamine	rat	10 µM	65
Transporter, Monoamine	rabbit	10 µM	97
Motilin	human	10 µM	88
Platelet activating factor (PAF)	human	10 µM	59
Potassium channel [K_A]	rat	10 µM	99
Progesterone Pr-B	human	10 µM	78
Serotonin (5-hydroxytryptamine)-5HT$_{5A}$	human	10 µM	71
Somatostatin sst1	human	10 µM	88
Somatostatin sst3	human	10 µM	76
Somatostatin sst2	human	10 µM	93
Somatostatin sst4	human	10 µM	90
Urotensin II	human	10 µM	79

Table 6: Binding IC_{50} values for receptors inhibited greater than 80% at 10 µM NVP-LDK378

Asssay	Binding IC_{50}
Transporter monoamine	0.331
K channel [Ka]	0.682
sst1	2.42
sst2	2.25
sst3	5.12
sst4	1.88
5-HT5A	5

(Tables excerpted from Applicant's submission)

The agonist and antagonist activities of NVP-LDK378 were evaluated for 10 GPCRs in Study RD-2008-50906 using a safety pharmacology screen at (b) (4)
A fluorometric imaging plate reader (FLIPR) assay was conducted with a four-fold, eight-point serial dilution of NVP-LDK378-NX-2 up to 10 µM to assess agonist and antagonist activity. Table 7 shows the EC_{50} and IC_{50} values for NVP-LDK378-NX-2 agonist or antagonist activity for the GPCRs tested. NVP-LDK378 showed weak agonist activity on the Dopamine D2 receptor with an EC_{50} potency of 6 µM, but did not exhibit significant activity against the other GPCRs tested.

Table 7: EC_{50} and IC_{50} values for NVP-LDK378 for GPCRs

Asssay	EC_{50} (µM); (Eff)	IC_{50} (µM)
Adenosine: A3	NA	> 10 µM
Adregenic: ADRA1A (α1A)	NA	> 10 µM
Dopamine: D2	6.0 (50%)	4.0* µM
Histamine: H2	NA	> 10 µM
Histamine: H3	NA	> 10 µM
Melanocortin: MC4	NA	> 10 µM
Opioid: OPRK1 (kappa)	NA	> 10 µM
Somatostatin: SST2 and SST4	NA	> 10 µM
Somatostatin: SST2 and SST4	NA	> 10 µM
Tackykinin: NK1	NA	> 10 µM

*Inhibition data collected for D2 was most likely caused by receptor desensitization.

(Excerpted from Applicant's submission)

A summary of the functional activities of NVP-LDK378 seen in all three studies is presented in Table 8. NVP-LDK378 antagonized Ad_3 (IC_{50} of 8.3 µM), MC_4 (35% at 10 µM), NK1 (79% at 30 µM), and $Ca2^{+}$(L)DHP (IC_{50} of 21 µM) and showed agonist activity on D_2 (EC_{50} of 6 µM), NK1 (35% at 30 µM), and the somatostatin sst2 receptor (147% at 30 µM) at concentrations 3.3- to 17-fold higher than that achieved in the clinic at the recommended dose (118- to 592-fold higher than the approximate achievable free drug concentration in the clinic). Thus, NVP-LDK378 is unlikely to exhibit functional agonism or antagonism on these off-target receptors at clinically achievable concentrations. LDK378 did not exhibit agonist or antagonist activity on H_2 at concentrations up to 10 µM; however, the Applicant stated that these studies should ideally be repeated.

Table 8: Summary of NVP-LDK378 activity against 42 receptors

	Binding			Functional		
Target	Batch AA-2 (IC_{50}, µM)	Batch NX-4* (IC_{50}, µM or % inh.)	Testing site	Agonism (EC_{50}, µM or % act.)	Antagonism (IC_{50}, µM or % inh.)	Testing site
Ad1	8.5	20	NVS			(b) (4)
Ad3	0.73	3.1	NVS		8.3	
Al1 (rat)	4.4	n.d	NVS			
Al1A	n.d	3.9	NVS			
Al2b		62% @ 10µM	(b) (4)			
Al2C	4.3	13	NVS			
Beta1	7.4	7.6	NVS			
Beta2	5.8	14	NVS			
CCKa	8.5	29	NVS			
D1	5.0	n.d	NVS			
D2L	1.2	6.7	NVS	6		
GAL2		59% @ 10µM	(b) (4)			
GHS	2.9	28	NVS			
H2	0.17	n.d	NVS			
MC3	0.67	3.4	NVS			
MC4	0.60	5.6	NVS		35% @ 10µM	
Motilin	8.5	3.5	NVS			
NK1	0.99	n.d	NVS	35% @ 30µM	79% @ 30µM	
OpK	0.57	4.5	NVS			
OpM	4.8	n.d	NVS			
PAF		59% @ 10µM	(b) (4)			
5HT2A	4.7	6.4	NVS			
5HT2B	8.5	5.0	NVS	14		NVS
5HT5A		5	(b) (4)			
sst1		2.4				
sst3		5.1				
sst2		2.2		147% @ 30µM		(b) (4)
sst4		1.9				
UTII		79% @ 10µM				
V1a	4	20	NVS			
Y1	5.4	20	NVS			
5HTT	8.6	7.4	NVS			
DAT	10	9.4	NVS			
NET	1.7	7.3	NVS			
VMAT2 (rabbit)		0.33	(b) (4)			
Ca2+(L) DHP site (rat)	4.3	9.3	NVS		21	
Ca2+(L) BZT site (rat)		1.8	(b) (4)			
Ca2+(L) PhA site (rat)		2.4				
K channel K_A (rat)		0.68				
PR		78% @ 10µM				
PXR	n.d	4.4	NVS			
PDE4d	4.1	1.8	NVS			

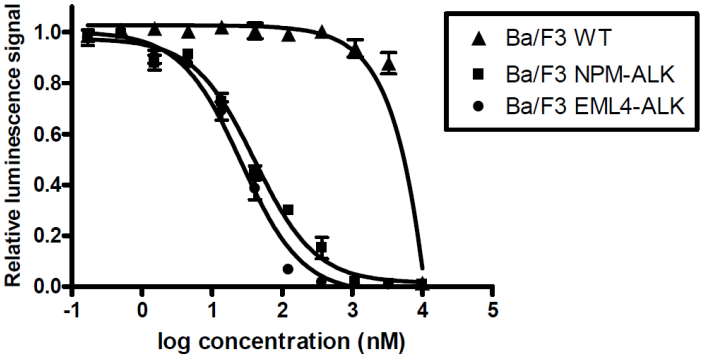
*Batch NX-2 was tested at (b) (4) Al1A, H2, OpK and sst4 cellular functional assays were run at (b) (4) but no agonist or antagonist activity was found up to 10 µM. These should ideally be repeated.

(Excerpted from Applicant's submission)

RD-2009-50672: In vitro antiproliferative activity of NVP-LDK378 in Ba/F3 cells transduced with ALK fusion kinases

ALK kinase is activated by fusion with the amino-terminal portion of Nucleophosmin1 (NPM) or echinoderm microtubule-associated protein like protein 4 (EML4) in anaplastic large cell lymphoma (ALCL) or NSCLC, respectively. The Applicant investigated the activity of NVP-LDK378 against constitutively active NPM-ALK and EML4-ALK fusion kinases. IL-3-dependent Ba/F3 (murine pro-B lymphoma) cells were stably transfected with NPM-ALK or EML4-ALK, thus rendering them IL-3-independent. Wild-type Ba/F3, Ba/F3-NPM-ALK, and Ba/F3-EML4-ALK cells were treated with control compounds or NVP-LDK378 serially diluted with DMSO, and cell viability was determined using the Bright-Glo™ luciferase bioluminescent assay. NVP-LDK378 inhibited proliferation of Ba/F3-NPM-ALK and Ba/F3-EML4-ALK cells with IC_{50} values of 35 nM and 27 nM, respectively, whereas it exhibited little anti-proliferative activity towards wild-type Ba/F3 cells (IC_{50} of 4.97 µM). Thus, NVP-LDK378 was effective at inhibiting proliferation of Ba/F3 cells whose proliferation was driven by NPM-ALK and EML4-ALK fusion proteins.

Figure 1: Activity of NVP-LDK378 against Ba/F3 cells expressing NPM-ALK or EML4-ALK



(Excerpted from Applicant's submission)

RD-2009-50673: In vitro antiproliferative activity of NVP-LDK378 in Ba/F3 cells transduced with TEL-FES and TEL-FER fusion kinases

The activity of NVP-LDK378 against the TEL-FER and TEL-FES fusion kinases was evaluated. IL-3-dependent Ba/F3 cells were stably transfected with TEL-FER and TEL-FES, thus rendering them IL-3-independent. Wild-type Ba/F3, Ba/F3-BMI/TEL-FES, Ba/F3-EV/TEL-FER, and Ba/F3-NPM-ALK cell lines were treated with control compounds or NVP-LDK378 serially diluted with DMSO, and cell viability was determined using the Bright-Glo™ luciferase bioluminescent assay. NVP-LDK378 inhibited proliferation of Ba/F3-NPM-ALK cells with an IC_{50} value of 26 nM, but was relatively inactive against wild-type Ba/F3, Ba/F3-BMI/TEL-FES, and Ba/F3-EV/TEL-FER cells (IC_{50} values of 2.435, 3.648, and 1.649 µM, respectively). Thus, NVP-LDK378 was selective for ALK fusion proteins and exhibited little in vitro activity against TEL-FES and TEL-FER.

RD-2009-50670: *In vitro* antiproliferative activity of NVP-LDK378 in Ba/F3 cells transduced with constitutively activated tyrosine kinases

The anti-proliferative activity of NVP-LDK378 against Ba/F3 cells stably transfected with 38 constitutively activated kinases was evaluated. Selected kinases were activated either by mutation or by fusion with the amino terminus of TEL. Cell viability following 48 hours of treatment with control compounds or NVP-LDK378 serially diluted with DMSO was determined using the Bright-Glo™ luciferase bioluminescent assay. NVP-LDK378 potently inhibited proliferation of Ba/F3-TEL-ALK-Q1 cells, as expected, with an IC_{50} of 56 nM. NVP-LDK378 also exhibited anti-proliferative activity towards Ba/F3 cells transduced with TEL-ROS, TEL-IGF-1R, and TEL-InsR (IC_{50} values of 180 nM, 220 nM, and 400 nM, respectively); ROS, IGF-1R, and InsR are all, like ALK, members of the insulin receptor tyrosine kinase family. NVP-LDK378 was relatively inactive against wild-type Ba/F3 cells and the other cell lines tested, exhibiting IC_{50} values from 1.23-3.02 µM. Thus, NVP-LDK378 exhibited greater specificity for ALK than for other members of the insulin receptor superfamily, and did not interact strongly with the other 34 tyrosine kinases tested.

Table 9: Anti-proliferative Activity of NVP-LDK378 against Ba/F3 cells transduced with constitutively activated tyrosine kinases

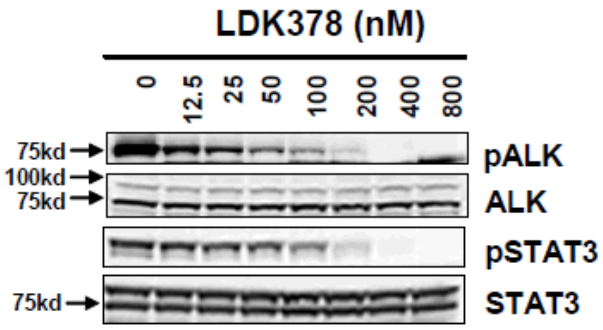
Cell Line	NVP-LDK378 IC_{50} (µM)	STDV (N=2)
Ba/F3 WT	1.88	0.32
Ba/F3-BCR-ABL	1.30	0.16
Ba/F3-BRAF-PIM1	1.98	0.31
Ba/F3-TEL-ALK-Q1	0.06	0.00
Ba/F3-TEL-BMX-1.2	3.02	0.17
Ba/F3-TEL-EphA3-4.2	1.26	0.09
Ba/F3-TEL-EphB2-Q2	1.39	0.10
Ba/F3-TEL-FGFR2-Q1	1.43	0.14
Ba/F3-TEL-FGFR3-Q2	1.37	0.09
Ba/F3-TEL-FGFR4-Q4	1.38	0.15
Ba/F3-TEL-FGR-2.1	1.41	0.10
Ba/F3-TEL-FLT1-Q1	1.47	0.10
Ba/F3-TEL-FLT3-1.2	1.58	0.14
Ba/F3-TEL-FLT4-Q2	1.24	0.09
Ba/F3-TEL-FMS-1.2	1.33	0.08
Ba/F3-TEL-IGF1R-N.1	0.22	0.02
Ba/F3-TEL-INSR-1.2*	0.40	0.05
Ba/F3-TEL-JAK1-S1.2	1.37	0.16
Ba/F3-TEL-JAK2-1.2	1.23	0.14
Ba/F3-TEL-JAK3-S1.2	1.44	0.13
Ba/F3-TEL-KDR-Q4	2.44	0.77
Ba/F3-TEL-KIT-Q1.2	1.63	0.15
Ba/F3-TEL-LCK-2.1	1.26	0.09
Ba/F3-TEL-LYN-1.2	2.35	0.53
Ba/F3-TEL-MER-3.2	1.40	0.13
Ba/F3-TEL-MET-3.1	2.13	0.54
Ba/F3-TEL-PDGFRb-Q2	2.23	0.48
Ba/F3-TEL-RET-Q1	1.30	0.07
Ba/F3-TEL-RON-4.1	1.28	0.10
Ba/F3-TEL-ROS-1.1	0.18	0.02
Ba/F3-TEL-SRC-3.1	1.56	0.12
Ba/F3-TEL-SYK-1.1	1.44	0.14
Ba/F3-TEL-TIE1-Q2	1.49	0.20
Ba/F3-TEL-TIE2-1.2	1.26	0.08
Ba/F3-TEL-TRKA-Q.3	2.88	0.32
Ba/F3-TEL-TRKB-Q2	1.95	0.29
Ba/F3-TEL-TRKC-Q2	1.41	0.23
Ba/F3-TEL-TYK2-1.3	1.37	0.09
Ba/F3-TEL-ZAP70-1.1	1.29	0.12

(Excerpted from Applicant's submission)

2008-50914: *In vitro* antiproliferative activity of NVP-LDK378 in Karpas299 (ALCL model expressing NPM-ALK) and NCIH2228 (NSCLC model expressing EML4-ALK)

The in vitro anti-proliferative activity of NVP-LDK378 was evaluated in the ALCL cell line Karpas299, which expresses NPM-ALK, and the NSCLC cell line NCI-H2228, which expresses EML4-ALK. The cell lines were treated for three days with 0.17 nM – 10 µM NVP-LDK378 or 0.1% DMSO, and cell proliferation was measured using the Bright-Glo™ luciferase bioluminescent assay. NVP-LDK378 inhibited the growth of Karpas299 and NCI-H2228 cells with average IC_{50} values of 45 nM (n=8) and 11 nM (n=5), respectively. Incubation of Karpas299 cells with NVP-LDK378 for two hours resulted in a concentration-dependent reduction in the phosphorylation of ALK at Tyr 1604 (equivalent to Tyr664 of NPM-ALK) and of its downstream signaling protein STAT3 at the activating tyrosine 705 without affecting total ALK or STAT3 protein levels. IC_{50} values for blocking phosphorylation of ALK and STAT3 were 46 nM and 150 nM, respectively. Thus, the anti-proliferative activity of NVP-LDK378 in Karpas299 cells appeared to correlate with inhibition of phosphorylated ALK and its downstream mediator STAT3.

Figure 2: Effect of NVP-LDK378 on ALK and STAT3 phosphorylation in Karpas299 cells



(Excerpted from Applicant's submission)

RD-2010-50442: *In vitro* antiproliferative activity of NVP-LDK378 in neuroblastoma cell line NB-1 with ALK amplification

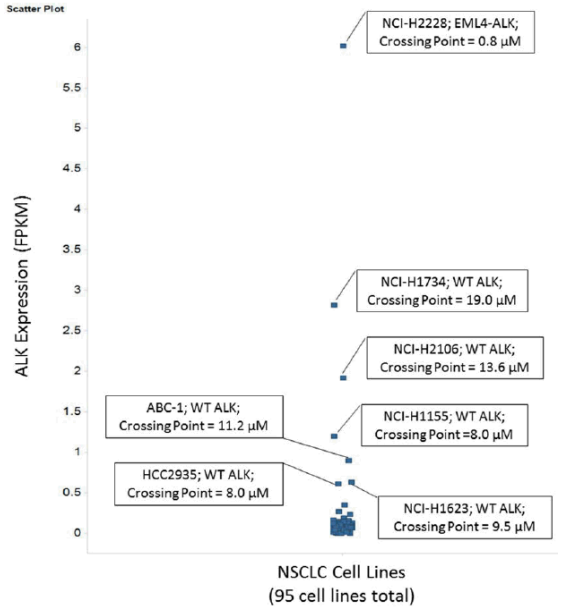
The *in vitro* anti-proliferative activity of NVP-LDK378 was evaluated in the ALK-amplified neuroblastoma cell line NB-1. NB-1-Luc-Blast cells were treated with 0.17 nM-10 µM of NVP-LDK378 or 0.1% DMSO for three days, and cell proliferation was measured using the BriteLite™ luciferase assay. NVP-LDK378 inhibited proliferation of NB-1 cells with an IC_{50} of 24 nM (n=3).

RD-2013-50316: Single agent activity of LDK378 and ALK expression levels in a panel of human non-small cell lung cancer cell lines

The growth inhibitory activity of NVP-LDK378 was investigated in a panel of 95 human NSCLC cell lines. NSCLC cell lines were incubated with NVP-LDK378 (0.3 nM to 30 µM or 2.5 nM to 8 µM) for 72 hrs, relative cell numbers were determined using Cell Titer Glo (Promega), and crossing point (CP) values were determined. CP values were defined as the concentration of NVP-LDK378 required to achieve Cell Titer Glo values that were -50%, or halfway between the

positive (MG132) and negative (vehicle) controls in the assay. Out of the 95 NSCLC cell lines tested, only one (NCI-H2228) had a CP value of < 1μM (0.8 μM) and was sensitive to NVP-LDK378. NCI-H2228 contains the EML4-ALK gene fusion, whereas the cell lines lacking this gene fusion were insensitive to NVP-LDK378 and exhibited CP values ranging from 5.8 to 30 μM. ALK expression levels were quantified in the 95 cell lines using Affymetrix Human Genome U133 Plus 2.0 microarrays and RNA-Seq analysis. The majority of NSCLC cell lines expressed low levels of ALK. Cell lines that expressed ALK but did not harbor ALK rearrangements were insensitive to NVP-LDK378, with CP values ≥ 8 μM. Thus, NVP-LDK378 sensitivity appears to correlate with the presence of ALK rearrangements but not total ALK expression, and NVP-LDK378 will likely to be most effective in cell lines expressing ALK translocations.

Figure 3: ALK expression levels in 95 NSCLC cell lines



(Excerpted from Applicant's submission)

RD-2008-50926: In vivo evaluation efficacy and exposure of NVP-LDK378-AZ-1 in a subcutaneous tumor model of the human non-small cell lung cancer cell line H2228 in SCID beige mice

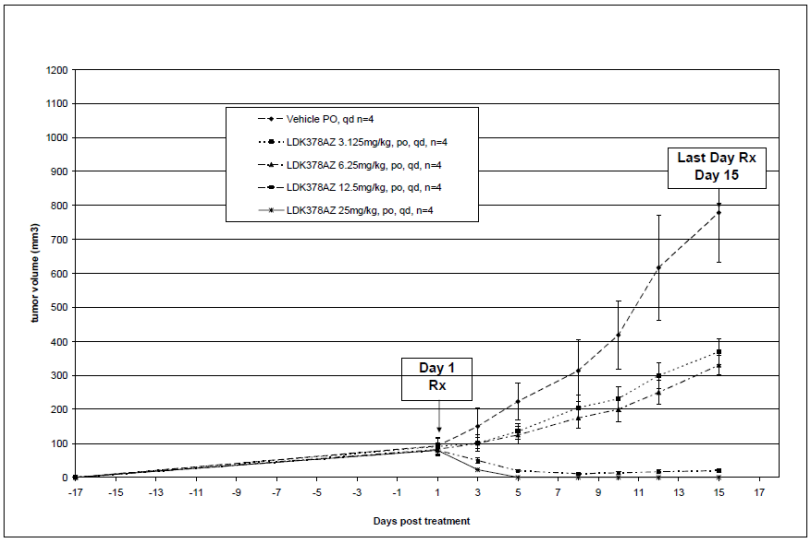
Methods

Drug:	NVP-LDK378-AZ-1
Doses:	3.125, 6.25, 12.5 and 25 mg/kg
Frequency of dosing:	Daily for 14 days
Route of administration:	Oral gavage
Dose Volume:	10 μL/g
Formulation/Vehicle:	0.5% methylcellulose (MC)/0.5% Tween 80
Species/Strain:	6-8 wk old female severe combined immunodeficient (SCID) beige mice
Number/Sex/Group:	4 mice/group
Study design:	5 x 10^7 NCI-H2228 cells/ml suspended in a 1:1 mixture of RPMI 1640 serum-free medium and matrigel were implanted

subcutaneously into the right hind flank of SCID mice. H2228 tumors were harvested and then passaged three consecutive times in mice. 2-3 pieces of 1-2 mm^3 stock H2228 tumor were subcutaneously implanted into the right flank of SCID beige mice in the matrigel mixture. Dosing was initiated when mean tumor volumes were ~85 mm^3. Tumors were measured three times per week using calipers, and body weight was monitored daily. Blood samples were collected from 3 mice/group at 1, 3, 7, 24, 36, and 48 hrs post-dose on Day 14. Percent change in tumor volume for treated (T) over control (C) group (% T/C) = ($\Delta T/\Delta C$) x 100 where $\Delta T > 0$; % T/C = ($\Delta T/\Delta T_i$) x 100 where $\Delta T < 0$.

The in vivo anti-tumor activity of NVP-LDK378 was investigated in mice bearing human NSCLC H2228 xenografts constitutively expressing EML4-ALK fusion gene activity. Daily treatment with 3.125, 6.25, 12.5, and 25 mg/kg NVP-LDK378 resulted in dose-dependent inhibition of H2228 xenograft growth (41%, 36%, -64%, and -100% T/C, respectively), which reached statistical significance at doses ≥ 6.25 mg/kg. Treatment with 25 mg/kg NVP-LDK378 resulted in complete tumor regression after 14 days of treatment.

Figure 4: Effect of NVP-LDK378 on human H2228 NSCLC xenografts in SCID mice



BEST AVAILABLE COPY

(Excerpted from Applicant's submission)

Treatment of tumor-implanted mice with NVP-LDK378 did not result in significant weight loss. NVP-LDK378 plasma exposure increased slightly more than dose proportionally, particularly in mice dosed with 25 mg/kg. Daily dosing with 25 mg/kg resulted in a mean Cmax of 2844 nM, which is ~1.6-fold greater than the mean Cmax achieved in the clinic at the recommended dose of NVP-LDK378, and a mean $AUC_{0\text{-}24h}$ of 40253 h*nM, which is achievable in the clinic. Overall, treatment with NVP-LDK378 exhibited significant anti-tumor activity against human NSCLC xenografts expressing activated EML4-ALK without inducing overt toxicity in female SCID mice.

Table 10: Mean Plasma Pharmacokinetic Parameters of NVP-LDK378 on Day 14

Dose (mg/kg)	Day of dosing	Plasma $AUC_{(0-24h)}$ (h*nM)	Plasma $AUC_{(0-48h)}$ (h*nM)	Fold change in $AUC_{(0-48h)}$	C_{max} (nM)	T_{max} (h)	$T_{1/2}$ (h)
3.125	14	3077	3544	1.0	219	4.3	9.4
6.25	14	6985	8762	2.5	440	7.0	11.8
12.5	14	14058	16123	4.5	895	5.7	8.3
25.0	14	40253	49601	14.0	2844	3.0	8.9

(Excerpted from Applicant's submission)

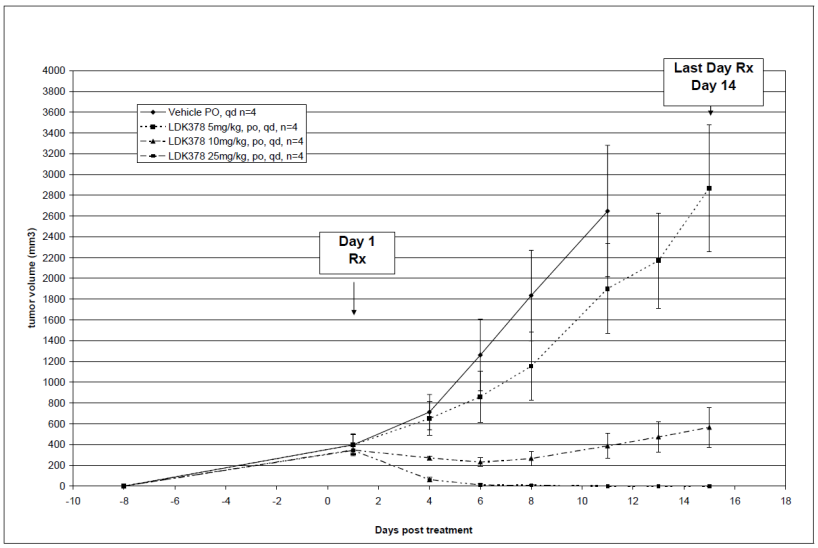
RD-2008-50933: In vivo evaluation of efficacy and exposure of NVP-LDK378-AZ-1 in a subcutaneous tumor model of the human non-small cell lung cancer cell line H2228 in nude rats

Methods

Drug:	NVP-LDK378-AZ-1
Doses:	5, 10, and 25 mg/kg
Frequency of dosing:	Daily for 14 days
Route of administration:	Oral gavage
Dose Volume:	10 µL/g
Formulation/Vehicle:	0.5% MC/0.5% Tween 80
Species/Strain:	7-8 wk old female RNU nude rats
Number/Sex/Group:	4 rats/group
Study design:	Nude rats were irradiated at 400-500 Rad three days before tumor implantation. 5-6 pieces of 2-3 mm^3 H2228 tumor were subcutaneously implanted into the right flank of nude rats in a 1:1 mixture of RPMI 1640 serum-free medium and matrigel. Dosing was initiated when mean tumor volumes were ~371 mm^3. Tumors were measured three times per week using calipers, and body weight was monitored daily. Blood samples from 2-3 rats/group were collected at 1, 3, 7, and 24 hrs post-dose on Day 14.

The in vivo anti-tumor activity of NVP-LDK378 was investigated in nude rats bearing human NSCLC H2228 xenografts. Daily treatment with 5, 10, and 25 mg/kg NVP-LDK378 resulted in dose-dependent inhibition of H2228 xenograft growth (67%, 2%, and -100% T/C, respectively), which reached statistical significance at doses ≥ 10 mg/kg. Treatment with 25 mg/kg NVP-LDK378 resulted in complete tumor regression after 14 days of treatment without significant weight loss. NVP-LDK378 plasma exposures were generally dose proportional, and daily dosing with 25 mg/kg resulted in a mean Cmax and AUC_{0-24h} of 916 nM and 13917 h*nM, respectively, which are achievable in the clinic. Overall, treatment with NVP-LDK378 exhibited significant anti-tumor activity against human NSCLC xenografts expressing the EML4-ALK fusion gene activity without inducing overt toxicity in female nude rats.

Figure 5: Effect of NVP-LDK378 on human H2228 NSCLC xenografts in nude rats



BEST AVAILABLE COPY

(Excerpted from Applicant's submission)

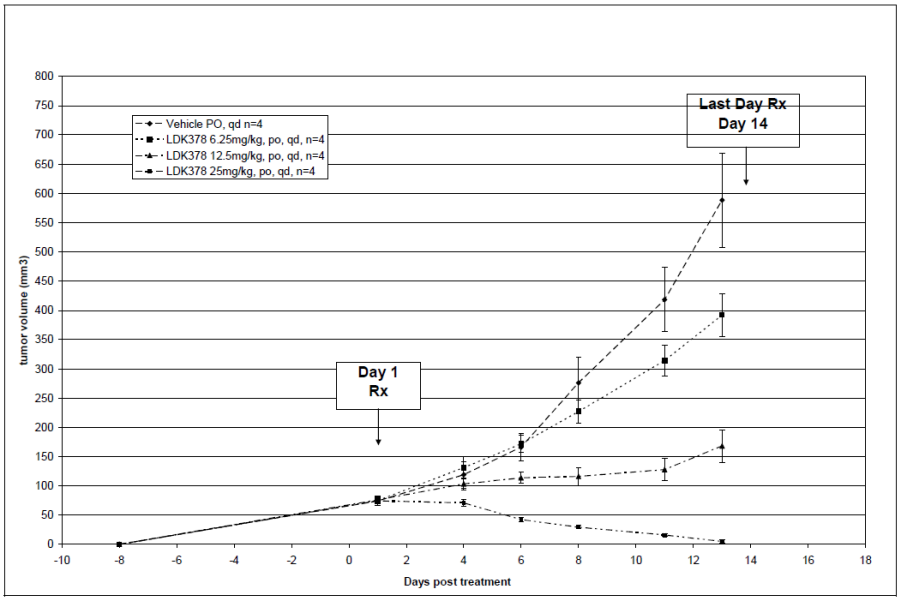
RD-2008-50927: In vivo evaluation efficacy and exposure of NVP-LDK378-AZ-1 in a subcutaneous tumor model of human anaplastic large-cell lymphoma Karpas299 in SCID beige mice

Methods

Drug:	NVP-LDK378-AZ-1
Doses:	6.25, 12.5 and 25 mg/kg
Frequency of dosing:	Daily for 14 days
Route of administration:	Oral gavage
Dose Volume:	10 µL/g
Formulation/Vehicle:	0.5% MC/0.5% Tween 80
Species/Strain:	6-8 wk old female SCID beige mice
Number/Sex/Group:	4 mice/group
Study design:	5 x 10^7 Karpas299 cells/ml suspended in a 1:1 mixture of RPMI 1640 serum-free medium and matrigel were implanted subcutaneously into the right hind flank of SCID mice. Karpas299 tumors were harvested and then passaged two consecutive times in mice. 2-3 pieces of 1-2 mm^3 stock Karpas299 tumor were subcutaneously implanted into the right flank of SCID beige mice in the matrigel mixture. Dosing was initiated when mean tumor volumes were ~74 mm^3. Tumors were measured three times per week using calipers, and body weight was monitored daily. Blood samples were collected from 3 mice/group at 3, 7, and 48 hrs post-dose on Day 14.

The in vivo anti-tumor activity of NVP-LDK378 was investigated in mice bearing human anaplastic large cell lymphoma (ALCL) Karpas299 xenografts constitutively expressing the NPM-ALK fusion gene. Daily treatment with 6.25, 12.5, and 25 mg/kg NVP-LDK378 resulted in dose-dependent inhibition of Karpas299 xenograft growth (62%, 18%, and -93% T/C, respectively), which reached statistical significance at doses ≥ 12.5 mg/kg. Treatment with 25 mg/kg NVP-LDK378 resulted in significant tumor regression after 14 days of treatment.

Figure 6: Effect of NVP-LDK378 on human Karpas299 ALCL xenografts in SCID mice



(Excerpted from Applicant's submission)

NVP-LDK378 did not result in significant weight loss. NVP-LDK378 plasma exposures increased slightly more than dose proportionally, particularly in mice dosed with 25 mg/kg. Daily dosing with 25 mg/kg resulted in a mean Cmax and $AUC_{0\text{-}24h}$ of 1735 nM and 24787 h*nM, respectively, which are achievable in the clinic. Overall, treatment with NVP-LDK378 exhibited significant anti-tumor activity against human ALCL xenografts expressing activated NPM-ALK without inducing overt toxicity in female SCID mice.

Table 11: Mean Plasma Pharmacokinetic Parameters of NVP-LDK378 on Day 14

Dose (mg/kg)	**Day of dosing**	**Plasma $AUC_{(0\text{-}24h)}$** (h*nM)	**Fold change in $AUC_{(0\text{-}48h)}$**	**C_{max}** (nM)	**T_{max}** (h)	**$T_{1/2}$** (h)
6.25	14	4002	1.0	364	3.0	5.8
12.5	14	10254	2.6	723	4.3	5.8
25.0	14	24787	6.2	1735	4.3	8.8

(Excerpted from Applicant's submission)

RD-2008-50942: In vivo evaluation of efficacy and exposure of NVP-LDK378-AZ-1 in a subcutaneous tumor model of the human anaplastic large-cell lymphoma Karpas299 in nude rats

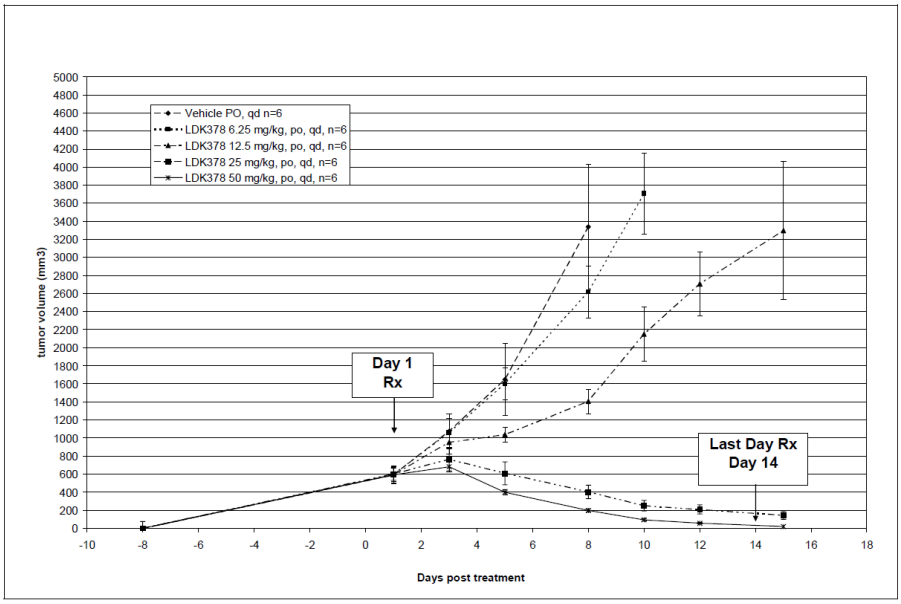
Methods

Drug:	NVP-LDK378-AZ-1
Doses:	6.25, 12.5, 25, and 50 mg/kg
Frequency of dosing:	Daily for 14 days
Route of administration:	Oral gavage
Dose Volume:	10 µL/g

Formulation/Vehicle:	0.5% MC/0.5% Tween 80
Species/Strain:	7-8 wk old female RNU nude rats
Number/Sex/Group:	6 rats/group
Study design:	Nude rats were irradiated at 400-500 Rad three days before tumor implantation. 5-6 pieces of 2-3 mm^3 Karpas299 tumor were subcutaneously implanted into the right flank of nude rats in a 1:1 mixture of RPMI 1640 serum-free medium and matrigel. Dosing was initiated when mean tumor volumes were ~595 mm^3. Tumors were measured three times per week using calipers, and body weight was monitored daily. Blood samples were collected from 2-3 rats/group at 1, 3, 7, and 24 hrs post-dose on Day 14.

The in vivo anti-tumor activity of NVP-LDK378 was investigated in mice bearing human ALCL Karpas299 xenografts constitutively expressing NPM-ALK fusion gene activity. Daily treatment with 6.25, 12.5, 25, and 50 mg/kg NVP-LDK378 resulted in dose-dependent inhibition of Karpas299 xenograft growth (74%, 30%, -33%, and -66% T/C on Day 11, respectively), which reached statistical significance at doses ≥ 12.5 mg/kg. Treatment with 25 and 50 mg/kg NVP-LDK378 resulted in significant tumor regression after 14 days of treatment (-76% and -97% T/C on Day 15, respectively). NVP-LDK378 did not result in significant weight loss. NVP-LDK378 plasma exposures increased less than dose proportionally at 50 mg/kg. Daily dosing with 25 and 50 mg/kg resulted in mean Cmax values of 895 nM and 1522 nM and mean $AUC_{0\text{-}24h}$ values of 13127 h*nM and 14006 h*nM, respectively, which are achievable in the clinic. Overall, treatment with NVP-LDK378 exhibited significant anti-tumor activity against human ALCL xenografts expressing activated NVP-ALK without inducing overt toxicity in female nude rats.

Figure 7: Effect of NVP-LDK378 on human Karpas299 ALCL xenografts in SCID mice



(Excerpted from Applicant's submission)

RD-2008-50976: In vivo pharmacodynamic and pharmacokinetic studies of NVP-LDK378-AZ-1 in an orthotopic model of human anaplastic large cell lymphoma Karpas299-Luc

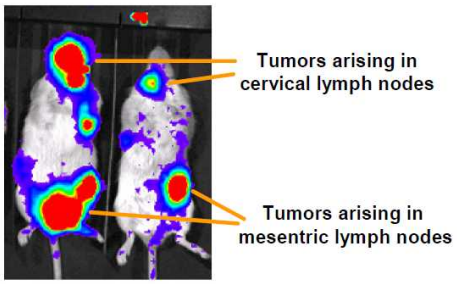
Methods

Drug:	NVP-LDK378-AZ-1
Doses:	5, 12.5, 25, 50, and 100 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% MC/0.5% Tween 80
Species/Strain:	6-8 wk old female SCID beige mice
Number/Sex/Group:	3 mice/group
Study design:	3 x 10^6 Karpas299 cells stably expressing luciferase (Karpas299-Luc) were inoculated in the bilateral tail vein of female SCID beige mice. Tumor development was monitored by bioluminescence using the Xenogen Imaging system. Four individual PK/PD studies were conducted (see Applicant's table below). Tumor-bearing mice were randomly grouped into dosing groups on Days 17-19 post-implantation, and then dosed with a single administration of vehicle or NVP-LDK378. Blood samples were collected after one single oral dose at the time points indicated below. Following euthanasia (~Days 24-26 post-implantation), tumor samples were collected for PK analysis and PD assays.

PD Study	PK Study	Study Date	Doses (mg/kg)	Time Points (h)
1st	EFF-122-214	10/30/2007	5, 12.5, 25, 50	3, 7, 16, 24
2nd	EFF-122-215	11/6/2007	25, 50, 100	24, 48, 72
3rd	EFF-122-221	12/10/2007	25, 50, 100	72, 96, 120
4th	EFF-122-234	3/2/2008	5, 12.5	24, 48, 72, 96, 120

The correlation between the pharmacokinetic (PK) and pharmacodynamic (PD) properties of NVP-LDK378 was investigated in an orthotopic mouse model of human ACLC expressing NPM-ALK fusion gene activity. Following intravenous inoculation of Karpas299-Luc cells, mice developed multiple lymphomas within three weeks of implantation. The mice typically exhibited a large tumor at the superficial cervical lymphatic area, and clusters of smaller tumors within the peritoneal cavity representing mesenteric lymphatic areas (see Figure 7). Cells isolated from the cervical lymphatic tumor were CD30+, characteristic of human ALCL cells.

Figure 8: Bioluminescence Imaging of the Orthotopic Karpas299 Mouse Model on Day 19 post-implantation

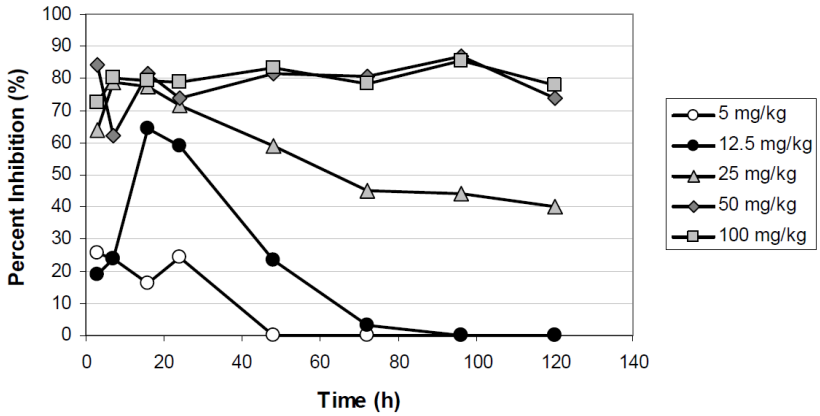


(Excerpted from Applicant's submission)

Phosphorylation of STAT3 at the activating Y705 was determined by immunoblotting of tumor lysates, and subsequently correlated with corresponding NVP-LDK378 concentrations in the plasma and tumor after a single dose of the drug. Dosing animals with 5 mg/kg NVP-LDK378 had little effect on STAT3 phosphorylation. Dosing animals with 12.5 mg/kg NVP-LDK378

reduced STAT3 phosphorylation by ~ 60% at 16-24 hrs post-dosing, but pSTAT3 levels quickly recovered. Dosing animals with 25 mg/kg inhibited STAT3 phosphorylation by ~60-80% at 3-48 hr post-dosing; at 120 hours post-dosing, there was only ~40% inhibition of pSTAT3. Dosing animals with 50 or 100 mg/kg NVP-LDK378 resulted in a ~70-90% inhibition of pSTAT3, which was maintained up to 120 hrs post-dosing. The percent inhibition of pSTAT3 was approximately linear between doses of 5 and 25 mg/kg, and plateaued at doses ≥ 25 mg/kg. Dosing with 50 or 100 mg/kg did not result in greater inhibition of pSTAT3 compared to 25 mg/kg, but did result in longer/sustained inhibition, corresponding with longer NVP-LDK378 half-lives in tumor tissue.

Figure 9: Dose- and time-dependent effect of NVP-LDK378 on STAT3 phosphorylation in the orthotopic Karpas299 Mouse Model



(Excerpted from Applicant's submission)

The plasma exposure of NVP-LDK378 increased more than dose proportionally, exhibiting a 56-fold increase in $AUC_{(0\text{-}120h)}$ at 100 mg/kg compared to 5 mg/kg with only a 20-fold increase between the doses. The plasma Cmax was relatively dose proportional, but increased less than dose proportionally at doses ≥ 50 mg/kg. The tumor exposure to LDK378 also increased more than dose proportionally, exhibiting a 46-fold increase in $AUC_{(0\text{-}120h)}$ at 100 mg/kg compared to 5 mg/kg despite only a 20-fold increase in dose. The tumor Cmax was generally dose-dependent. Plasma concentrations peaked at 3-7 hrs post-dosing, whereas tumor concentrations peaked between 7-24 hrs post-dosing; mice dosed with 100 mg/kg exhibited the highest tumor half-life. NVP-LDK378 exposure was higher in the tumor than in plasma, exhibiting a mean tumor to plasma ratio of 53 and 30 for $AUC_{(0\text{-}120h)}$ and Cmax, respectively. Thus, NVP-LDK378 preferentially distributed in tumor tissue versus plasma. Mean NVP-LDK378 concentrations in tumor tissue at 120 hrs post-dosing with 25, 50, and 100 mg/kg were 0.82, 3, and 19 μM, respectively, indicating that tumor concentrations remained high for a prolonged period of time with higher doses, correlating with sustained inhibition of pSTAT3 at 120 hr in mice dosed with 50 and 100 mg/kg. Dosing animals with 50 and 100 mg/kg resulted in similar pharmacodynamic profiles, despite differences in the NVP-LDK378 tumor concentrations and half-lives.

Table 12: Pharmacokinetic parameters of NVP-LDK378 in plasma in the orthotopic Karpas299 mouse model

Table	$AUC_{(0\text{-}120h)}$ (h*nM)	AUC/Dose (h*nM)/(mg/kg)	C_{max} (nM)	C_{max}/Dose (nM)/(mg/kg)	T_{max} (h)	$T_{1/2}$ (h)
5	4,713	943	202	40	3	11.5
12.5	17,219	1,378	446	36	7	14.2
25	34,338	1,374	964	39	3	17.8
50	71,898	1,438	1,523	30	3	11.6
100	261,964	2,620	2,479	25	7	17.5

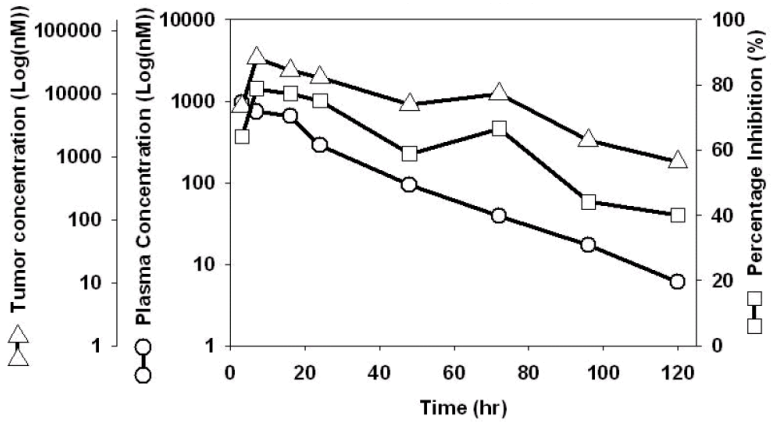
Table 13: Pharmacokinetic parameters of NVP-LDK378 in tumor in the orthotopic Karpas299 mouse model

Dose (mg/kg)	$AUC_{(0\text{-}120h)}$ (h*nM)	AUC/Dose (h*nM)/(mg/kg)	C_{max} (nM)	C_{max}/Dose (nM)/(mg/kg)	T_{max} (h)	$T_{1/2}$ (h)
5	230,281	46,056	4,445	889	24	17.5
12.5	772,980	61,838	12,869	1,030	24	13.3
25	1,856,350	74,254	37,255	1,490	7	13.3
50	5,374,000	107,480	44,033	881	16	14.3
100	10,690,500	106,905	78,926	789	24	24.0

(Excerpted from Applicant's submission)

Overall, NVP-LDK378 induced a dose-dependent and time-dependent inhibition of ALK-STAT3 signaling in Karpas299 tumors expressing activated NPM-ALK. NVP-LDK378 plasma concentrations did not appear to be as closely correlated with NVP-LDK378-induced inhibition of STAT3 phosphorylation (see Figure 10 below) as tumor concentration of the drug. Single doses of ≥ 25 mg/kg NVP-LDK378 resulted in ≥70% inhibition of pSTAT3. Daily dosing for 14 days with 25 mg/kg NVP-LDK378 resulted in significant regression of Karpas299 xenografts (-93% T/C; Study RD-2008-50927 reviewed above) in female SCID mice, suggesting that an approximately 70% reduction in tumor levels of pSTAT3 at Y705 may be necessary for tumor regression. Different methods of tumor cell inoculation were utilized in this study and RD-2008-50927, however (tail vein injection versus subcutaneous implantation), so definitive conclusions cannot be drawn.

Figure 10: PK/PD correlation of 25 mg/kg NVP-LDK378



(Excerpted from Applicant's submission)

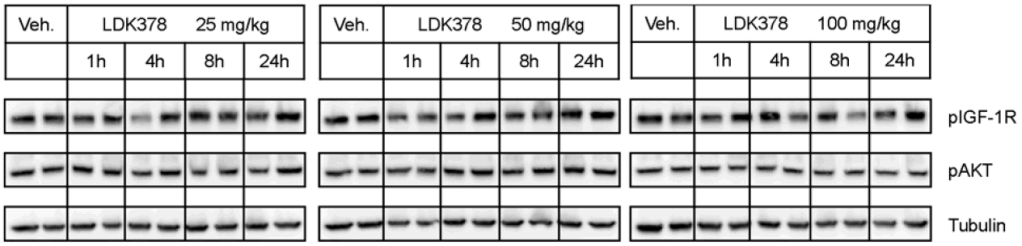
RD-2013-00413: Addendum to RD-2008-50976 NVP-LDK378 does not inhibit the IGF-1R pathway *in vivo*

Methods

Drug:	NVP-LDK378-NX-4
Doses:	25, 50, or 100 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose Volume:	10 mL/kg
Formulation/Vehicle:	0.5% MC: 99% deionized water
Species/Strain:	6-8 wk old female Hsd: Athymic Nude-nu mice
Study design:	NIH3T3 cells stably expressing human IGF-1R and IGF-2 were generated. NIH3T3:IGF-1R:IGF-2 cell proliferation and survival is dependent on the IGF-1R/IGF-2 pathway. 2.5 x 10^6 NIH3T3:IGF-1R:IGF-2 clone 2 cells resuspended in HBSS were injected subcutaneously into female nude mice. Dosing was initiated when mean tumor volumes were ~200 mm^3. Mice were sacrificed after 1, 4, 8, and 24 hrs and tumor samples were lysed and subjected to western blot analysis for phosho-IGF-1Rβ/InsRβ (Tyr1150/1151), phospho-AKT (Ser473), and tubulin.

The in vivo activity of NVP-LDK378 against IGF-1R was investigated in an IGF-1R-driven mouse model. Slight decreases in phosphorylated IGF-1R/InsR were observed in one out of two tumors treated with 25 mg/kg NVP-LDK378 for 4 hrs and 100 mg/kg NVP-LDK378 for 4 hrs and 8 hrs; however, overall, single pharmacodynamically active doses of 25, 50, or 100 mg/kg NVP-LDK378 did not significantly suppress phosphorylated IGF-1R/InsR or phosphorylated AKT in tumor samples, suggesting that NVP-LDK378 may not significantly inhibit the IGF-1R signaling pathway in vivo. Total protein levels of IGF-1R and AKT were not assessed.

Figure 11: Effects of NVP-LDK378 on the IGF-1R signaling pathway in the NIH3T3:IGF-1R:IGF-2 mouse model



(Excerpted from Applicant's submission)

RD-2010-50507: Evaluation of effects of ALK inhibitor LDK378 on glucose tolerance and indicators of insulin resistance in wild-type C57BL/6J mice

The Applicant evaluated the effects of NVP-LDK378 on normal glucose metabolism and insulin resistance in adult male wild-type C57BL/6J mice. 8 mice/group were dosed orally once per day for 7 days with vehicle or 25, 50, or 100 mg/kg NVP-LDK378. On Day 7, NVP-LDK378 was administered 180 minutes before a 3 g/kg glucose bolus. Oral glucose tolerance tests (OGTT)

were performed on fasted mice at baseline and after the glucose bolus; blood was obtained via tail bleeding and glucose levels were measured using a glucometer. The ALK inhibitor NVP-TAE684 was included as an assay control for impaired glucose tolerance. Separate blood samples were collected for insulin analysis three hours prior to dosing on Days 0 and 7. Homeostatic model assessment of insulin resistance (HOMA-IR) was used to assess insulin resistance from fasting glucose and insulin or C-peptide concentrations. Daily dosing with 25, 50, or 100 mg/kg NVP-LDK378 for seven days did not significantly alter blood glucose excursion compared to vehicle in the glucose tolerance test, fasting plasma insulin levels, or HOMA-IR. Thus, NVP-LDK378 inhibition of InsR and IGF-1R did not result in off-target effects on glucose metabolism or insulin resistance, suggesting that InsR/IGF-1R inhibition is unlikely to have functional consequences in humans. Despite this, hyperglycemia has been observed in the clinic with LDK378.

Figure 12: Oral Glucose Tolerance Test in C57BL/6J mice following 7 days of treatment with NVP-LDK378

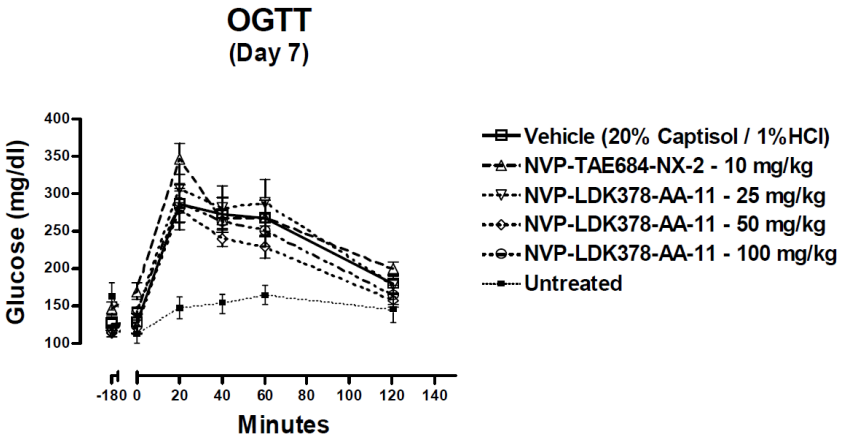


Figure 13: Fasting plasma insulin levels on Day 0 and Day 7

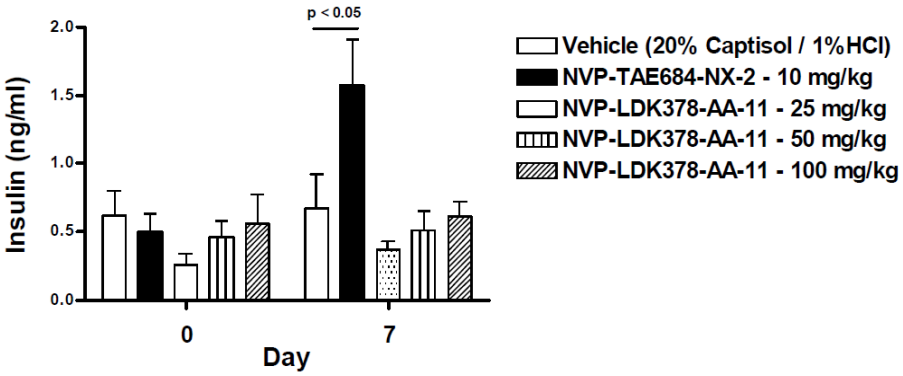
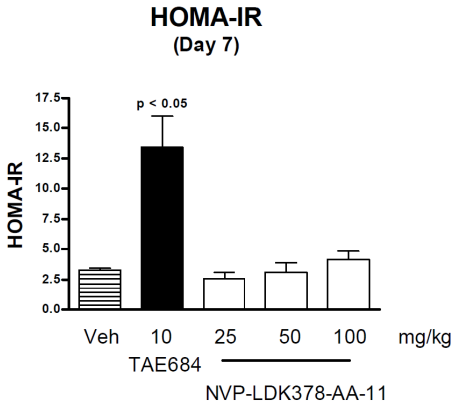


Figure 14: Homeostatic Model Assessment of Insulin Resistance following 7 days of treatment with NVP-LDK378



(Excerpted from Applicant's submission)

RD-2010-50501: Cytochrome p450 3A4 induction potential of the ALK inhibitor NVP-LDK378 by measuring activation of human PXR in a reporter gene assay

The potential ability of NVP-LDK378 to induce cytochrome p450 3A4 (CYP3A4) transcription through activation of the human pregnane X receptor (PXR) was evaluated using a luciferase-based reporter gene assay marketed by (b) (4) and DPX-2 cells, which are HepG2 cells stably transfected with the full-length human PXR and a reporter construct containing the human CYP3A4 proximal promoter and distal enhancer elements cloned upstream of a luciferase reporter gene. This luciferase reporter construct contains PXR response elements in the CYP3A4 regulatory region. Thus, luciferase activity provides a measurement of PXR-mediated CYP3A4 transcription. DPX-2 cells were treated for 24 hrs with 3-fold serial dilutions of NVP-LDK378-AA-1 and the PXR agonist NVP-LDV652 (rifampicin), which was used as a positive control in this assay. In order to measure PXR activation, luciferase activity was measured using Steady-Glo (Promega). The maximum response of NVP-LDV652 was defined as 100% efficacy, and the percent efficacy at each NVP-LDK378 concentration was calculated. NVP-LDV652 stimulated PXR activation, as expected, with an average EC_{50} value of 1.63 ± 0.2 µM. At 3.3 µM, NVP-LDK378 weakly stimulated luciferase activity, exhibiting 9.8% of the maximum response elicited by NVP-LDV652. At 10 µM NVP-LDK378, luciferase activity dropped below basal levels. The Applicant proposed that this may be due to cellular toxicity in the assay. Thus, these data suggest that NVP-LDK378 is unlikely to cause significant induction of CYP3A4 expression in vivo.

Figure 15: Effects of NVP-LDK378 and NVP-LDV652 on PXR activation using a luciferase reporter gene assay

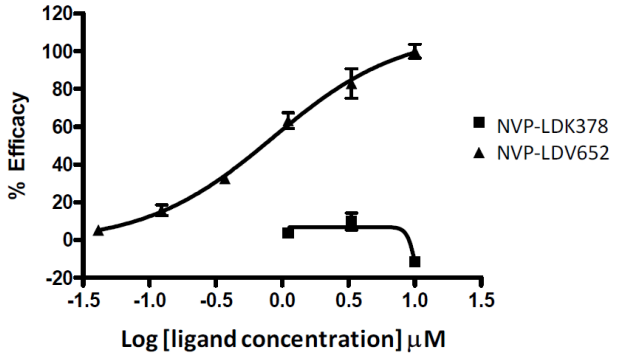


Table 14: Effects of NVP-LDK378 and NVP-LDV652 on PXR activation using a luciferase reporter gene assay

Ligand	Concentration (μM)	%Efficacy
DMSO	--	0
NVP-LDV652	10	96.5; 103.6
NVP-LDV652	3.3	75.1; 90.7
NVP-LDV652	1.1	58.9; 67.4
NVP-LDV652	0.37	34.7; 30.1
NVP-LDV652	0.12	12.9; 18.5
NVP-LDV652	0.04	5.6; 4.6
NVP-LDK378	10	-11.2; -11.9
NVP-LDK378	3.3	14.2; 5.4
NVP-LDK378	1.1	3.5; 4

(Excerpted from Applicant's submission)

RD-2013-50375: *In vitro* antiproliferative activity of NVP-LDK378 in Ba/F3 cells transduced with crizotinib resistant mutant EML4-ALK fusion kinases

The anti-proliferative activity of NVP-LDK378 and NVP-LDQ718-AA-4 (crizotinib) was determined in a panel of Ba/F3 cell lines stably transduced with wild-type EML4-ALK or EML4-ALK expressing secondary ALK mutations found in patients who developed acquired resistance to crizotinib. Cell viability following 48 hours of treatment with NVP-LDK378 or crizotinib serially diluted with DMSO was determined using the Bright-Glo™ luciferase bioluminescent assay. NVP-LDK378 and crizotinib were inactive against wild-type Ba/F3 cells, as expected. NVP-LDK378 and crizotinib inhibited proliferation of Ba/F3-EML4-ALK wild-type cells, with IC_{50} values of 0.031 μM and 0.16 μM, respectively. NVP-LDK378 was ~5-fold more potent than crizotinib at inhibiting Ba/F3-EML4-ALK wild-type cells. Both NVP-LDK378 and crizotinib exhibited less anti-proliferative activity against Ba/F-EML4-ALK cell lines with crizotinib-resistant ALK mutations compared to those expressing wild-type EML4-ALK. NVP-LDK378 inhibited Ba/F3-EML4-ALK mutant cell lines with IC_{50} values of 0.16, 0.69, and 0.94 μM for C1156Y, the gatekeeper mutant L1196M, and G1202R, respectively, compared to IC_{50} values of 0.44, 1.46, and 1.37 μM observed with crizotinib. Thus, ALK mutations associated with acquired resistance to crizotinib in patients appear to be more sensitive to NVP-LDK378 than to crizotinib. This was also true for Ba/F3-EML4-ALK cell lines harboring an ALK mutation associated with in vitro crizotinib resistance (I1171T), or ALK mutations with the potential to induce crizotinib resistance in patients (S1206A and G1269S). Overall, these data demonstrate that while NVP-LDK378 is less

active against ALK mutants than wild-type ALK, it may still retain some activity against ALK mutants associated with acquired crizotinib resistance.

Table 15: Anti-proliferative Activity of NVP-LDK378 against Ba/F3 cells expressing wild-type or mutant EML4-ALK fusion kinases

Cell Line	NVP-LDK378 IC_{50} (μM)	STDV (N=4)
Ba/F3 WT	> 7.16	3.28
Ba/F3 EML4-ALK WT	0.031	0.002
Ba/F3 EML4-ALK C1156Y	0.16	0.01
Ba/F3 EML4-ALK L1196M	0.069	0.007
Ba/F3 EML4-ALK G1202R	0.94	0.27
Ba/F3 EML4-ALK I1171T	0.0376	0.0005
Ba/F3 EML4-ALK S1206A	0.16	0.03
Ba/F3 EML4-ALK G1269S	0.25	0.07

Table 16: Anti-proliferative Activity of Crizotinib against Ba/F3 cells expressing wild-type or mutant EML4-ALK fusion kinases

Cell Line	Crizotinib IC_{50} (μM)	STDV (N=4)
Ba/F3 WT	4.24	1.34
Ba/F3 EML4-ALK WT	0.16	0.02
Ba/F3 EML4-ALK C1156Y	0.44	0.08
Ba/F3 EML4-ALK L1196M	1.46	0.23
Ba/F3 EML4-ALK G1202R	1.37	0.10
Ba/F3 EML4-ALK I1171T	0.34	0.08
Ba/F3 EML4-ALK S1206A	0.27	0.04
Ba/F3 EML4-ALK G1269S	2.14	0.38

(Excerpted from Applicant's submission)

RD-2013-50301: Comparison of *in vivo* efficacy of NVP-LDK378-NX-4 with Crizotinib in the H2228 lung cancer xenograft model

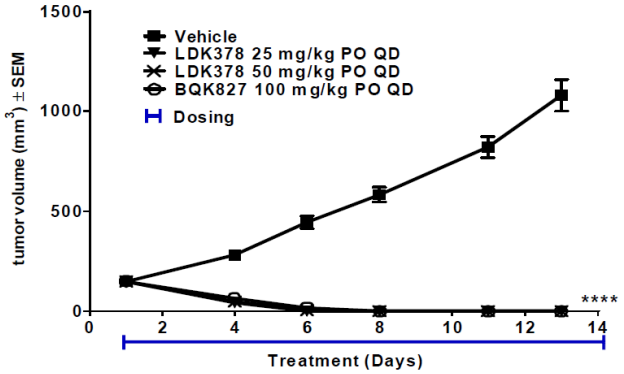
Methods

Drug:	NVP-LDK378-NX-4 NVP-BQK827-AA-4 (crizotinib)
Doses:	NVP-LDK378-NX-4: 25 and 50 mg/kg Crizotinib: 100 mg/kg
Frequency of dosing:	Daily for 14 days
Route of administration:	Oral gavage
Dose Volume:	10 mL/kg
Formulation/Vehicle:	0.5% MC/0.5% Tween 80
Species/Strain:	6-8 wk old female Fox Chase SCID beige mice
Number/Sex/Group:	8 mice/group
Study design:	5 x 10^7 NCI-H2228-luc cells/ml suspended in a 1:1 mixture of RPMI 1640 serum-free medium and matrigel were implanted subcutaneously into the right hind flank of mice. H2228 tumors 300-400 m^3 were harvested and then passaged in mice. 1-2 pieces of tumor tissue were subcutaneously implanted into the

right flank of mice with matrigel. Dosing was initiated on Day 9 following implantation when mean tumor volumes were ~150 mm^3, and mice were dosed for 14 days. Tumors were measured three times per week using calipers, and body weight was monitored daily. Tumors were followed for relapse from Days 15 to 198. Mice were euthanized when the tumors became too large.

The in vivo anti-tumor activity of NVP-LDK378 compared to that of crizotinib was examined in an EML4-ALK-expressing H2228 NSCLC xenograft model. NVP-LDK378-treated mice exhibited body weight loss ≤ 3% and crizotinib-treated mice exhibited ~7% body weight loss on Day 14, compared to ~14% body weight loss in vehicle-treated mice (presumably due to tumor burden). Daily dosing with NVP-LDK378 (25 and 50 mg/kg) or 100 mg/kg crizotinib resulted in 100% tumor regression on Day 13.

Figure 16: Effect of NVP-LDK378 and Crizotinib on H2228 NSCLC xenografts in SCID mice

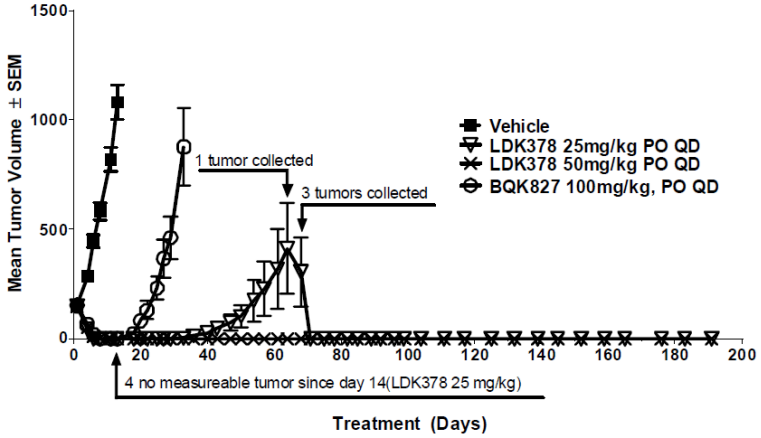


****, P ≤ 0.0001

(Excerpted from Applicant's submission)

After treatment was discontinued, the mice were monitored for up to 198 days post-treatment for tumor relapse. Tumors initially treated with crizotinib relapsed at Day 20. Tumors treated with 25 mg/kg NVP-LDK378 relapsed around Day 60, although four mice in this group had no measureable tumor on Days 14 through 198. 7/8 mice dosed with 50 mg/kg NVP-LDK378 had no tumors or metastases on Day 198; one mouse was euthanized on Day 151 due to labored breathing and was found to have a metastatic tumor in the thoracic cavity. Thus, although NVP-LDK378 and crizotinib exhibited similar anti-tumor activity against H2228 xenografts, treatment with NVP-LDK378 resulted in a more sustained anti-tumor response.

Figure 17: Effect of NVP-LDK378 and Crizotinib on H2228 tumor relapse in SCID mice



(Excerpted from Applicant's submission)

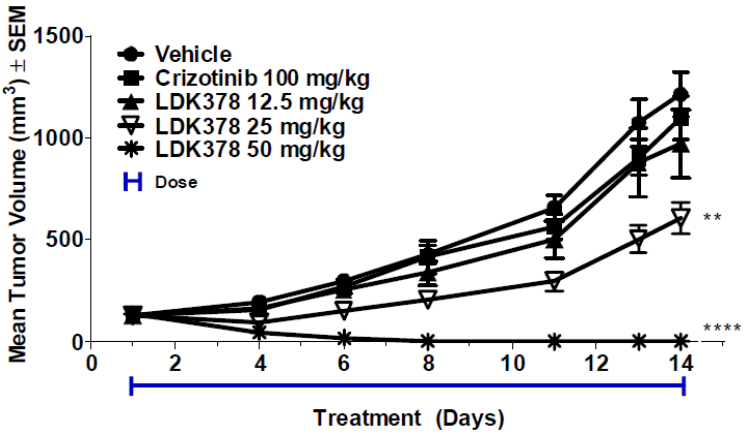
RD-2013-50300: In Vivo efficacy of NVP-LDK378-NX-4 in Crizotinib resistant H2228 lung cancer xenograft model carrying the I1171T mutation

Methods

Drug:	NVP-LDK378-NX-4 NVP-BQK827-AA-3 (crizotinib)
Doses:	NVP-LDK378-NX-4: 12.5, 25, and 50 mg/kg Crizotinib: 100 mg/kg
Frequency of dosing:	Daily for 14 days
Route of administration:	Oral gavage
Dose Volume:	10 mL/kg
Formulation/Vehicle:	0.5% MC/0.5% Tween 80
Species/Strain:	6-8 wk old female SCID beige mice
Number/Sex/Group:	6 mice/group
Study design:	A NCI-H2228 tumor treated with escalating doses of crizotinib (50-100 mg/kg) for 61 days and rendered crizotinib-resistant was collected from a mouse and frozen down to generate tumor stocks; expression of the I1171T mutation was confirmed. The tumor stock was passaged twice through mice treated continuously with 100 mg/kg crizotinib. Mice were then subcutaneously implanted with 2-3 pieces of frozen tumor with matrigel, achieving a >90% tumor take rate. Dosing was initiated on Day 9 when mean tumor volumes were ~130 mm^3. Tumors were measured three times per week using calipers, and body weight was monitored daily. Blood samples were collected from 2 mice/group at 3, 7, and 24 hrs post-dose on Day 14. RNA was extracted from H2228 samples collected at the end of treatment. 2 µg of RNA was reverse-transcribed to cDNA using the (b) (4), the nucleotide sequence of the EML4-ALK kinase domain was determined by (b) (4) and sequence data was analyzed with the Mutation Surveyor Program (SoftGenetics LLC). % T/C =($\Delta T/\Delta C$) x 100 where $\Delta T > 0$; % Regression =($\Delta T/\Delta T_i$) x 100 where $\Delta T < 0$.

The in vivo anti-tumor activity of NVP-LDK378 was examined in a crizotinib-resistant EML4-ALK-expressing H2228 NSCLC xenograft model with an ALK I1171T mutation. NVP-LDK378-treated mice exhibited body weight loss ≤13% and crizotinib-treated mice exhibited ~12% body weight loss on Day 14 of treatment, compared to 18% in vehicle-treated mice. Body weight loss decreased with increasing doses of NVP-LDK378, suggesting it was correlated with tumor burden. Daily dosing with 12.5, 25, or 50 mg/kg NVP-LDK378 resulted in dose-dependent inhibition of crizotinib-resistant xenograft growth (78% T/C, 44% T/C, and 100% regression on Day 14, respectively), which reached statistical significant at doses ≥ 25 mg/kg. In contrast, crizotinib was ineffective (89% T/C), as expected. ALK sequencing analysis confirmed the presence of the ALK I1171T mutation in tumor samples collected at the end of treatment from mice dosed with 100 mg/kg crizotinib or 25 mg/kg NVP-LDK378.

Figure 18: Effect of NVP-LDK378 and Crizotinib on Crizotinib-resistant H2228 NSCLC xenografts with ALK I1171T mutation



**, P ≤ 0.05; ****, P ≤ 0.0001
(Excerpted from Applicant's submission)

NVP-LDK378 plasma exposures increased approximately dose proportionally, and tumor exposure was approximately 25-fold higher than plasma exposure, indicating high tumor distribution. Crizotinib tumor exposure was also approximately 25-fold higher than plasma exposure. Overall, these data demonstrate that NVP-LDK378 exhibits in vivo anti-tumor activity against NSCLC xenografts resistant to crizotinib.

Table 17: Pharmacokinetic parameters of NVP-LDK378 in crizotinib-resistant H2228 ALK I1171T xenografts

Day of dosing	Dose mg/kg	Sample	C_{max} nM	AUC_{0-24h} h*nM	C_{max}/ Dose nM/(mg/kg)	AUC_{0-24h}/ Dose h*nM/(mg/kg)	Tumor/ Plasma AUC_{0-24h} Ratio
14	12.5	Plasma	581	10161	46.5	812.9	
	25	Plasma	1007	15863	40.3	634.5	
	50	Plasma	2433	44111	48.7	882.2	
14	12.5	Tumor	12074	241520	965.9	77.3	23.8
	25	Tumor	21037	404250	841.5	33.7	25.5
	50	Tumor	NA	NA	NA	NA	NA

Table 18: Pharmacokinetic parameters of Crizotinib in crizotinib-resistant H2228 ALK I1171T xenografts

Dose	Day of dosing	Sample	Cmax	$AUC_{0-24\ h}$	Animal	Plasma Conc. (nM)		
mg/kg			*nM*	*h*nM*	*ID*	3 h	7 h	24 h
100	14	Plasma	6930	104310	7	7384.9		
					8	6474.2		
					9		4352.0	
					10		5936.8	
					11			3237.1
					12			2890.7
100	14	Tumor	174265	3097300	7	91159.3		
					8	112027.8		
					9		153140.5	
					10		195387.0	
					11			62211.4
					12			152367.1

(Excerpted from Applicant's submission)

RD-2013-50302: In Vivo efficacy of NVP-LDK378-NX-4 in the Crizotinib resistant H2228 xenograft model carrying C1156Y mutation

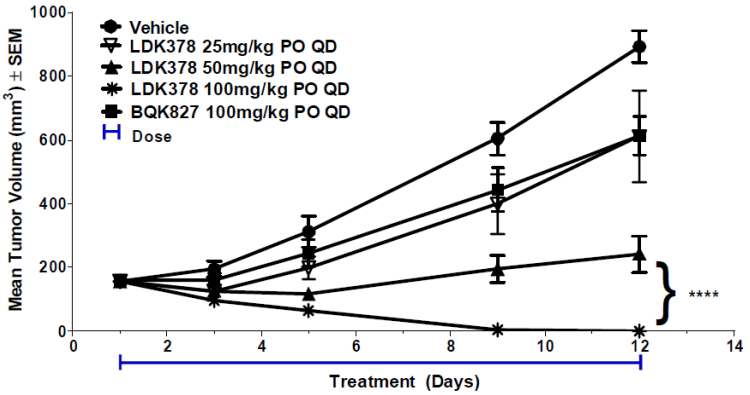
Methods

Drug:	NVP-LDK378-NX-4 NVP-BQK827-AA-4 (crizotinib)
Doses:	NVP-LDK378-NX-4: 25, 50, and 100 mg/kg Crizotinib: 100 mg/kg
Frequency of dosing:	Daily for 12 or 137 days
Route of administration:	Oral gavage
Dose Volume:	10 mL/kg
Formulation/Vehicle:	0.5% MC/0.5% Tween 80
Species/Strain:	6-8 wk old female Fox Chase SCID beige mice
Number/Sex/Group:	8 mice/group
Study design:	A NCI-H2228 tumor treated with 50-100 mg/kg crizotinib for 48 days and rendered crizotinib-resistant was collected from a mouse and frozen down to generate tumor stocks; expression of the C1156Y mutation was confirmed. The tumor stock was passaged through mice treated continuously with 100 mg/kg crizotinib. Mice were then subcutaneously implanted with 2-3 pieces of frozen tumor plus matrigel, achieving a >90% tumor take rate. Dosing was initiated on Day 11 when mean tumor volumes were ~155 mm^3. Tumors were measured three times per week using calipers, and body weight was monitored daily. Blood samples were collected from 3 mice/group at 1, 3, 7, and 24 hrs post-dose on Day 11. Blood samples were also collected from the animals dosed with 100 mg/kg on Day 46. The nucleotide sequence of the EML4-ALK kinase domain was determined from H22288 samples collected at the end of treatment from mice dosed with vehicle, 25 and 50 mg/kg NVP-LDK378, and 100 mg/kg crizotinib as described in Study RD-2013-50300.

The in vivo anti-tumor activity of NVP-LDK378 was examined in a crizotinib-resistant H2228 NSCLC xenograft model with an ALK C1156Y mutation. Treatment with 25, 50, and 100 mg/kg

NVP-LDK378 resulted in 3.07, 4.86, and 10.63% body weight loss on Day 12 of treatment, respectively, compared to 11.72% body weight loss with vehicle and 5.94% body weight loss with crizotinib. According to the Applicant, mice treated with 100 mg/kg NVP-LDK378 exhibited 13.6% body weight loss on Day 79, but body weight loss appeared to quickly recover within the next couple of days. Daily dosing with 25, 50, and 100 mg/kg NVP-LDK378 resulted in dose-dependent inhibition of crizotinib-resistant xenograft growth (61.8% T/C, 11.7% T/C, and 100% regression on Day 12, respectively), which reached statistical significant at doses ≥ 50 mg/kg. In contrast, crizotinib was less effective (61.2% T/C), as expected.

Figure 19: Effect of NVP-LDK378 and Crizotinib on Crizotinib-resistant H2228 NSCLC xenografts with ALK C1156Y mutation



****, P ≤ 0.0001

(Excerpted from Applicant's submission)

The increase in plasma exposures of NVP-LDK378 was roughly dose proportional, correlating with dose-dependent tumor inhibition (see Table below). The mean Cmax and AUC of 100 mg/kg crizotinib on Day 11 were 3863 ng/ml and 49059 h*ng/ml, respectively, despite a lack of tumor inhibition.

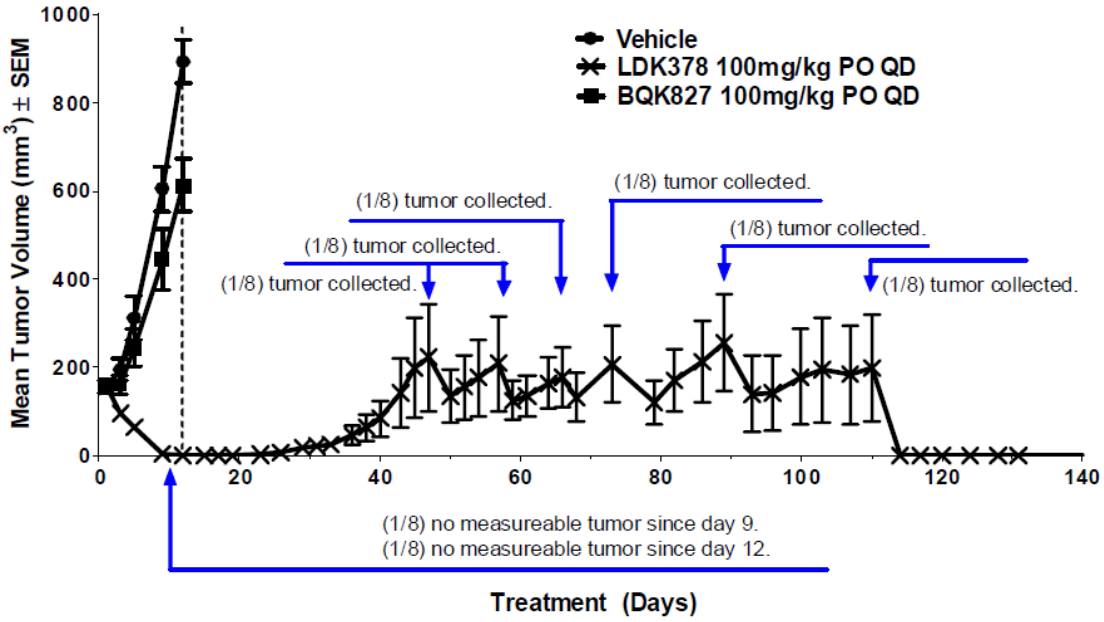
Table 19: Plasma Pharmacokinetic parameters of NVP-LDK378 in crizotinib-resistant H2228 ALK C1156Y xenografts

Day of Dosing	Dose *mg/kg*	Cmax (nM) Mean	Cmax (nM) SD	$AUC_{0\text{-}24h}$ (h*nM) Mean	$AUC_{0\text{-}24h}$ (h*nM) SD	Cmax/ Dose *nM/(mg/kg)*	$AUC_{0\text{-}24h}$ / Dose *h*nM/ (mg/kg)*
11	25	1176	153	22408	1865	47	896
	50	3022	160	53306	4037	60	1066
	100	8479	4034	103578	29533	85	1036
46	100	4631	1692	84247	29980	46	842

(Excerpted from Applicant's submission)

Tumors were collected on Day 12 from mice dosed with vehicle, 25 mg/kg NVP-LDK378, or 100 mg/kg crizotinib, and on Day 23 from mice dosed with 50 mg/kg NVP-LDK378. Mice were treated with 100 mg/kg NVP-LDK378 for up to 137 days in order to monitor tumor relapse. Tumors began to relapse after ~40 days of treatment with 100 mg/kg NVP-LDK378 and were reportedly collected for sequencing when they reached ≥ 500 mm^3. 2/8 mice had no measurable tumor from Day 12 to Day 137 of the study.

Figure 20: Crizotinib-resistant H2228 NSCLC xenograft relapse



(Excerpted from Applicant's submission)

ALK sequencing analysis confirmed the presence of the ALK C1156Y mutation in tumor samples collected from 7/8 mice dosed with 25 or 50 mg/kg NVP-LDK378 or 100 mg/kg crizotinib; mutational status was listed as "not determined" for the other three mice. Vehicle-treated mice did not exhibit the ALK C1156Y mutation at the end of the study, suggesting that constant selection pressure is needed to maintain the C1156Y mutation. Notably the mutation appeared to be preserved by treatment with NVP-LDK378, and not just crizotinib.

RD-2013-50303: In vivo efficacy of NVP-LDK378-NX-4 in non-ALK mutation based Crizotinib resistant H2228 xenograft model

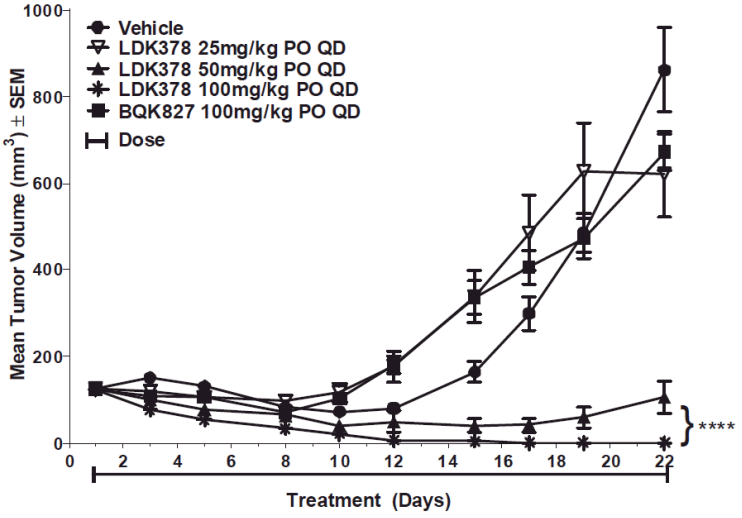
Methods

Drug:	NVP-LDK378-NX-4 NVP-BQK827-AA-4 (crizotinib)
Doses:	NVP-LDK378-NX-4: 25, 50, and 100 mg/kg Crizotinib: 100 mg/kg
Frequency of dosing:	Daily for 22 days. 50 and 100 mg/kg NVP-LDK378 groups were treated for 147 days.
Route of administration:	Oral gavage
Dose Volume:	10 mL/kg
Formulation/Vehicle:	0.5% MC/0.5% Tween 80
Species/Strain:	6-8 wk old female Fox Chase SCID beige mice
Number/Sex/Group:	8 mice/group
Study design:	A NCI-H2228 tumor treated with 50-100 mg/kg crizotinib for 30 days and rendered crizotinib-resistant was collected from a mouse and frozen down to generate tumor stocks. The tumor stock was passaged through mice treated continuously with 100 mg/kg crizotinib. Mice were then subcutaneously

implanted with 2-3 pieces of frozen tumor plus matrigel, achieving a >90% tumor take rate. Dosing was initiated on Day 11 when mean tumor volumes were ~123.57 mm^3. Tumors were measured three times per week using calipers, and body weight was monitored daily. The nucleotide sequence of the EML4-ALK kinase domain was determined from H2228 samples collected at the end of the study as described in Study RD-2013-50300.

The in vivo anti-tumor activity of NVP-LDK378 was examined in a crizotinib-resistant H2228 NSCLC xenograft model for which the mechanism of acquired crizotinib resistance was unknown. NVP-LDK378-treated mice exhibited body weight loss ≤ 8% and crizotinib-treated mice exhibited 37% body weight loss on Day 22, compared to ~2% body weight loss in vehicle-treated mice. Daily dosing with 25, 50, and 100 mg/kg NVP-LDK378 resulted in dose-dependent inhibition of crizotinib-resistant xenograft growth (67.24% T/C, 15.97% regression, and 100% regression on Day 22, respectively), which reached statistical significance at doses ≥ 50 mg/kg. In contrast, crizotinib was less effective (74.6% T/C), as expected.

Figure 21: Effect of NVP-LDK378 and Crizotinib on Crizotinib-resistant H2228 NSCLC xenografts in SCID mice



****, P ≤ 0.0001
(Excerpted from Applicant's submission)

Tumors were collected on Day 22 from mice dosed with vehicle, 25 mg/kg NVP-LDK378, or 100 mg/kg crizotinib. Mice in the 50 or 100 mg/kg NVP-LDK378 dose groups were treated with NVP-LDK378 for up to 147 days in order to monitor tumor relapse; tumors were reportedly collected when mice showed signs of sickness or metastases. Tumors began to relapse after ~20 days of treatment with 50 mg/kg NVP-LDK378; 1/8 mice had no measurable tumor from Day 12 to Day 147 of the study. Tumors began to relapse after ~55 days of treatment with 100 mg/kg NVP-LDK378; 3/8 mice had no measurable tumor from Day 17 to Day 147 of the study.

Figure 22: H2228 tumor relapse in SCID mice treated with 50 mg/kg NVP-LDK378

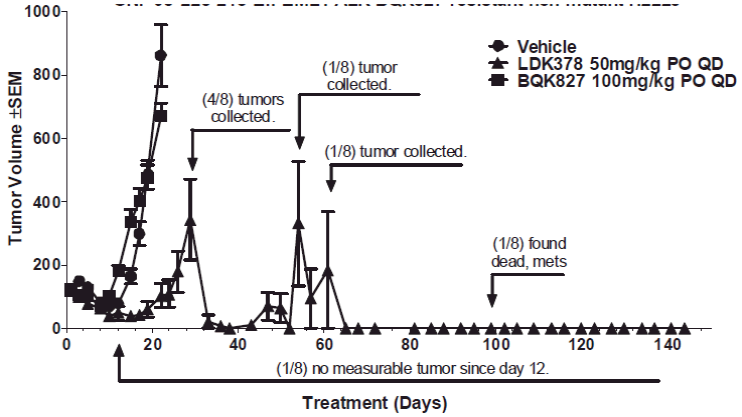
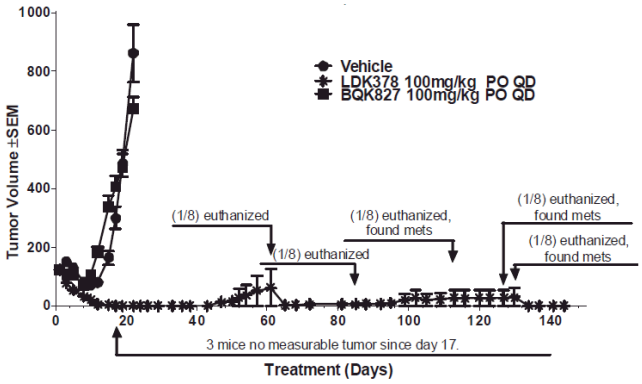


Figure 23: H2228 tumor relapse in SCID mice treated with 100 mg/kg NVP-LDK378



(Excerpted from Applicant's submission)

Mutations in the kinase domain of the EML4-ALK fusion gene were not detected in the majority of the tumors collected from the 25 mg/kg dose group on Day 22, with the following exceptions: 3/8 vehicle-treated tumors and 2/7 tumors treated with 25 mg/kg NVP-LDK378 exhibited an R1113Q mutation, and 1/7 tumors treated with 25 mg/kg NVP-LDK378 exhibited an F1174L mutation. The F1174L mutation has been associated with acquired resistance to crizotinib in patients, but a potential link between R1113Q and crizotinib resistance is unknown. Sequencing data was not provided for tumors treated with 50 and 100 mg/kg NVP-LDK378. Together these data suggest that NVP-LDK378 also exhibits anti-tumor activity against H2228 xenografts with alternate mechanisms of acquired resistance to crizotinib.

RD-2012-50420: Evaluation of NVP-LDK378/NVP-AUY922 combination in the HLUX-1787 human lung primary tumor xenograft model

Methods

Drug:	NVP-LDK378-NX-5
	NVP-AUY922-AG-4
Doses:	NVP-LDK378-NX-5: 10 or 25 mg/kg
	NVP-AUY922-AG-4: 50 mg/kg

Frequency of dosing:	NVP-LDK378-NX-5: Daily for 20 or 13 days, oral gavage NVP-AUY922-AG-4: Once or twice a week for 20 or 13 days, IV
Dose Volume:	NVP-LDK378-NX-5: 10 mL/kg NVP-AUY922-AG-4: 5 mL/kg
Formulation/Vehicle:	NVP-LDK378-NX-5: 0.5% MC/0.5% Tween 80 NVP-AUY922-AG-4: 5% dextrose in water
Species/Strain:	Female nu/nu (nude) mice
Number/Sex/Group:	4 (TRP-0318) or 5 (TRP-0335) mice/group
Study design:	HLUX-1787 primary tumors were harvested, cut into 3 x 3 x 3 mm^3 pieces, and implanted subcutaneously into nude mice. For study TRP-0318, dosing was initiated on Day 27 when mean tumor volume was ~240 mm^3, and continued for 20 days. For study TRP-0335, dosing was initiated on Day 24 when mean tumor volume was ~240 mm^3, and continued for 13 days. Tumors were measured twice per week and body weight was recorded twice per week. After a single dose, blood samples were collected from 3 mice/group at 3, 6, 24, and 48 hrs post-dose (3 and 24 hrs for vehicle groups).

The in vivo anti-tumor activity of NVP-LDK378, both alone and in combination with the non-geldanamycin based HSP90 inhibitor NVP-AUY922, was evaluated in the human lung primary tumor xenograft model HLUX-1787, which harbors an EML4-ALK variant 2 translocation. HSP90s are chaperone proteins involved in the folding and stabilization of numerous client proteins, including EML4-ALK. Treatment with NVP-LDK378, NVP-AUY922-AG, or the combination was well-tolerated in nude mice; once weekly administration of NVP-AUY922 was slightly better tolerated than twice weekly administration.

Table 20: Body weight changes and anti-tumor effects associated with NVP-LDK378 and NVP-AUY922 on Day 47 (Study TRP-0318)

Treatment	Dose	Schedule	Tumor Response		Host Response	
			T/C (%)	T/T0 (%)	% BW change	Survival
D5W	5 ml/kg	2qw iv			4.1%	4
0.5% MC/ 0.5% Tween 80	10 ml/kg	qd po				
LDK378	10 mg/kg	qd, po	50.9%		3.5%	4
AUY922	50 mg/kg	2qw, iv	19.2%*		-6.8%	4
LDK378 AUY922	10 mg/kg 50 mg/kg	qd, po 2qw, iv		-6.8%*	-5.2%	4

*p<0.05 compared to Vehicle by one way ANOVA post hoc Tukey test.

(Excerpted from Applicant's submission)

Table 21: Body weight changes and anti-tumor effects associated with NVP-LDK378 and NVP-AUY922 on Day 37 (Study TRP-0335)

Treatment	Dose	Schedule	Tumor Response		Host Response	
			T/C (%)	T/T0 (%)	% BW change	Survival
D5W 0.5% MC/ 0.5% Tween 80	5 ml/kg 10 ml/kg	2qw iv qd po			1.5%	5
LDK378	25 mg/kg	qd, po	45.3%		3.0%	5
AUY922	50 mg/kg	qw, iv	19.3%*		5.0%	5
AUY922	50 mg/kg	2qw, iv	20.0%*		-2.2%	5
LDK378 AUY922	25 mg/kg 50 mg/kg	qd, po qw, iv	16.0%*		1.1%	5
LDK378 AUY922	25 mg/kg 50 mg/kg	qd, po 2qw, iv		-34%**	-0.1%	5

*p<0.05 compared to Vehicle by one way ANOVA post hoc Tukey test.
**p<0.001 compared to Vehicle by one way ANOVA post hoc Tukey test.

(Excerpted from Applicant's submission)

In Study TRP-0318, daily dosing with 10 mg/kg NVP-LDK378 resulted in limited anti-tumor activity (50.9% T/C on Day 47). Administration of 50 mg/kg NVP-AUY922 twice a week resulted in statistically significant tumor growth inhibition (19.2% T/C), whereas combined treatment with NVP-LDK378 and NVP-AUY922 resulted in tumor stasis (-6.8% T/T0). Although combined treatment with NVP-LDK378 and NVP-AUY922 resulted in greater anti-tumor activity than either agent alone, combination therapy was not statistically superior to either monotherapy. In Study TRP-0335, daily dosing with 25 mg/kg NVP-LDK378 resulted in modest anti-tumor activity (45.3% T/C on Day 37). Administration of 50 mg/kg NVP-AUY922 once or twice a week resulted in statistically significant tumor growth inhibition (19.3% or 20% T/C, respectively). Combined treatment with NVP-LDK378 and NVP-AUY922 administered once or twice a week, however, resulted in greater tumor inhibition than either agent alone (16% T/C and -34% T/T0, respectively). Again, combination treatment was not statistically significant with respect to single agent treatment. Together, these data suggest that combined inhibition of ALK and HSP90 results in enhanced anti-tumor activity in a lung cancer xenograft model harboring an EML4-ALK variant 2 translocation.

Figure 24: Effect of NVP-LDK378 in combination with NVP-AUY922 on HLUX-1787 xenograft growth (Study TRP-0318)

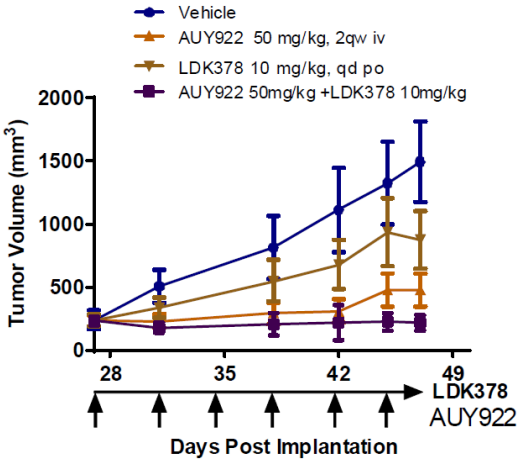
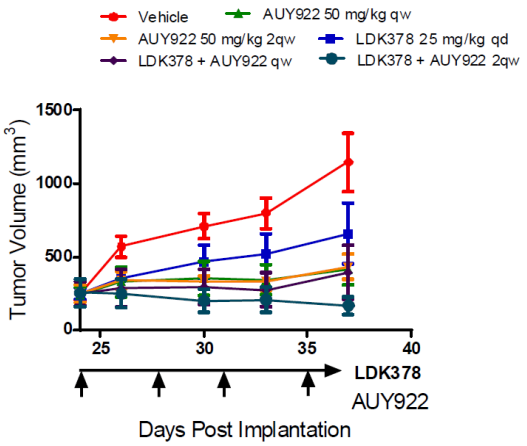


Figure 25: Effect of NVP-LDK378 in combination with NVP-AUY922 on HLUX-1787 xenograft growth (Study TRP-0335)



(Excerpted from Applicant's submission)

PK analysis following single doses of NVP-LDK378 and NVP-AUY922 revealed that plasma concentrations for each drug were generally similar between groups receiving monotherapy or combination treatments with the exception of an NVP-LDK378 plasma concentration that was 5-fold higher at 24 hr post-dose in the combination group compared to the monotherapy group. According to the Applicant, it is unknown whether the discrepancy at the 24 hour time point was due to sampling or assay variability.

Table 22: NVP-LDK378 plasma concentrations following a single dose

Treatment	LDK378 (ng/ml)			
	3h	6h	24h	48h
LDK378 25 mg/kg	859 ± 141	601 ± 231.6	41 ± 15.9	8 ± 6.9
LDK378 25 mg/kg + AUY922 50 mg/kg	639 ± 42.1	929 ± 331.3	204 ± 125.9	3 ± 1.1

Table 23: NVP-AUY922 plasma concentrations following a single dose

Treatment	AUY922 (ng/ml)			
	3h	6h	24h	48h
AUY922 50 mg/kg	64.2 ± 6.8	49.8 ±16.1	1.7 ± 1.4	0
LDK378 25 mg/kg+ AUY922 50 mg/kg	52.3 ± 12.8	33.3 ± 1.1	1.8 ± 2	0

(Excerpted from Applicant's submission)

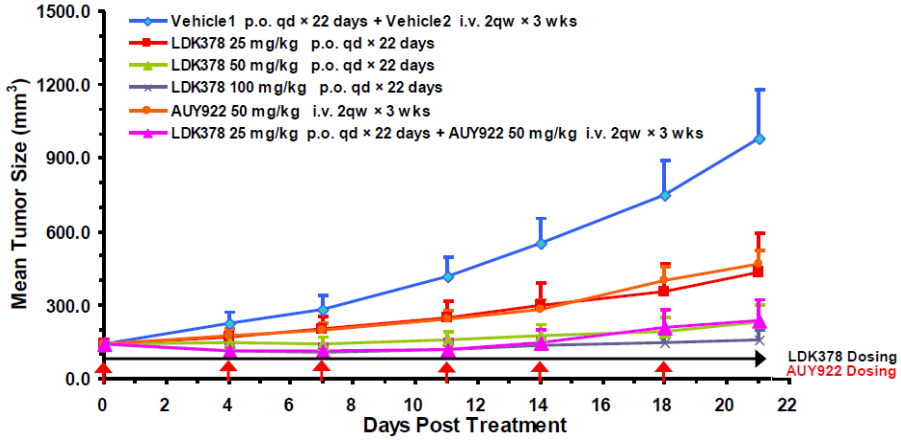
RD-2012-50517: Evaluation of NVP-LDK378/NVP-AUY922 combination in the LUF-1656 human lung primary tumor xenograft model

Methods	
Conducting laboratory and location:	(b) (4)
Drug:	NVP-LDK378-NX-5 NVP-AUY922-AG

Doses:	NVP-LDK378-NX-5: 10, 25, 50, and 100 mg/kg NVP-AUY922-AG-4: 50 mg/kg
Frequency of dosing:	NVP-LDK378-NX-5: Daily for 21 days, oral gavage NVP-AUY922-AG-4: Twice a week for 3 weeks, IV
Dose Volume:	NVP-LDK378-NX-5: 10 mL/kg NVP-AUY922-AG-4: 5 mL/kg
Formulation/Vehicle:	NVP-LDK378-NX-5: 0.5% MC/0.5% Tween 80 NVP-AUY922-AG-4: 5% dextrose in water
Species/Strain:	7-8 wk old female nu/nu mice
Number/Sex/Group:	8 mice/group
Study design:	LUF-1656 primary tumors were harvested and cut into 3 x 3 x 3 mm^3 fragments. One tumor fragment was implanted subcutaneously into the right flank of each nude mouse. Dosing was initiated when mean tumor volume was ~140 mm^3. Tumors were measured twice weekly using calipers and body weight was recorded twice per week.

The in vivo anti-tumor activity of NVP-LDK378, both alone and in combination with the HSP90 inhibitor NVP-AUY922-AG, was evaluated in the human lung primary tumor xenograft model LUF-1656, which harbors an EML4-ALK variant 1 translocation. Treatment with NVP-LDK378, NVP-AUY922, and the combination was well-tolerated in nude mice; there were no significant effects on mean body weight. Daily treatment with 25, 50, and 100 mg/kg NVP-LDK378 resulted in dose-dependent inhibition of LUF-1656 xenograft growth (35.1%, 10.9%, and 1.9% T/C, respectively on Day 21), which reached statistical significance at doses ≥ 50 mg/kg. IV administration of 50 mg/kg NVP-AUY922 twice weekly for three weeks resulted in modest anti-tumor activity (38.7% T/C). Combination treatment with 25 mg/kg NVP-LDK378 and 50 mg/kg NVP-AUY922 significantly inhibited LUF-1656 xenograft growth (11.4% T/C; P = 0.001) and resulted in greater tumor inhibition than treatment with 25 mg/kg NVP-LDK378 or 50 mg/kg NVP-AUY922 alone; however, combination therapy was not statistically superior to either monotherapy, and was not more efficacious than daily dosing with 50 or 100 mg/kg NVP-LDK378 as single-agents. Daily dosing with 100 mg/kg NVP-LDK378 resulted in greatest tumor inhibition.

Figure 26: Effect of NVP-LDK378 in combination with NVP-AUY922 on human lung primary tumor LUF-1656 xenograft growth in nude mice



(Excerpted from Applicant's submission)

4.2 Secondary Pharmacology

None submitted.

4.3 Safety Pharmacology

Study title: Effects of LDK378 on cloned hERG potassium channels expressed in human embryonic kidney cells

Study no.:	0970418
Study report location:	Electronic submission, M4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 1, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, batch #, and % purity:	LDK378, batch #0850001, purity 98.9%

Methods

Concentrations:	0, 0.3, 0.4, 1 and 2.4 μM
Vehicle control:	HEPES-buffered physiological saline (HB-PS) + 0.3% DMSO
Positive control:	Terfenadine
Reference Solution rationale:	E-4031 selectively inhibits hERG potassium current with IC_{50}=12nM

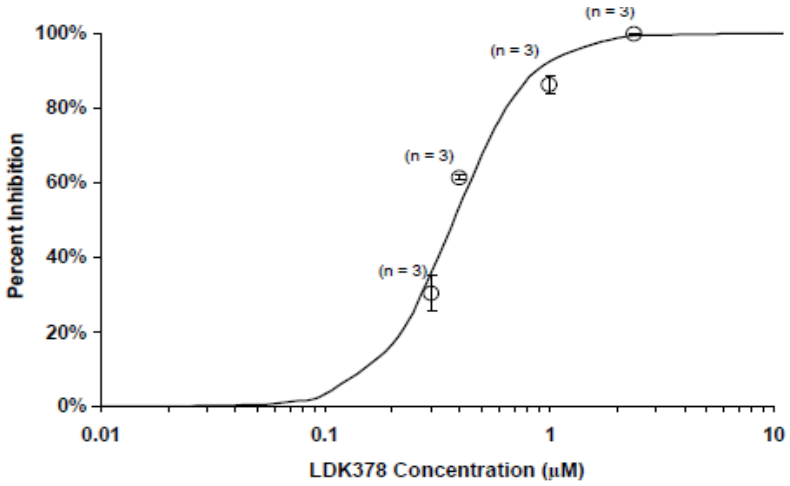
Key Study Findings:

● LDK378 significantly inhibited hERG channel activity when tested at concentrations ≥ 0.3 μM.

Study Summary

hERG potassium channel inhibition was evaluated by manual patch-clamp electrophysiology using HEK293 cells transfected with hERG cDNA. Four concentrations of LDK378 (0.3, 0.4, 1 and 2.4 μM) significantly inhibited hERG potassium current by 30.2, 61.3, 86.2 and 99.7% compared to the 0.3% vehicle control value. Inhibition was significant at $p<0.05$ for all concentrations. The IC_{50} for the inhibitory effect of LDK378 on hERG potassium current was 0.4 μM. See following figure excerpted from Applicant's submission.

Figure 27 Inhibition of hERG channel by LDK378 (concentration-response relationship)



Percent inhibition of hERG potassium current after application of each concentration of LDK378 (Mean ± SEM, open circles) was fit to a binding equation (solid line) with IC_{50} = 0.4 µM and Hill coefficient = 2.6. The number of cells at each concentration is given in parentheses.

(Excerpted from Applicant's submission)

Study title: LDK378: Telemetry study of cardiovascular effects in male monkeys after a single oral (gavage)administration

Study no.:	0770889
Study report location:	Electronic submission, M4.2.1.3
Conducting laboratory and location:	Novartis Pharma
Date of study completion:	May 13, 2008
GLP compliance:	No
QA statement:	No
Drug, batch #, and % purity:	LDK378, batch #TRD 1427-46, purity 98.9%

Methods

Doses:	250 (294) mg/kg (base/salt ratio of 1.176)
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Vehicle control:	HEPES-buffered physiological saline (HB-PS) + 0.3% DMSO
Species/strain (Number/sex):	Cynomolgus monkey (2/male)

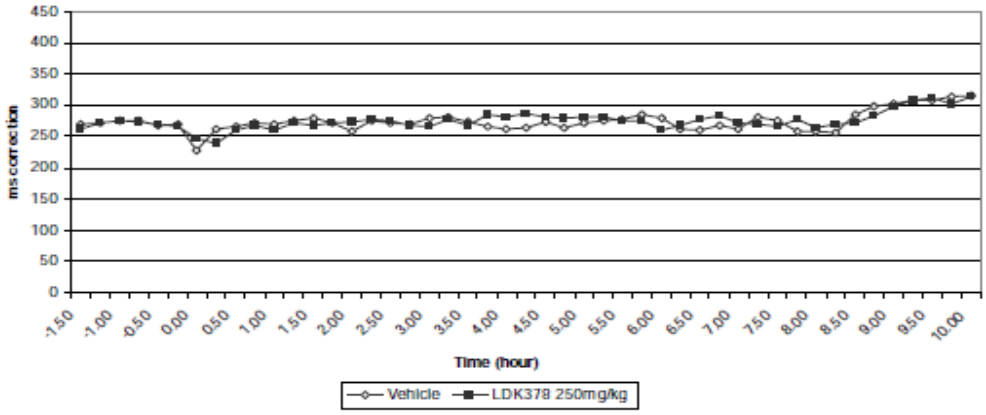
Key Study Findings:

●.No electrocardiography changes (including QTc prolongation) following a single dose of 250 mg/kg LDK378 to cynomolgus monkeys

Study Summary

Parameter	Dose (250 mg/kg)
Mortality	None
Clinical observations	Emesis (6, 24 and 46h following dosing), Soft feces, ↓ food consumption
EKG	♦ No effects on blood pressure, heart rate or body temperature ♦ No remarkable changes in ECG durations or intervals

Figure 28 Average QTcVW in monkeys administered a single 250 mg/kg of LDK378



(Excerpted from Applicant's submission)

Study title: Telemetry study of cardiovascular effects in male monkeys after a single oral (gavage) administration at multiple dose levels

Study no.:	0970420
Study report location:	Electronic submission, M4.2.1.3
Conducting laboratory and location:	Novartis Pharma
Date of study completion:	June 2, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, batch #, and % purity:	LDK378, batch #0850001, purity 98.9%

Methods

Doses:	0, 10, 30, 100 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Vehicle control:	HEPES-buffered physiological saline (HB-PS) + 0.3% DMSO
Species/strain (Number/sex):	Cynomolgus monkey (4/males), 3-4 years; 3.0 - 3.5 kg) [Each dose administered to same 4 monkeys with a 7 day rest period between doses

Key Study Findings:

●. QTc prolongation in 1 of 4 animals 10-19h following administration of 100 mg/kg LDK378
● No drug-related changes in BP, HR or body temperature

Study Summary

Parameter	Doses (10, 30, 100 mg/kg)
Mortality	None
Clinical observations	Emesis: 2/4 animals administered doses ≥ 30 mg/kg Soft feces: 3/4 monkeys administered doses ≥ 10 mg/kg
EKG	♦ QT/QTc prolongation observed 10-19 hours following administration of 100 mg/kg in 1 of 4 monkeys (animal #1003) (14.3 – 43.5 ms above predose baseline)* ♦ ↑ heart rate or decrease in RR interval) + secondary ↓ in PR and QT interval within ~30m following dosing in vehicle and all LDK378-treatment groups. Findings were considered a result of excitement caused by the dosing procedure. ♦ No drug-related effects on blood pressure, heart rate, body temperature, PR or QRS intervals, or arrhythmias

*Note that monkey exhibiting QT/QTc prolongation following administration of 100 mg/kg LDK378 had previously been administered 10 and 30 mg/kg with 7-day rest period between doses.

Figure 29 QT interval data in animal #1003

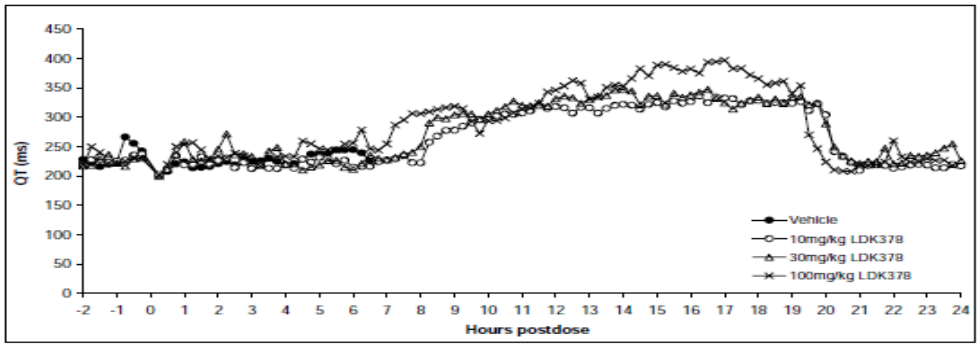
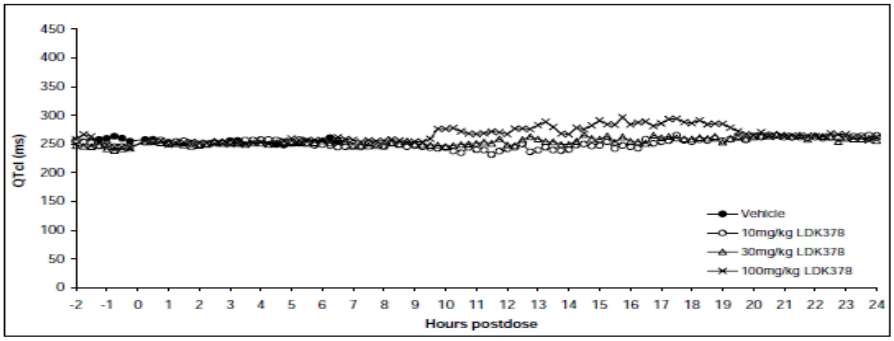


Figure 30 QTc data in animal #1003



(Excerpted from Applicant's submission)

Study title: Single-dose oral (gavage)safety pharmacology study in rats (nervous system and respiratory functions)

Study no.:	0970415
Study report location:	Electronic submission, M4.2.1.3
Conducting laboratory and location:	Novartis Pharma AG
Date of study completion:	April 20, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, batch #, and % purity:	LDK378, batch #0850001, purity 98.9%

Methods

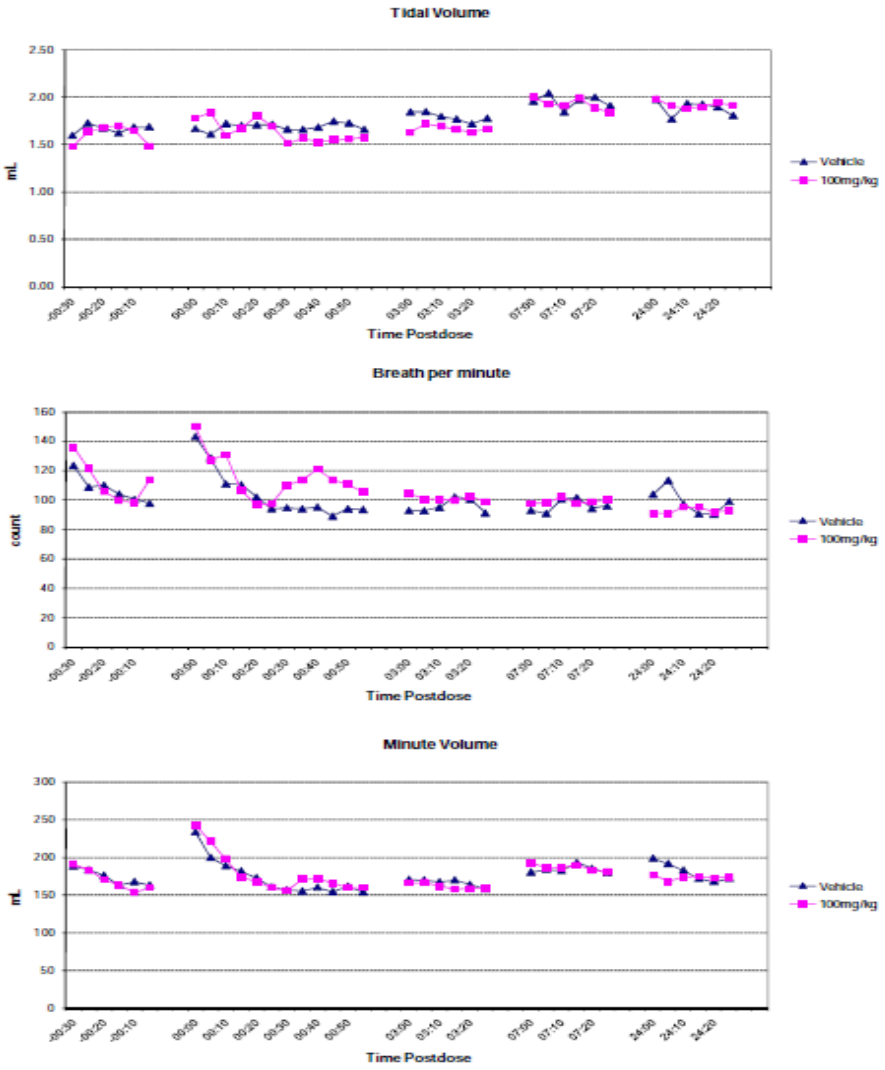
Doses:	100 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Vehicle control:	HEPES-buffered physiological saline (HB-PS) + 0.3% DMSO
Species/strain (Number/sex):	Rat (RccHan:WIST)
Age/Weight/Number/Sex/Group:	9-10 weeks; 237-318 g; 10 males/vehicle control and test group

Key Study Findings:

●. No significant neurologic changes from controls were observed during functional observational battery (FOB) conducted 3 and 24 hours following single dose of 100 mg/kg LDK378 though some initial inactivity was observed.

● Significantly higher breaths per minute (BPM) values (~20%) observed from 35-60 min post dose; effects were transient. Changes in 5m values considered to be a result of sensitivity of plethysmograph and in animal behavior. (See figure below).

Figure 31: Respiratory function of animals administered 100 mg/kg LDK378



(Excerpted from Applicant's submission)

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

DMPK R0900773a: Absorption, metabolism and excretion of LDK378 in intact and bile duct-cannulated rats following an intravenous or oral dose of [14C]LDK378

Methods

Drug: [^{14}C]LDK378

Batch, specific activity, radiochemical purity: (excerpted from Applicant's submission)

Dose/route	Batch #	Specific Activity (mCi/mg	Radiochemical purity (%)
ADME 10 mg/kg i.v.	LU 12105 53B	20	> 98
ADME 25 mg/kg p.o.	LU12150 53	8	> 98
Bile 10 mg/kg i.v.	LU12105 65	~ 20	> 98
Bile 25 mg/kg p.o.	LU12150 65	~ 8	> 98

Dose, ROA, dose volume:	25 mg/kg (8 µCi/kg), oral gavage, 10 mL/kg 10 mg/kg (20 µCi/kg), IV, 2 mL/kg
Formulation/Vehicle:	Oral: 0.5% MC in deionized water IV: 30% propylene glycol/5% solutol buffer in phosphate buffered saline, adjusted to pH 4
Species/Strain:	~8 wk old HanWistar male rats
Number/Group:	No cannulation: IV, 3 for PK + 3 for metabolite profiling; oral, 2 for PK + 3 for metabolite profiling Cannulation: IV, 2/group; oral, 3/group
Sample collection:	Blood/plasma: 0.083 (IV only), 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, and 168 hr post-dose Urine/feces: 0-24, 24-48, 48-72, 72-96, and 96-168 hr post-dose; 24 hr intervals up to 72 hrs in cannulated rats Bile: 0-1, 1-2, 2-4, 4-8, 8-24, 24-48, and 48-72 hrs post-dose Carcass: at termination (168 hr)
Sample collection for metabolic group:	Plasma: 0.083 (IV only), 0.25, 0.5, 1, 2, 4, 8, 24, and 48 hr post-dose

The Applicant examined the absorption, metabolism, and excretion of LDK378 following a single IV or oral dose of [^{14}C]LDK378 in intact and bile duct-cannulated rats. This study investigated blood and plasma levels of radioactivity, excretion of radioactivity in urine and feces, LDK378 and metabolite levels in selected plasma, bile and feces samples, and metabolite structure/patterns in plasma, bile and excreta. The radioactivity of all dose, blood, plasma, urine, feces, and cage wash samples was determined by liquid scintillation counting in a Packard Liquid Scintillation Counter. Serial plasma samples were analyzed for LDK378 by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. LDK378 concentrations in excreta were determined by high pressure liquid chromatography (HPLC) with radioactivity detection. The limit of detection (LOD) for radioactivity analysis in this study was 0.225 and 0.563 ngEq/mL for IV and oral samples, respectively.

Following a single 10 mg/kg IV dose of [^{14}C]LDK378, the LDK378 plasma concentration profile appeared to be monophasic, with a relatively long terminal half-life (9.7 hrs). LDK378 had moderate plasma clearance (1.49 L/h/kg). The steady-state volume of distribution (19.9 L/kg) greatly exceeded total body water (~0.67 L/kg for rats), indicating extensive distribution into tissues. Following a single 25 mg/kg oral dose of [^{14}C]LDK378, the Tmax values for blood and plasma radioactivity were 10 hrs and 12 hrs respectively, indicating a slow absorption rate. The apparent terminal half-life of LDK378 in plasma was relatively long (13.2 hrs). Further, [^{14}C]LDK378 was moderately absorbed in intact rats, exhibiting 37.1% absorption and 48.3% bioavailability. The absorption was estimated based on the AUC_{inf} of the oral and IV plasma radioactivity. Since the absorption and bioavailability values were comparable, these data indicate that there was a minimal first-pass effect.

Table 24: Summary of pharmacokinetic parameters and excretion data following a single IV or oral dose of [^{14}C]LDK378 to rats

Species/strain/gender:	Rat/HanWistar/male			
Bile cannulation	No	No	Yes	Yes
Route/formulation:	Intravenous / solution	Oral / suspension	Intravenous / solution	Oral / suspension
Dose (mg/kg):	10	25	10	25
Plasma LDK378 concentration[b]				
Cmax (ng/mL):	975 ± 139[a] [1750 ± 250 nM]	363 [650 nM]	-[c]	-[c]
Tmax (h):	0.083 ± 0[a]	12.0	-[c]	-[c]
AUClast (ng•h/mL):	6890 ± 1510 [12300 ± 2700 nM]	8330 [14900 nM]	-[c]	-[c]
AUCinf (ng•h/mL):	6950 ± 1470 [12400 ± 2630 nM]	8390 [15000 nM]	-[c]	-[c]
Terminal T1/2 (h):	9.7 ± 1.2	13.2	-[c]	-[c]
CL (L/h/kg):	1.49 ± 0.342	NA	-[c]	-[c]
Vss (L/kg):	19.9 ± 0.49	NA	-[c]	-[c]
Plasma [^{14}C]radioactivity[b]				
Cmax (ngEq/mL):	2030 ± 242[a]	367	-[c]	-[c]
Tmax (h):	0.083 ± 0[a]	12.0	-[c]	-[c]
AUClast (ngEq•h/mL):	9750 ± 2020	8320	-[c]	-[c]
AUCINF (ngEq•h/mL):	10200 ± 2150	9470	-[c]	-[c]
Apparent terminal T1/2 (h)	15.4 ± 4.0	13.8	-[c]	-[c]
Blood [^{14}C]radioactivity[b]				
Cmax (ngEq/mL):	2800 ± 153[a]	820	-[c]	-[c]
Tmax (h):	0.083 ± 0[a]	10.0	-[c]	-[c]
AUClast (ngEq•h/mL):	20100 ± 3440	19900	-[c]	-[c]
AUCINF (ngEq•h/mL):	20600 ± 3350	20400	-[c]	-[c]
Apparent terminal T1/2 (h)	20.7 ± 4.3	12.7	-[c]	-[c]
Excretion in urine (% dose): radioactivity				
0-24 h:	0.128 ± 0.114	0.0498	0.41	0.59 ± 0.644
0-168 h or 0-72 h:	0.241 ± 0.174	0.180	0.62	1.05 ± 1.03
LDK378 in urine:	ND	ND	ND	ND
Excretion in feces (% dose): radioactivity				
0-24 h:	43.2 ± 8.14	33.2	10.2	27.6 ± 20.1
0-168 h or 0-72 h:	107 ± 7.8	101	29.8	65.0 ± 17.0
LDK378 in feces:	82.7	80.4	12.1	51.8
Excretion in bile (% dose): radioactivity				
0 - 2 h	-[c]	-[c]	2.99	0.284 ± 0.258
0 - 4 h	-[c]	-[c]	6.21	0.906 ± 0.866
0 - 24 h	-[c]	-[c]	29.7	11.2 ± 4.09
0 - 72 h	-[c]	-[c]	65.4	24.3 ± 8.52
LDK378 in bile:	-[c]	-[c]	34.9	9.19
Cage wash (% dose)	0.795 ± 0.874	0.202	0.032	0.0372 ± 0.029
Total radioactivity recovery (% dose):	108 ± 6.84	101	95.8	90.4 ± 17.2
Absorption (%):	-[c]	37.1[d]	-[c]	38.4[e]
Bioavailability (%):	-[c]	48.3	-[c]	-[c]

ND = not detectable

[a] The first sampling was 0.083 h

[b] The value was estimated by non-compartment analysis

[c] Not applicable

[d] Absorption was estimated based on the plasma radioactivity AUC

[e] Absorption was estimated based on the radioactivity recovery in the urine and bile in biliary excretion study following i.v. and p.o. doses

(Excerpted from Applicant's submission)

Regardless of the route of administration, fecal excretion was dominant and accounted for ~100% of the radioactivity dose in intact rats, whereas urinary excretion was minor and accounted for <1% of the dose (see table above). Recovery was nearly complete in the excreta by 168 hrs post-dose for both routes of administration. Unchanged LDK378 was detected in the

feces, but not in the urine. Following IV administration of [^{14}C]LDK378 to bile-duct cannulated rats, excretion into the urine, feces, and bile was <1%, 29.8%, and 65.4% of the radioactivity dose, suggesting that fecal excretion was the result of both biliary and GI excretion. Biliary excretion of unchanged LDK378 accounted for approximately 34.9% of the IV dose of [^{14}C]LDK378, and fecal excretion of unchanged LDK378 accounted for 12.1% of the dose. Absorption in bile-duct cannulated rats was estimated based on the radioactivity recovery in the urine and bile. Following oral administration of [^{14}C]LDK378 to bile-duct cannulated rats, excretion into the urine, feces, and bile was 1%, 65%, and 24.3% of the radioactivity dose. Combined biliary and urinary recoveries suggested that ~38.4% of the oral dose of LDK378 was absorbed, which was similar to that estimated in intact rats. Together, these data suggest that hepatic clearance through biliary excretion is an important route of elimination of IV administered [^{14}C]LDK378.

Unchanged LDK378 was the major circulating component in plasma regardless of route of administration, accounting for 100% of the $AUC_{0\text{-}24h}$. Unchanged LDK378 was also the major component in feces, accounting for ~80% of the oral and IV dose in intact rats. The major fecal metabolite in intact rats was M33.4 (oxygenation on the benzyl group), which accounted for 7.2% and 6.1% of the IV and oral doses, respectively. Unchanged LDK378 accounted for 12.1% and 71.77% of the IV and oral doses in bile duct-cannulated rat feces. The major fecal metabolite in bile duct-cannulated rats was M23.6 (mono-oxygenatation), which accounted for 4.6% and 5.9% of the IV and oral doses, respectively. In addition, unchanged LDK378 was the major component in the bile of bile duct-cannulated rats, accounting for 34.9 and 9.2% of the IV and oral doses, respectively. Metabolites in the bile accounted for <5% of the dose, with M36.8 being the most abundant.

Table 25: LDK378 and its metabolites in the feces and bile of intact and bile duct-cannulated rats following IV and oral doses of [^{14}C]LDK378

Metabolites	Excretion (% of dose)					
	Intact Rats		Bile duct-cannulated rats			
	Feces		Feces		Bile	
	IV	Oral	IV	Oral	IV	Oral
M21.6	1.62	1.42	3.75	4.54	ND[a]	1.71
M23.6	1.74	1.44	4.56	5.88	ND	0.84
M26.9	ND	ND	ND	ND	ND	1.52
M29.5	1.81	1.57	2.69	ND	1.75	1.31
M30.6	ND	1.43	ND	ND	ND	ND
M32.9	3.26[b]	2.89[b]	ND	ND	1.09	0.72
M33.4	7.20[b]	6.13[b]	ND	2.82	3.26	1.46
M35.8	3.89	3.46	ND	ND	0.83	0.52
M36.8	4.35	1.86	ND	ND	4.44	2.95
LDK378	82.65	80.40	12.05	51.77	34.89	9.19

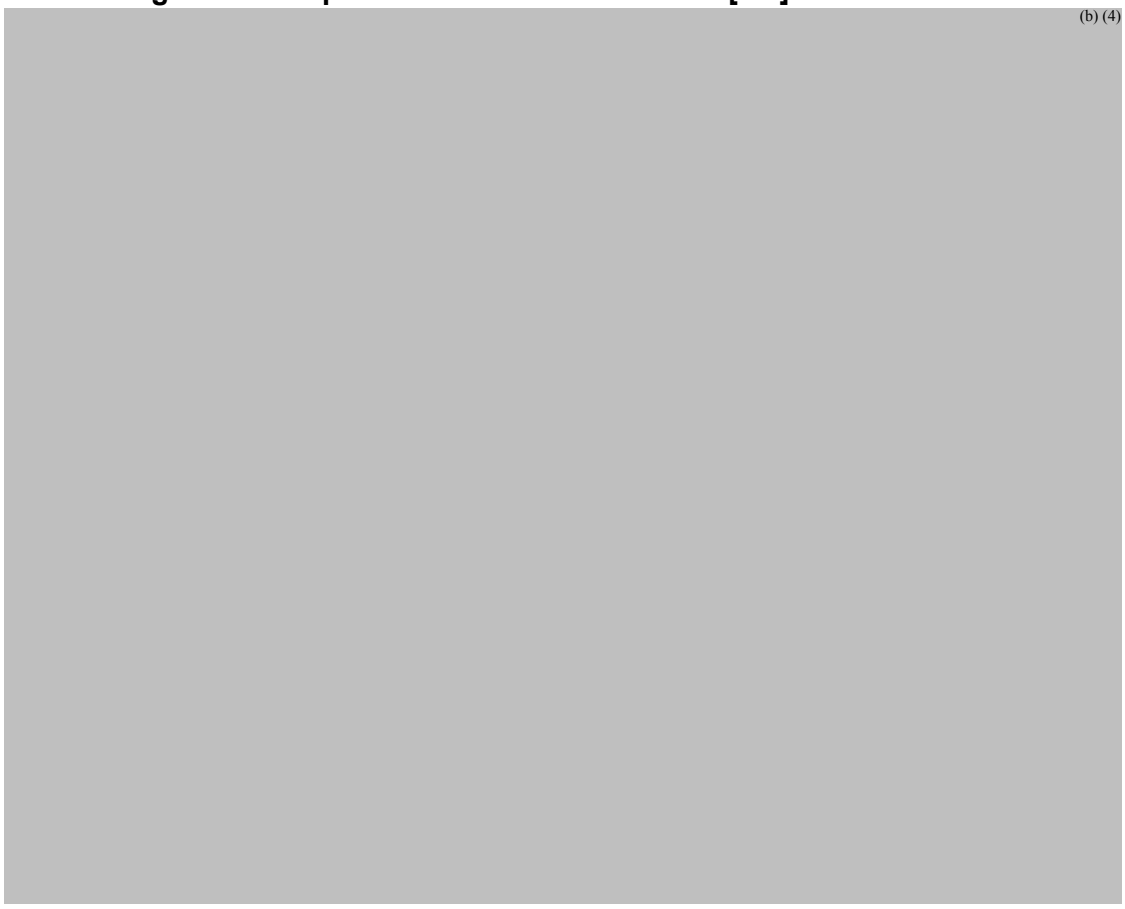
[a] ND = Not detected

[b] M32.9 and M33.4 were poorly resolved. Samples we run on a separate gradient to resolve the M32.9 and M33.4 peaks and determine percent of dose for each. Data not shown.

(Excerpted from Applicant's submission)

LC-MS/MS was used for structural characterization of LDK378 metabolites in rat plasma, bile, and feces. The molecular ions were determined by LC/MS, with off-line radioactivity detection for peak correlation with mass spectral data. According to the Applicant, LDK378 metabolites were the result of the several biotransformation reactions, including dealkylation, mono- and di-oxygenation, glucuronidation, and sulfation. Metabolites were also formed from combinations of these biotransformations. The proposed metabolic pathway for LDK378 in rats is depicted below.

Figure 32: Proposed Metabolic Schema for [^{14}C]LDK378 in the rat



(Excerpted from Applicant's submission)

In conclusion, radioactivity was excreted predominantly in the feces. LDK378 was not extensively metabolized, and remained the major component in plasma, feces, and bile.

DMPK R1200422: Absorption, metabolism and excretion of LDK378 in the monkey following an intravenous or oral dose of [^{14}C]LDK378

Methods

Drug, % purity:	[^{14}C]LDK378, >96% radiochemical purity
Dose, ROA, batch #:	30 mg/kg (1.67 µCi/mg), oral gavage, PB2-190612-B 10 mg/kg (4.97 µCi/mg), IV, PB2-190612-A
Dose volume:	30 mg/kg: 5 mL/kg 10 mg/kg: 2 mL/kg
Formulation/Vehicle:	Oral: 0.5% MC in deionized water IV: 30% propylene glycol/5% solutol buffer in phosphate buffered saline
Species/Strain, weight:	Male cynomolgus monkeys, 3.3-5.0 kg
Number/Group:	3 for IV, 2 for oral
Sample collection for	IV: pre-dose, 5, 15, 30 min, 1, 2, 4, 7, 10, 24, 48, 72, 96, and

Blood: 168 hr post-dose (~2 mL of venous whole blood)
Oral: pre-dose, 15, 30 min, 1, 2, 4, 7, 10, 24, 48, 72, 96, and 168 hr post-dose

Sample collection for Excreta: IV and oral: 0-24, 24-48, 48-72, 72-96, and 96-168 hrs

The Applicant examined the absorption, PK, bioavailability, metabolism, and excretion of LDK378 following a single IV or oral dose of [^{14}C]LDK378 in male cynomolgus monkeys. This study investigated blood and plasma levels of radioactivity, excretion of radioactivity in urine and feces, LDK378 levels in selected plasma samples, and metabolite structure/profiles in plasma and excreta. The radioactivity of all formulation, blood, plasma, urine, feces, and cage wash samples were determined by liquid scintillation counting in a Packard Liquid Scintillation Counter. Plasma concentrations of LDK378 were determined by LC-MS/MS. LDK378 concentrations in excreta were determined by HPLC with radioactivity detection.

Following a single 10 mg/kg IV dose of [^{14}C]LDK378 to monkeys, radioactivity concentrations were higher in the blood than in the plasma, suggesting preferred distribution of LDK378 to the blood. The Cmax of unchanged LDK378 in the plasma was 78% of peak plasma radioactivity, and the AUCinf was ~58% of the plasma radioactivity AUC. The estimated systemic plasma clearance of LDK378 (0.366 L/h/kg) was low, and the mean terminal half-life of LDK378 in plasma was long (14.5 hrs). The steady-state volume of distribution (6.53 L/kg) exceeded total body water (~0.69 L/kg for monkeys), indicating extensive distribution into tissues. Following a single 30 mg/kg oral dose of [^{14}C]LDK378 to monkeys, radioactivity concentrations were again higher in blood than in plasma. The rate of absorption was relatively slow, and LDK378 exhibited a terminal half-life in plasma of 12.1 hrs. Bioavailability was calculated to be 43% in monkeys. Absorption was calculated to be 25.4% in blood and 15.7% in plasma based on oral and IV radioactivity data.

Table 26: Summary of pharmacokinetic parameters and excretion data following a single IV or oral dose of [^{14}C]LDK378 to monkeys

Parameters	Oral (mean ± SD)			Intravenous (mean)
Radioactivity in blood				
Cmax (ngEq/mL):	1020	±	127	5520
Tmax (h):	13.7	±	9.1	0.083 [a]
AUClast (ngEq•h/mL):	40900	±	10000	52900
AUCinf (ngEq•h/mL):	43000	±	10400	56400
Terminal T1/2 (h):	15.7	±	1.7	61.8
Radioactivity in plasma				
Cmax (ngEq/mL)	499	±	19.1	4090
Tmax (h):	18.3	±	9.81	0.083 [a]
AUClast (ngEq•h/mL)	20400	±	2070	43400
AUCinf (ngEq•h/mL)	22600	±	2460	47800
Terminal T1/2 (h):	19.2	±	4.08	72.1
LDK378 in plasma				
Cmax (ng/mL)	881	±	12.5	3190
Tmax (h):	18.3	±	9.81	0.083 [a]
AUClast(ng•h/mL)	35500	±	3520	27400
AUCinf (ng•h/mL)	35800	±	3460	27800
T1/2 (h):	12.1	±	2.05	14.5
CL ((L/h)/kg):	- [b]	±	- [b]	0.366
Vss (L/kg):	- [b]	±	- [b]	6.53
Bioavailability (%):	43.0	±	4.17	- [b]
Absorption (%):	>40			

Parameter	Oral (mean ± SD)	Intravenous (mean)
Excretion in urine		
Radioactivity, 0-24 h (% dose):	0.41 ± 0.43	0.28
Radioactivity, 0-168 h (% dose):	0.71 ± 0.45	0.59
LDK378, 0-72 h (% dose):	- [b] ± - [b]	
Excretion in feces		
Radioactivity, 0-24 h (% dose):	18.4 ± 0.95	25.5
Radioactivity, 0-168 h (% dose):	92.3 ± 6.94	105
LDK378, 0-72 h (PO) or 0-96 h (IV) (% dose):	60.2	55.1
Total recovery of radioactivity in excreta and cage wash, 0-168 h (% dose):	98.8 ± 4.18	106

[a] First sampling time point; [b] Not available

(Excerpted from Applicant's submission)

The predominant route of excretion was feces, regardless of the route of administration. Approximately 92.3% and 105% of radioactivity was excreted in the feces following oral and IV administration of [^{14}C]LDK378, respectively, whereas <1% of radioactivity was excreted in the urine (see table above). Recovery was nearly complete in the excreta by 168 hrs post-dose (7 days) for both routes of administration. Unchanged LDK378 was detected in the feces, but not in the urine. Fecal excretion of unchanged LDK378 accounted for 60.2% and 55.1% of the oral and IV doses of [^{14}C]LDK378, respectively.

Unchanged LDK378 was the major circulating component in plasma regardless of route of administration, accounting for 90% of the radioactivity $AUC_{0\text{-}24h}$ following IV administration and 84.4% of the radioactivity $AUC_{0\text{-}48h}$ following oral dosing. Eight metabolites were identified, but they accounted for <4% of the radioactivity AUC following IV and oral dosing.

Table 27: Concentrations of LDK378 and its metabolites in monkey plasma following a 10 mg/kg IV dose of [^{14}C]LDK378

Metabolite	Concentration (ngEq/mL)					Cmax	Tmax	AUClast	% AUC
Time (h)	1	2	4	7	24	ngEq/mL	h	ngEq/h*mL	
M21.6	14.2	22.4	22.0	4.90	21.4	22.5	2	163	1.40
M26.9	2.2	3.40	6.20	0.20	0.40	6.20	4	13.6	0.10
M27.6	43.9	78.3	82.7	5.40	3.10	82.6	4	213	1.80
M32.9	16.6	14.4	31.1	1.50	2.20	31.0	4	70.6	0.60
M33.4	21.2	39.4	56.3	2.10	6.80	56.2	4	145	1.20
M34.5	10.2	13.5	18.4	2.30	3.20	18.4	4	60.8	0.50
LDK378	2100	2020	1360	1080	371	2100	1	10700	89.9
M43.1	84.9	35.5	41.4	12.9	5.80	41.4	4	189	1.60
M46.1	146	61.6	53.1	25.2	18.1	53.3	4	353	3.00
Total	2440	2290	1670	1130	432	2420	3.40	11900	100

Table 28: Concentrations of LDK378 and its metabolites in the monkey plasma following a 30 mg/kg oral dose of [^{14}C]LDK378

Metabolite Time (h)	AUC0-48h (ng·h/mL)	% AUC 0-48h
M21.6	617	3.60
M26.9	376	2.20
M27.6	421	2.50
M32.9	61.5	0.40
M33.4	272	1.60
M34.5	92.3	0.50
LDK378	14400	84.4
M43.1	303	1.80
M46.1	526	3.10
Total	17100	100.0

(Excerpted from Applicant's submission)

Unchanged LDK378 was also the major component in feces, accounting for 55.1% and 60.2% of the IV and oral dose in monkeys, respectively. The most abundant fecal metabolites identified were M33.4 (mono-oxygenation; 10.9% of the IV dose and 5.9% of the oral dose) and M35.8 (mono-oxygenation; 17.9% of the IV dose and 8.7% of the oral dose). Urine samples were not analyzed for metabolites due to low dose recovery in the urine.

Table 29: Concentrations of LDK378 and its metabolites in monkey feces following IV and oral doses of [^{14}C]LDK378

Metabolite	Feces (0-96h)	
	IV (% of Dose)	PO (% of Dose)
M21.6	1.80	2.70
M23.6	2.80	2.20
M30.4	6.40	3.40
M30.6	0.30	0.60
M29.5	0.70	0.80
M32.9	3.70	2.70
M33.4	10.9	5.90
M34.5	0.90	0.40
M35.8	17.9	8.70
LDK378	55.1	60.2
M46.1	0.30	0.40
Total	100	88.1

(Excerpted from Applicant's submission)

LC-MS/MS was used for structural characterization of the LDK378 metabolites in monkey plasma and feces. The molecular ions were determined by LC/MS, with either off-line or on-line radioactivity detection for peak correlation with mass spectral data. According to the Applicant, the primary biotransformation reactions of LDK378 observed in the monkey were O- and S-dealkylation and mono-oxygenation; secondary biotransformation reactions of the primary biotransformation products included additional oxygenations, dehydrogenation, glucuronidation, and sulfation. Further, a thiol conjugate of O-dealkylated LDK378 was also observed. The proposed metabolic pathway for LDK378 in monkeys is depicted below.

Figure 33: Proposed Metabolic Schema for [^{14}C]LDK378 in the monkey

(Excerpted from Applicant's submission)

DMPK R0800227-01: Pharmacokinetics of LDK378 following an intravenous or oral dose in the monkey

Methods

Drug, batch #:	LDK378 (free base), TRD 1427-43
Doses, route of administration:	60 mg/kg, oral gavage 5 mg/kg, IV
Formulation/Vehicle:	IV: 30% propylene glycol/5% solutol buffer in phosphate buffered saline Oral: 0.5% MC
Species/Strain, weight:	Male cynomolgus monkeys, 3.1-3.7 kg
Number/Sex/Group:	3 for oral, 2 for IV
Sample Collection:	Blood samples were collected at 0, 0.083 (IV only), 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72, and 144 hrs post-dose

The PK parameters of LDK378 following IV or oral administration of a single dose of LDK378 as its free base were investigated in cynomolgus monkeys. Plasma concentrations of LDK378 were determined by LC-MS/MS. Following a single 5 mg/kg IV dose to monkeys, the plasma clearance was moderate (0.78 L/h/kg), and the estimated half-life was very long (29 hrs). The steady-state volume of distribution (13.5 L/kg) greatly exceeded total body water (~0.69 L/kg for monkeys), indicating extensive distribution into tissues. Following a single 60 mg/kg oral dose of LDK378, the rate of absorption was very slow, as signified by a Tmax of 13 hrs. LDK378 also exhibited a long apparent mean half-life after oral dosing (16 hrs), although not as long as that estimated for IV dosing. The calculated absolute bioavailability was moderate at 58%. Further, the Applicant stated that LDK378 exposure from the free base formulation in this study was

comparable to or slightly higher than that achieved with its phosphate salt at 60 mg/kg in a toxicokinetic study (DMPK R0770888; study not reviewed).

Table 30: Plasma concentrations and pharmacokinetic parameters of LDK378 after a single IV or oral dose to monkeys

Animal number	1	2	Mean	3	4	5	Mean ± SD
Body Weight (kg)	3.7	3.3	3.5	3.2	3.2	3.1	3.2 ± 0.06
time（h）	Intravenous dose (5 mg/kg)			Oral dose (60 mg/kg)			
0.083	1560	1260	1410				
0.25	551	456	504	7.27	14.7	2.66	8.21 ± 6.07
0.5	380	374	377	28.1	27.7	23.0	26.3 ± 2.84
1	307	320	314	35.0	46.1	57.4	46.2 ± 11.2
2	286	262	274	99.0	81.6	128	103 ± 23.4
4	266	230	248	445	265	375	362 ± 90.7
8	266	242	254	976	992	795	921 ± 109
24	85.9	43.5	64.7	663	1070	786	840 ± 209
48	18.8	13.0	15.9	371	546	389	435 ± 96.3
72	7.81	2.91	5.36	139	202	108	150 ± 47.9
144	2.12	1.53	1.83	6.40	7.10	4.73	6.08 ± 1.22
t_{max} (h)	0.083	0.083	0.083	8	24	8	13 ± 9.2
C_{max} (ng/mL)	1560	1260	1410	976	1070	795	947 ± 140
C_{max}/Dose (ng/mL)/(mg/kg)	312	252	282	16.3	17.8	13.3	15.8 ± 2.33
$AUC_{0-\infty}$ (ng.h/mL)	7400	5660	6530	40500	55500	39800	45300 ± 8860
AUC_{0-last} (ng.h/mL)	7300	5600	6450	40300	55300	39700	45100 ± 8840
AUC_{0-last}/dose(ng.h/mL)/(mg/kg)	1460	1120	1290	672	922	662	752 ± 147
$t_{1/2}$ (h)	32	26	29	16.2	15.0	15.8	16 ± 0.61
CL (L/h/kg)	0.68	0.89	0.78	-[a]	-	-	-
V_{ss} (L/kg)	13.3	13.7	13.5	-	-	-	-
Absolute bioavailability (%)	-	-	-	-	-	-	58

[a] Not applicable

(Excerpted from Applicant's submission)

RD-2008-50924: Pharmacokinetics of NVP-LDK378 after intravenous and oral single dose administration to mice

Methods

Drug:	NVP-LDK378-AA-10 and NVP-LDK378-AA-11
Doses, route of administration:	20 mg/kg, oral gavage 5 mg/kg, IV
Formulation/Vehicle:	75% Polyethylene glycol 300 and 25% of 5% dextrose in distilled water
Species/Strain, weight:	Male Balb/C mice, 22-25 g
Number/Sex/Group:	3/group for PK study; 8 for brain distribution study (2/time point)
Sample Collection:	PK: Blood samples (50 µL) were collected at 0.03, 1, 3, 7, and 24 hrs post-IV dose, and 0.5, 1, 3, 7, and 24 hrs post-oral dose Brain: Terminal brain and blood samples were collected at 0.5, 1, 3, and 7 hrs post-dose

The PK of LDK378 was evaluated following a single 5 mg/kg IV or 20 mg/kg oral administration of LDK378 to mice. Brain distribution of LDK378 was evaluated in a separate study following administration of 20 mg/kg LDK378 to mice by oral gavage. Plasma and homogenized brain samples were analyzed by LC-MS/MS. The lower limits of quantitation (LLOQ) were 1 ng/mL and 11 ng/g in plasma and brain, respectively. Following IV administration of 5 mg/kg LDK378, systemic total clearance was low to moderate (26.6 mL/min/kg). The steady-state volume of

distribution (9.7 L/kg) exceeded total body water (~0.72 L/kg for mice), indicating extensive distribution into tissues. The 20 mg/kg oral dose of LDK378 was slowly absorbed, reaching maximum plasma levels at 7 hrs after dosing. Bioavailability (F) was determined to be 54.6% in mice.

Table 31: Pharmacokinetic parameters of LDK378 following a 5 mg/kg IV dose to male Balb/c mice

Mouse ID	$AUC_{(0\text{-}24h)}$ (h*nM)	$AUC_{(0\text{-}\infty)}$ (h*nM)	AUC/dose (h*nM)/(mg/kg)	$T_{1/2}$ (h)	CL (mL/min/kg)	V_{ss} (L/kg)	C_{max} (nM)	$C_{(24\ h)}$ (nM)
1	5675	5991	1198	6.5	24.9	9.1	2299	34
2	5480	5770	1154	6.5	25.9	10.0	1667	30
3	4944	5141	1028	5.7	29.0	10.1	1293	24
Mean	5366	5634	1127	6.2	26.6	9.7	1753	29
SD	379	441	88	0.5	2.2	0.6	509	5

Table 32: Pharmacokinetic parameters of LDK378 following a 20 mg/kg oral dose to male Balb/c mice

Mouse ID	$AUC_{(0\text{-}24h)}$ (h*nM)	$AUC_{(0\text{-}\infty)}$ (h*nM)	$AUC_{(0\text{-}\infty)}$/Dose (h*nM)/(mg/kg)	C_{max} (nM)	T_{max} (h)	$C_{(24\ h)}$ (nM)	F (%)
4	11367	13416	671	724	7.0	173	59.5
5	9462	11585	579	663	7.0	170	51.4
6	10172	11888	594	697	7.0	153	52.8
Mean	10334	12296	615	695	7.0	165	54.6
SD	963	981	49	31	0.0	11	4.4

(Excerpted from Applicant's submission)

Following a single oral dose of 20 mg/kg LDK378 to mice, the $AUC_{(0\text{-}7h)}$ and Cmax of LDK378 in the brain were 12% and 13% of that in plasma, respectively. Cmax was achieved in the brain and plasma three hours after dosing. This study suggests that LDK378 crosses the blood-brain barrier and has the potential to reach exposure levels in the brain 12-13% of plasma exposure levels.

Table 33: Pooled plasma and brain exposure of LDK378 following a 20 mg/kg oral dose to male Balb/c mice

Mouse Matrix	$AUC_{(0\text{-}7\ h)}$ (h*nM)	AUC/Dose (h*nM)/(mg/kg)	C_{max} (nM/g or nM)	T_{max} (hrs)	$C_{(7\ h)}$ (nM/g or nM)
Brain (B)	365	19.3	66.9	3	48.0
Plasma (P)	3082	154.1	532.3	3	516.8
B/P ratio	0.12		0.13		0.09

(Excerpted from Applicant's submission)

DMPK R0900773b: Tissue distribution following a single oral or intravenous dose of [^{14}C]LDK378 in the rat

Methods

Drug, % purity: [^{14}C]LDK378, >98%

Dose, ROA, batch #: batch: 25 mg/kg (8 µCi/mg), oral gavage, LU12105-53

	10 mg/kg (20 µCi/mg), IV, LU12105-53B
Dose Volume:	25 mg/kg: 10 mL/kg 10 mg/kg: 2 mL/kg
Formulation/Vehicle:	Oral: 0.5% MC in deionized water IV: 30% propylene glycol/5% solutol buffer in phosphate buffered saline, adjusted to pH 4
Species/Strain:	~7 wk old Long Evans Hooded (LEH) pigmented rats
Number/Sex/Group:	1 rat per time point

The Applicant evaluated radioactivity distribution into tissues, organs, and body fluids following administration of a single 25 mg/kg oral dose of [^{14}C]LDK378 or a single 10 mg/kg IV dose of [^{14}C]LDK378 to LEH pigmented rats, using quantitative whole-body autoradiography (QWBA). Blood samples were collected from orally dosed rats at 1, 4, 8, 24, 72, and 168 hrs post-dose prior to termination, and from IV-dosed rats at 0.25 hrs post-dose prior to termination. One HanWistar (albino) rat administered a single 25 mg/kg oral dose of [^{14}C]LDK378 was sacrificed at 168 hrs post-dose for comparison. Tissue radioactivity concentrations were determined by digital analysis of the phosphor image autoradiograms. The lower limit of quantification (LLOQ) in this study ranged from 7.61 to 47.3 ngEq/g of tissue.

[^{14}C]LDK378-related radioactivity was distributed to most rat tissues after a single oral dose of [^{14}C]LDK378, as seen in Table 34. The Tmax for most tissues was 4 hrs post-dose (Table 35), but the Tmax for some tissues was 8, 24 (eye, pituitary gland, uveal tract), or 168 (harderian gland and testis) hrs post-dose. Tissue concentrations decreased over time, and were not distinguishable from surrounding tissues and/or background in many tissues by 168 hrs post-dose. Radioactivity concentrations were still high in the harderian gland, pituitary gland, and uveal tract 168 hrs post-dose; later time points were not evaluated. Radioactivity concentrations were still quantifiable in the epididymis, kidney, liver, lung, skin, spleen, and testis 168 hrs post-dose. Radioactivity concentrations in tissue were higher than those observed in blood for most tissues except for the brain, epididymis, eye, seminal vesicles, spinal cord, and testis, where the tissue-to-blood ratio based on Cmax was < 1. Tissue-to-blood ratios based on AUC_{inf} were ≤ 1 for the brain, eye, spinal cord, and white fat. The highest tissue-to-blood ratios (> 50) based on AUC_{inf} were observed in the colon wall, small intestine wall, bile, adrenal cortex, liver, harderian gland, pituitary gland, and uveal tract.

There was low distribution of [^{14}C]LDK378 to the brain, indicating that [^{14}C]LDK378 crossed the blood-brain barrier. The radioactivity tissue-to-blood ratios in the brain were 0.0689 and 0.148 based on Cmax and AUC_{inf}, respectively. [^{14}C]LDK378 concentrations in the brain peaked at 4 hrs and were not distinguishable from the surrounding tissues and/or background by 72 hrs post-dose. Further, the radioactivity concentration in the uveal tract of LEH pigmented rats at 168 hrs post-dose was 8160 ngEq/g, whereas it was not distinguishable from the surrounding tissues and/or background in the HanWistar albino rat. As a result, the Applicant concluded that the drug-related radioactivity showed significant retention to melanin in the uveal tract.

Table 34: Tissue distribution following a single 25 mg/kg oral dose of [^{14}C]LDK378 in rats

	Radioactivity concentration (ngEq/g)						
	1 h	4 h	8 h	24 h	72 h	168 h	168 h
	Rat #1	Rat #2	Rat #3	Rat #4	Rat #5	Rat #6	Rat #7
Sample	(LEH)	(LEH)	(LEH)	(LEH)	(LEH)	(LEH)	(HW)
Adrenal cortex	1870	37000	11000	4100	41.4	NM	NM
Adrenal medulla	890	21000	2100	1300	48.1	NM	NM
Bile	NM	74000	29000	11000	NM	NM	NM
Blood	63.4	600	220	48.0	NM	NM	NM
Bone marrow	198	7600	3000	2700	23.3	NM	NM
Bone mineral	41.1	1800	240	160	NM	NM	NM
Brain	29.8	42.0	20.0	13.0	NM	NM	NM
Brown fat	244	4100	1300	460	NM	NM	NM
Colon wall	336	3300	370000	1600	NM	NM	NM
Epididymis	52.8	420	100	150	31.5	68.0	57.7
Esophagus	829	5300	2200	380	157	NM	NM
Eye	LLOQ	13.0	LLOQ	22.0	LLOQ	NM	NM
Harderian gland	31.5	1700	1800	2600	1060	2990	NM
Heart	449	7700	2000	350	10.2	NM	NM
Kidney cortex	693	17000	6400	1300	20.3	28.3	NM
Kidney medulla	887	16000	5400	1600	22.2	29.1	NM
Kidney pelvis	389	6600	2400	520	16.6	23.8	NM
Liver	2720	33000	8100	1800	78.7	130	51.3
Lung	607	18000	5900	2100	28.9	21.2	NM
Lymph node	200	19000	2900	4100	183.0	NM	NM
Muscle	181	3300	1100	240	NM	NM	NM
Pancreas	388	13000	4000	1300	6.6	NM	NM
Pituitary gland	229	14000	6600	15000	1810	2590	NM
Salivary gland	208	9700	3500	1700	NM	NM	NM
Seminal vesicles	85.8	540	160	190	NM	NM	NM
Skin	67.3	990	470	730	65.8	24.4	NM
Small intestine wall	164000	490000	11000	730	NM	NM	NM
Spinal cord	LLOQ	38.0	LLOQ	14.0	NM	NM	NM
Spleen	1040	27000	6400	2500	42.3	41.6	NM
Stomach cutaneous	NM	NM	5500	280	NM	NM	NM
Stomach glandular	13400	5200	1900	310	NM	NM	NM
Testis	22.7	120	44.0	92.0	45.8	134	51.6
Thymus	120	4300	1400	2400	86.7	NM	NM
Thyroid gland	843	18000	7300	1200	NM	NM	NM
Uveal tract	108	6500	1100	8500	2880	8160	NM
White fat	22.7	800	220	18.0	NM	NM	NM
LLOD (ngEq/g)	14.2	7.93	10.5	4.92	3.80	8.02	23.7
LLOQ (ngEq/g)	28.4	15.9	21.0	9.85	7.61	16.0	47.3

[a] LEH = Long Evans Hooded pigmented rats; HW = HanWistar albino rats

NM = not measured, tissue concentration not distinguishable from surrounding tissues and/or background

NS = tissue not sectioned

Trace = concentration below the limit of quantification and greater than the limit of detection

LLOD = Lower limit of detection; LLOQ = Lower limit of quantification

(Excerpted from Applicant's submission)

Table 35: Pharmacokinetic parameters following a single 25 mg/kg oral dose of [^{14}C]LDK378 in rats

	Cmax		Tmax	AUCinf	T1/2	Ratio (tissue-to-blood)	
Sample	(µgEq/g)	(nmol/g)	(h)	(µgEq•h/g)	(h)	Cmax	AUCinf
Adrenal cortex	36.6	64.2	4	377	7.8	60.8	72.4
Adrenal medulla	20.8	36.5	4	138	11	34.6	26.5
Bile	74.1	130	4	807	8.2	123	155
Blood	0.602	1.06	4	5.21	5.9	1.00	1.00
Bone marrow	7.60	13.3	4	146	8.5	12.6	28.0
Bone mineral	1.80	3.16	4	11.9	7.5	2.99	2.28
Brain	0.0415	0.07	4	0.770	14	0.0689	0.148
Brown fat	4.12	7.23	4	36.3	7.2	6.84	6.97
Colon wall	368	645	8	NA	NA	611	710*
Epididymis	0.416	0.73	4	30.3	180	0.691	5.82
Esophagus	5.30	9.30	4	62.7	19	8.80	12.0
Eye	0.0218	0.0382	24	NA	NA	0.0362	0.0424*
Harderian gland	2.99	5.24	168	NA	NA	4.97	62.8
Heart	7.73	13.6	4	59.8	8.6	12.8	11.5
Kidney cortex	17.2	30.2	4	171	22	28.6	32.8
Kidney medulla	16.0	28.1	4	168	22	26.6	32.2
Kidney pelvis	6.61	11.6	4	67.3	26	11.0	12.9
Liver	32.8	57.5	4	274	23	54.5	52.6
Lung	18.0	31.6	4	194	20	29.9	37.2
Lymph node	18.5	32.4	4	235	14	30.7	45.1
Muscle	3.27	5.74	4	26.3	5.8	5.43	5.05
Pancreas	12.9	22.6	4	128	6.8	21.4	24.6
Pituitary gland	14.9	26.1	24	1100	68	24.8	211
Salivary gland	9.72	17.0	4	105	9.3	16.1	20.2
Seminal vesicles	0.541	0.949	4	10.7	20	0.899	2.05
Skin	0.987	1.73	4	38.6	32	1.64	7.41
Small intestine wall	486	852	4	2150	2.5	807	413
Spinal cord	0.0378	0.0663	4	NA	NA	0.0628	0.0468*
Spleen	26.8	47.0	4	248	22	44.5	47.6
Stomach cutaneous	5.55	9.73	8	NA	NA	9.22	13.2*
Stomach glandular	13.4	23.5	1	68.3	5.2	22.3	13.1
Testis	0.134	0.235	168	NA	NA	0.223	2.60*
Thymus	4.26	7.47	4	111	13	7.08	21.3
Thyroid gland	18.1	31.7	4	158	5.4	30.1	30.3
Uveal tract	8.51	14.9	24	NA	NA	14.1	176*
White fat	0.798	1.40	4	5.24	3.8	1.33	1.01

*Tissue-to-blood ratio of AUC was calulated with AUClast, tissue due to indisernible terminal phase. The actual AUC ratio may be higher

(Excerpted from Applicant's submission)

Drug-related radioactivity was distributed to most rat tissues after a single 10 mg/kg IV dose of [^{14}C]LDK378, as seen in Table 36. The radioactivity concentrations were higher in tissue than in blood for most tissues; however, the tissue-to-blood ratio was < 1 in the bone mineral, brain, epididymis, eye, skin, spinal cord, testis, and white fat. In general the tissue-to-blood ratios were lower in the major target tissues following a single 10 mg/kg IV dose of NVP-LDK378 as compared to the 25 mg/kg oral dose The tissue-to-blood ratios in the colon wall, small intestine wall, and liver were ~159-, 164-, and 2.6-fold lower following the 10 mg/kg IV dose compared to the 25 mg/kg oral doses.

Table 36: Tissue distribution following a single 10 mg/kg IV dose of [^{14}C]LDK378 in LEH rats

	Rat 8 (LEH) 0.25 h	
Sample	Radioactivity Concentration (ngEq/g)	Tissue/Blood
Adrenal cortex	48700	34.3
Adrenal medulla	14600	10.3
Bile	48800	34.4
Blood	1420	1.00
Bone marrow	9220	6.50
Bone mineral	1170	0.824
Brain	137	0.096
Brown fat	11500	8.12
Colon wall	5450	3.84
Epididymis	859	0.61
Esophagus	5040	3.55
Eye	51.1	0.04
Harderian gland	5040	3.55
Heart	18000	12.7
Kidney cortex	23700	16.7
Kidney medulla	19800	14.0
Kidney pelvis	10800	7.58
Liver	29500	20.8
Lung	23300	16.4
Lymph node	8720	6.14
Muscle	11700	8.24
Pancreas	14200	9.98
Pituitary gland	15000	10.6
Salivary gland	8740	6.16
Skin	1120	0.79
Small intestine wall	6980	4.92
Spinal cord	119	0.084
Stomach cutaneous	1820	1.280
Stomach glandular	6590	4.64
Testis	263	0.19
Thymus	3860	2.72
Thyroid gland	35100	24.7
Uveal tract	8600	6.06
White fat	695	0.49
LLOD (ngEq/g)	6.47	
LLOQ (ngEq/g)	12.9	

LEH = Long Evans Hooded pigmented rats
LLOD = lower limit of detection; LLOQ = lower limit of quantification

(Excerpted from Applicant's submission)

DMPK R0900777: In vitro distribution of ^{14}C-labeled LDK378 to blood cells, serum and plasma proteins in the rat, dog, monkey and human

The Applicant evaluated the distribution of [^{14}C]LDK378 to blood cells, in vitro binding to human serum, and the fraction of [^{14}C]LDK378 bound to rat, dog, monkey, and human plasma proteins. The distribution of [^{14}C]LDK378 (batch LU-12104-50-15; specific activity 200 µCi/mg) between blood cells and plasma was determined over a range of [^{14}C]LDK378 concentrations (50, 100,

500, 1000, and 10,000 ng/mL dissolved in 190 proof ethanol). Rat, dog, and monkey samples were prepared in triplicate, and individual human samples were obtained from three human volunteers. Blood and plasma radioactivity was determined using Formula 989® and a liquid scintillation spectrometer. [^{14}C]LDK378 distributed slightly more to red blood cells than to plasma in all species tested, as indicated by blood:plasma concentration ratios > 1. The fraction of [^{14}C]LDK378 distributed to blood cells (f_{bc}) was highest in the monkey, followed by the dog, rat, and human; f_{bc} values were independent of [^{14}C]LDK378 concentration over the range tested. The mean f_{bc} value of 0.582 suggested that [^{14}C]LDK378 distributes slightly more equally between blood cells and plasma in humans as compared to the other species tested.

Table 37: Blood:plasma concentration ratio of [^{14}C]LDK378 at 37ºC

[^{14}C]LDK378	Rat [a]			Monkey [a]			Dog [a]			Human [b]		
(ng/mL)	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
50	1.80	±	0.023	2.71	±	0.135	1.34	±	0.062	1.35	±	0.150
100	1.75	±	0.127	2.64	±	0.022	1.30	±	0.013	1.38	±	0.203
500	1.62	±	0.175	2.61	±	0.181	1.35	±	0.015	1.38	±	0.285
1000	1.57	±	0.057	2.56	±	0.063	1.35	±	0.030	1.35	±	0.212
10000	1.63	±	0.064	2.45	±	0.042	1.57	±	0.060	1.32	±	0.222
Average ± SD	1.72	±	0.137	2.59	±	0.128	1.38	±	0.106	1.35	±	0.186

[a] pooled blood (n ≥ 3) and data obtained from triplicate analyses;
[b] mean data obtained from three individual human subjects

Table 38: Fraction of [^{14}C]LDK378 distributed to blood cells (f_{bc}) at 37ºC

[^{14}C]LDK378	Rat [a]			Monkey [a]			Dog [a]			Human [b]		
(ng/mL)	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
50	0.688	±	0.004	0.787	±	0.011	0.666	±	0.016	0.582	±	0.035
100	0.677	±	0.024	0.782	±	0.002	0.656	±	0.003	0.591	±	0.037
500	0.649	±	0.037	0.778	±	0.015	0.670	±	0.004	0.586	±	0.055
1000	0.641	±	0.013	0.775	±	0.005	0.670	±	0.007	0.581	±	0.053
10000	0.653	±	0.013	0.764	±	0.004	0.716	±	0.011	0.569	±	0.048
Average ± SD	0.671	±	0.028	0.777	±	0.011	0.676	±	0.023	0.582	±	0.040

Average hematocrit values were 0.44, 0.42, 0.55 and 0.44 in rat, monkey, dog and human, respectively.
[a] pooled blood (n ≥ 3) and data obtained from triplicate analyses;
[b] mean data obtained from the three individual human subjects

$F_{bc} = 1-[(1-H)(C_p/C_b)]$; H = hematocrit; C_p = radioactivity concentration (disintegrations per minute; DPM) in plasma; C_b = radioactivity concentration in blood

(Excerpted from Applicant's submission)

In order to determine the fraction of [^{14}C]LDK378 bound to plasma proteins, radioactivity was determined in rat, dog, monkey, and human plasma before and after ultracentrifugation. The plasma protein binding of [^{14}C]LDK378 was high in all the species tested over 50-10,0000 ng/mL. [^{14}C]LDK378 was 98.5%, 98.3%, 97.2%, and 94.7% bound to plasma protein in the dog, rat, human, and monkey, respectively.

Table 39: Fraction of [^{14}C]LDK378 bound to plasma protein at 37ºC

[^{14}C]LDK378 (ng/mL)	Rat [a] Mean ± SD	Monkey [a] Mean ± SD	Dog [a] Mean ± SD	Human [b] Mean ± SD
50	0.979 ± 0.002	0.944 ± 0.005	0.988 ± 0.0005	0.988 ± 0.005
100	0.981 ± 0.003	0.945 ± 0.009	0.988 ± 0.0029	0.966 ± 0.024
500	0.984 ± 0.000	0.945 ± 0.004	0.983 ± 0.0021	0.972 ± 0.014
1000	0.985 ± 0.001	0.944 ± 0.002	0.983 ± 0.0013	0.968 ± 0.020
10000	0.984 ± 0.001	0.952 ± 0.002	0.984 ± 0.0008	0.967 ± 0.018
Average ± SD	0.983 ± 0.003	0.946 ± 0.005	0.985 ± 0.0027	0.972 ± 0.017

[a] pooled blood (n ≥ 3) and data obtained from triplicate analyses
[b] mean data obtained from the three individual human subjects

Fraction of drug bound to plasma protein (β) = $(T_t-T_s)/T_t$; T_t = radioactivity in uncentrifuged sample; T_s = radioactivity in supernatant after centrifugation
(Excerpted from Applicant's submission)

[^{14}C]LDK378 binding to human serum proteins was determined at 100 and 10,000 ng/mL. Mean serum protein binding was similar to plasma protein binding at 100 ng/mL, and slightly higher than plasma protein binding at 10,000 ng/mL. The mean percentage of [^{14}C]LDK378 bound to human serum protein at the concentrations tested was 98.1%, which is overlapping with the mean percent bound to plasma protein (97.2% ± 0.017). As a result, the anticoagulant heparin did not appear to significantly affect [^{14}C]LDK378 protein binding.

Table 40: Fraction of [^{14}C]LDK378 bound to human serum protein at 37ºC

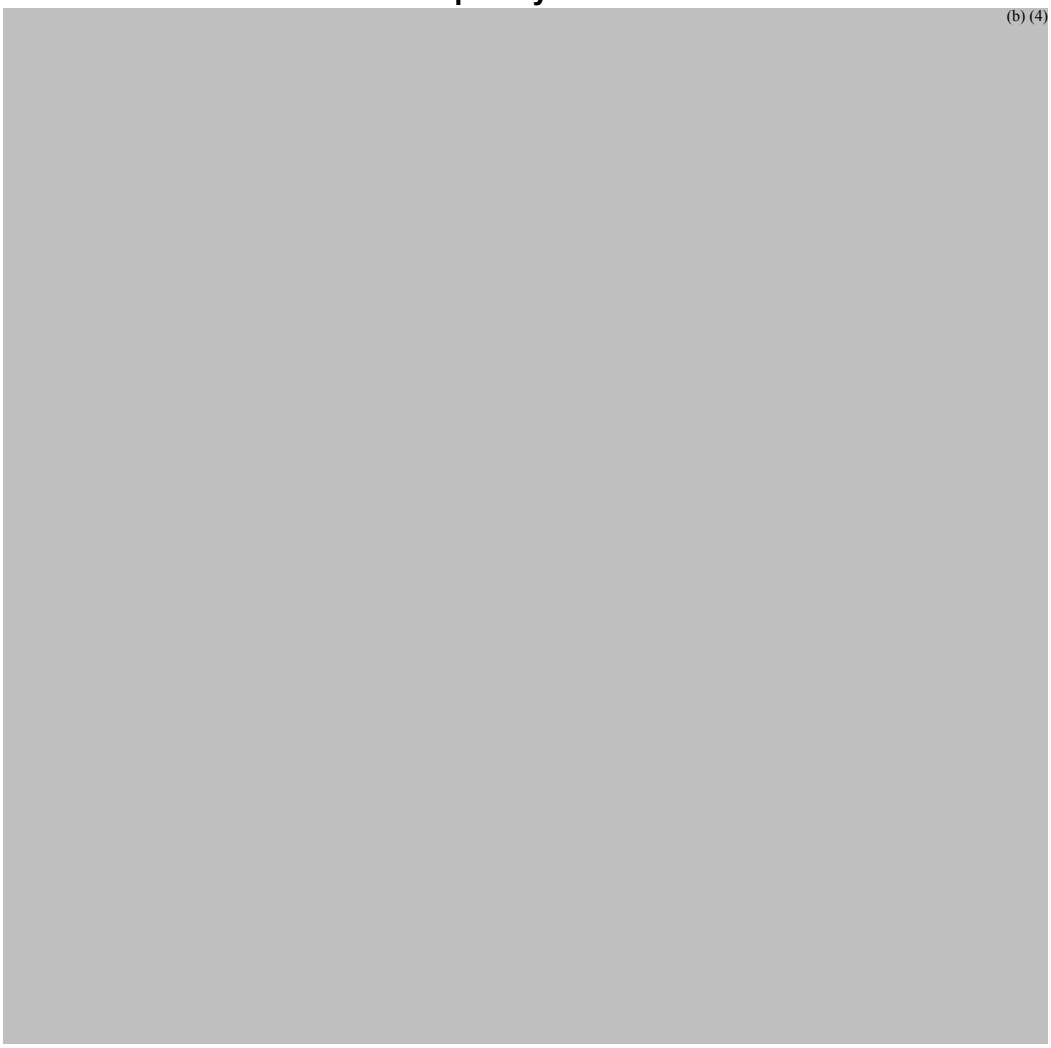
[^{14}C]LDK378 Concentration (ng/mL)	Subj.	Serum DPM/0.05 mL	Supernatant DPM/0.05 mL	Bound fraction Individual values	Mean ± SD
100	A	1654	28	0.983	0.976 ± 0.006
	B	1928	50	0.974	
	C	1763	49	0.972	
10000	A	214468	2966	0.993	0.986 ± 0.008
	B	200049	5572	0.986	
	C	213788	9532	0.978	
Overall average					0.981 ± 0.0079

(Excerpted from Applicant's submission)

DMPK R1000033: Preliminary in vitro metabolism of [^{14}C]LDK378 in human, monkey and rat hepatocytes

The Applicant evaluated the in vitro metabolism of [^{14}C]LDK378 using rat, monkey, and human hepatocytes. The hepatocytes were incubated with two nominal concentrations (2.5 µM or 12.5 µM) of [^{14}C]LDK378 (batch LU-12104-82p; specific activity 100 µCi/mg; radiochemical purity 99%) for up to 24 hrs; control incubations were conducted at both concentrations in the absence of hepatocytes. [^{14}C]LDK378 and its metabolites in the hepatocyte extracts were analyzed by HPLC with off-line radioactivity detection, and the metabolite structures were characterized using LC-MS/MS. Cell viability was assessed during hepatocyte incubation with [^{14}C]LDK378 using the (b) (4), and the metabolism of [^{14}C]terfenadine and [^{14}C]7-ethoxycoumarin were used as positive controls for hepatocyte metabolism. Monkey hepatocytes metabolized LDK378 the most, followed by human hepatocytes; no significant metabolism was observed in rat hepatocytes. The proposed in vitro metabolic pathway for LDK378 is depicted below.

Figure 34: Proposed Metabolic Schema for [^{14}C]LDK378 in monkey, human, and rat hepatocytes



(Excerpted from Applicant's submission)

The major metabolites in the monkey hepatocytes were M27.5 (oxygenated metabolite)/M27.6 (secondary glucuronide), which accounted for 25% and 9.3% of radioactivity at 2.5 and 12.5 µM of [^{14}C]LDK378 following 24 hrs of incubation, respectively, and M25.7 (secondary glucuronide), which accounted for 11.5% and 8.1% of radioactivity at 2.5 and 12.5 µM of [^{14}C]LDK378 following 24 hrs of incubation, respectively. Unchanged LDK378 accounted for 32% and 62% of radioactivity in monkey hepatocytes at 2.5 and 12.5 µM, respectively. The major metabolites observed in human hepatocytes were M27.5/M27.6, which accounted for 9% at 2.5 µM and 3% at 12.5 µM, respectively, and M32.9, which accounted for 3% at 2.5 µM and 4% at 12.5 µM, respectively. Unchanged LDK378 accounted for the majority of radioactivity in human hepatocytes (81.4 and 90.8% at 2.5 and 12.5 µM, respectively). M32.9 is the *O*-dealkylated metabolite corresponding to the reference standard for LFV037. According to the Applicant, LDK378 metabolites were the result of the several biotransformation reactions, including dealkylation, oxygenation, glucuronidation, and sulfation. Metabolites were also formed from combinations of these biotansformations.

Table 41: [^{14}C]LDK378 metabolites following incubation with monkey hepatocytes expressed as % of radioactivity in the sample

Nominal concentration of 2.5 µM									
Time (hr)	DS[a]	0.25	0.5	1	2	4	8	18	24
M21.6		0.23	0.28	0.81	1.96	2.37	2.97	4.06	7.11
M23.6		0.31	0.59	0.95	1.26	2.13	3.01	2.40	4.42
M25.7		ND[c]	ND	ND	ND	2.20	4.27	5.11	11.5
M27.5/M27.6[b]		3.33	4.68	8.67	10.6	13.4	14.8	20.4	25.0
M29.5		ND	ND	ND	1.00	1.46	2.69	2.68	5.23
M32.9		0.68	0.77	0.58	5.15	7.15	8.12	8.21	8.77
M33.4		1.20	1.77	3.58	ND	ND	ND	ND	ND
M35.8		ND	ND	ND	ND	ND	ND	2.51	2.15
M37.3		ND	ND	ND	ND	ND	ND	2.91	2.5
LDK378		92.5	89.9	83.8	76.9	69.1	61.5	50.6	32.0
Nominal concentration of 12.5 µM									
Time (hr)	DS[a]	0.25	0.5	1	2	4	8	18	24
M21.6		NA[c]	0.23	0.34	1.68	1.12	2.16	2.10	4.05
M23.6		NA	0.24	0.56	0.97	1.21	1.34	1.43	1.81
M25.7		NA	ND	ND	1.09	1.56	4.06	5.61	8.11
M27.5/M27.6[b]		NA	1.95	3.11	6.05	5.32	5.96	6.81	9.31
M29.5		NA	0.20	0.41	1.17	1.81	3.48	3.34	5.82
M32.9		NA	0.37	0.53	5.60	6.12	5.90	5.07	5.41
M33.4		NA	1.92	3.09	ND	ND	ND	ND	ND
M35.8		NA	ND	ND	ND	ND	ND	0.89	0.87
M37.3		NA	ND	ND	ND	ND	0.93	1.69	1.40
LDK378		NA	92.7	89.3	80.7	80.6	74.2	71.2	61.9

[a] DS: Drug stability, samples incubated at 37°C for 24 hr in the absence of hepatocytes
[b] M27.5/M27.6 co-elute under the LC/MS conditions used
[c] NA: not available; ND: not detected

Table 42: [^{14}C]LDK378 metabolites following incubation with human hepatocytes expressed as % of radioactivity in the sample

Nominal concentration of 2.5 µM									
Time (hr)	DS[a]	0.25	0.5	1	2	4	8	18	24
M27.5/M27.6[b]		0.78	0.77	1.28	2.22	3.11	4.52	8.17	8.94
M32.9		0.75	0.98	1.35	1.18	1.46	1.47	1.61	2.62
M33.4		1.76	1.19	1.14	1.02	1.26	1.55	1.52	1.38
M37.3		ND[c]	ND	ND	ND	ND	1.11	1.59	2.28
LDK378		90.4	90.9	91.8	91.6	89.9	87.6	83.2	81.4
Nominal concentration of 12.5 µM									
Time (hr)	DS[a]	0.25	0.5	1	2	4	8	18	24
M27.5/M27.6[b]		NA[c]	ND	ND	ND	ND	ND	7.05	2.96
M32.9		NA	ND	ND	ND	ND	ND	ND	3.93
M33.4		NA	3.60	4.47	4.55	4.26	4.81	7.89	0.67
M37.3		NA	ND	ND	ND	ND	ND	ND	1.65
LDK378		NA	96.4	95.5	95.5	95.7	95.2	85.1	90.8

[a] DS: Drug stability, samples incubated at 37°C for 24 hr in the absence of hepatocytes
[b] M27.5/M27.6 co-elute under the LC/MS conditions used
[c] NA: not available; ND: not detected.

(Excerpted from Applicant's submission)

The intrinsic clearance of LDK378 was calculated based on the disappearance of LDK378 during the hepatocyte incubations as a function of time, and the in vitro data was used to estimate in vivo hepatic clearance and hepatic extraction ratios. The intrinsic clearance values (CL_{int}) were low in monkey, human, and rat hepatocytes (see table below), resulting in low

predicted in vivo hepatic clearance values (CL_H). Use of the positive control [^{14}C]terfenadine demonstrated that the freshly isolated rat and monkey hepatocytes had satisfactory metabolic capacity; however, the rate of [^{14}C]terfenadine metabolism was lower than that observed in previous studies, suggesting that the CL_{int} estimations in this study may underestimate the extent of LDK378 metabolism in human hepatocytes.

Table 43: Intrinsic clearance of [^{14}C]LDK378 in monkey, rat, and human hepatocytes

Species	Concentration (μM)	Time points used (h)	$t_{½}$ (h)	CL_{int} (μL/h/10^6 cells)	CL_H [a] (mL/h/kg)	Extraction Ratio[b]
Rat	2.5	0-18	NC [c]	NC	NC	NC
	12.5	0-24	NC	NC	NC	
Monkey	2.5	0-4	7.02	98.7	312.9	0.12
	12.5	0-8	18.0	38.5	131.7	
Human [d]	2.5	0.25-18	100.4	6.90	17.3	0.014
	12.5	0-18	66.95	10.4	25.8	

[a] Scaled to estimated hepatic clearance using parameters listed in Table 2-3.
[b] Extraction ratio (CL_H/Q_H) was calculated only at the lower substrate concentration to minimize saturation effect.
[c] NC= not calculated
[d] calculated values are likely to underestimate the values observed in vivo due to the low metabolic activity of this hepatocyte preparation

(Excerpted from Applicant's submission)

Cell viability measurements following 0.5, 1, 2, 4, and 24 hrs of incubation with [^{14}C]LDK378 demonstrated that with the exception of rat hepatocytes, which exhibited 47.1% hepatocyte viability at 24 hrs (2.5 μM [^{14}C]LDK378), the viability of hepatocytes treated with [^{14}C]LDK378 was not significantly different than that of untreated controls, indicating that there was no significant toxicity during the course of incubation.

DMPK R1000487: In vitro assessment of covalent protein binding potential for LDK378 in rat and human liver microsomes and hepatocytes

The potential for [^{14}C]LDK378 to bind covalently to protein when incubated with cryopreserved rat and human liver microsomes or hepatocytes was determined by assessing the relative amounts of nonextractable radioactivity associated with liver microsomal protein after incubation with [^{14}C]LDK378 (batch LU-12104-58-9; specific activity 200 μCi/mg; radiochemical purity >99%) or the positive control reference compound [^{14}C]L746530 (5-lipoxygenase inhibitor; 101 μCi/mg). The extent of apparent covalent binding was estimated by incubating [^{14}C]LDK378 or [^{14}C]L746530 with rat or human liver microsomes for 60 minutes, precipitating the protein, and measuring drug-related radioactivity associated with the washed protein isolate by liquid scintillation counting. There were low levels of non-extractable [^{14}C]LDK378 radioactivity associated with rat and human microsomal protein (10.0 – 21.4 pmol/mg protein), which appeared to be NADPH dependent. In contrast, the levels of covalent binding for [^{14}C]L746530 in the presence of NADPH were 34-fold higher in rat liver microsomes and 12.4-fold higher in human liver microsomes compared to [^{14}C]LDK378. Apparent covalent binding of [^{14}C]LDK378 appeared to be relatively similar between rats and humans in the presence of NADPH, but differences were noted with the addition of UDPGA and GSH to NADPH.

Table 44: Apparent covalent binding in liver microsomes

	Cofactors included			
	GSH	NADPH	NADPH+UDPGA	NADPH+UDPGA +GSH
LDK378	Drug equivalents bound (pmol/mg protein[a])			
Rat	10.0 ± 1.2	21.2 ± 1.3	21.4 ± 0.9	15.0 ± 1.9
Human	10.4 ± 1.3	20.5 ± 0.5	12.3 ± 10.7	11.3 ± 0.9
L746530				
Rat	64.4 ± 1.7	715.9 ± 35.7	579.8 ± 35.0	590.0 ± 49.5
Human	52.8 ± 2.6	254.3 ± 5.2	245.0 ± 24.1	354.1 ± 33.8

[a]Based on nominal microsomal protein included in incubation; values are mean ± SD (n=3); all values were rounded to three significant figures or to the nearest 0.1.

(Excerpted from Applicant's submission)

The apparent covalent binding of [^{14}C]LDK378 increased with time in rat and human hepatocytes, and was 65.17 and 75.11 pmol/mg of liver tissue in rat and human hepatocytes after a 4 hr incubation, respectively. The apparent covalent binding of [^{14}C]L746530 also increased with time, and was approximately 1.8-fold and 1.5-fold higher compared to [^{14}C]LDK378 after a 4 hr incubation in rat and human hepatocytes, respectively. The Applicant stated that these results only provide an indication of potential covalent binding, not definitive proof.

Table 45: Apparent covalent binding in human hepatocytes

	Drug equivalents bound (pmol/10^6 cells[a])			
Incubation time	**0.083 h**	**0.5 h**	**1 h**	**4 h**
LDK378	105.80 ± 7.02	281.74 ± 0.68	504.46 ± 16.86	702.25 ± 57.12
L746530	173.98 ± 8.8	523.11 ± 0.5	684.59 ± 70.4	1057.12 ± 76.3
	Drug equivalents bound (pmol/mg tissue[b])			
LDK378	11.32 ± 0.75	30.13 ± 0.07	53.95 ± 1.80	75.11 ± 6.11
L746530	18.61 ± 0.94	55.95 ± 0.05	73.22 ± 7.53	113.06 ± 8.16

[a]Based on viable cells.

[b]Based on 107 x 10^6 hepatocytes per gram of tissue (Wilson, et al 2003); values are mean ± range (n=2) and rounded to three significant figures or to the nearest 0.01.

Table 46: Apparent covalent binding in rat hepatocytes

	Drug equivalents bound (pmol/10^6 cells[a])			
Incubation time	**0.083 h**	**0.5 h**	**1 h**	**4 h**
LDK378	108.39 ± 2.34	324.73 ± 17.09	426.58 ± 62.86	609.30 ± 32.39
L746530	168.29 ± 1.3	460.89 ± 22.3	709.78 ± 43.0	1068.22 ± 17.5
	Drug equivalents bound (pmol/mg tissue[b])			
LDK378	11.59 ± 0.25	34.73 ± 1.83	45.62 ± 6.72	65.17 ± 3.46
L746530	18.00 ± 0.14	49.29 ± 2.38	75.91± 4.60	114.25 ± 1.88

[a]Based on viable cells.

[b]Based on 107 x 10^6 hepatocytes per gram of tissue (Wilson, et al 2003); values are mean ± range (n=2) and rounded to three significant figures or to the nearest 0.01.

(Excerpted from Applicant's submission)

6 General Toxicology

6.1 Single-Dose Toxicity

Single-dose pilot studies were not reviewed.

6.2 Repeat-Dose Toxicity

Study title: 4-week oral (gavage) toxicity study in rats with a 4-week recovery period

Study no.:	0970416
Study report location:	Electronic submission, M4.2.3.2
Conducting laboratory and location:	Novartis Pharmaceuticals
Date of study initiation:	October 28, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, batch #, and % purity:	LDK378, batch #0850001, purity 98.9%

Methods

Doses:	0, 7.5, 25, 75/50 mg/kg/day
Frequency of dosing:	Daily for 4 weeks
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	Formulated in 0.5% (w/v) methylcellulose (400 cPs) in reverse osmosis water
Species/Strain:	Rat/IGS Wistar Hannover (9-10 weeks in age; weight: M: 315-374 g; F: 179-237 g
Number/Sex/Group:	

Group	No. of animals		Dose level
	M	F	
Control	10	10	0
Low	10	10	7.5
Mid	10	10	25
High	10	10	75/50

Study Summary

This 4-week repeat-dose toxicology study in Wistar rats was originally reviewed by Sachia Khasar, Ph.D. in October, 2010 following submission of IND 109272. LDK378 doses of 7.5, 25 and 75/50 were administered daily for 4 weeks. The high dose level was reduced from 75 to 50 mg/kg/day on D13 due to significant weight loss; HD animals were not dosed on D9-12. Following 4 weeks of LDK378 administration, the primary target sites included the extra-hepatic bile duct (dilatation, inflammation), biliopancreatic duct (inflammation, erosion/necrosis), liver (vacuolation of intra-hepatic bile duct epithelium), pancreas (atrophy, inflammation) and mesenteric lymph nodes (↑accumulation of macrophage aggregates). Findings observed in the bile duct were generally observed following recovery. Findings were focused within the biliary drainage system, and were exhibited at all doses tested. In addition, HD males exhibited pulmonary phospholipidosis which was confirmed microscopically.

The LDK378 batch used for this study (as well as the 4-week exploratory pilot toxicity study noted below) was used by the Applicant to qualify two impurities (b) (4) at (b) (4)

Two-week pilot studies in rats were not reviewed. A 4- week exploratory pilot toxicity study in male rats was also not reviewed. These studies were not GLP compliant.

Study title: 13-week oral gavage toxicity and toxicokinetic study with LDK378 in rats with a 8-week recovery phase

Study no.:	1270164
Study report location:	Electronic submission, M4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 16/17, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, batch #, and % purity:	LDK378, batch #1251005, purity 100%

Key Study Findings

● The primary target site in rats following LDK378 administration was the biliopancreatic duct with chronic inflammation, degeneration/necrosis, erosion, hyperplasia, dilatation, and vacuolation at all doses. Findings persisted following the 8-week recovery period.
● Additional target organs included the duodenum (necrosis, hyperplasia, dilatation, inflammation, and vacuolation), liver (bile duct vacuolation), mesenteric lymph nodes (increased macrophage aggregates) and lung (macrophage aggregates)
● Increased amylase during recovery in HD males and during dosing and recovery in HD females; no changes in lipase
● Increased fibrinogen and platelets in HD animals
● Increased thyroid indices (TSH, T3, T4) were not accompanied by organ weight or histologic changes; the cause of LDK378-related changes on thyroid hormones is undetermined. Thyroid weight changes were also observed in monkeys administered LDK378 for 4 weeks.
● LDK378 exposure increased with dose with minor accumulation observed following repeat dosing.

Methods

Doses:	3, 10, 30 mg/kg/day
Frequency of dosing:	Daily for 92 days (13 weeks)
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	Formulated in 0.5% (w/v) methylcellulose (400 cPs) in reverse osmosis water
Species/Strain:	Rat, Crl:WI(Han)

Number/Sex/Group:

Group	No. of animals		Dose level (mg/kg/day)	Dose concentration
	M	F		
Control	16	16	0	0
Low	10	10	3	0.6
Mid	10	10	10	2
High	16	16	30	6
Sentinel	0	8	ND	N

Age: 7-8 weeks
Weight: M: 195-257 g; F: 156-202 g
Satellite groups: None
Unique study design: 8 week recovery period: 6 animals/control + HD groups. Sentinel animals were not dosed; animals used for veterinary purposes only (serology and health monitoring). Dose levels were justified by 4 week study performed in rats of the same strain.
Deviation from study protocol: A large number of protocol deviations were noted, although deviations do not appear to have had a major impact on the study.

Observation	Time of assessment
Mortality	2x/d
Clinical observations	Pretest and ~3 hours postdose during dosing; daily during recovery
Body weight	Pretest, weekly thereafter
Food consumption	Weekly
Ophthalmoscopy	Pretest and D87/88
Hematology	♦Blood collection Weeks 2, 5, 9 for hematology and clinical chemistry ♦Blood collection dosing Week 13 + Recovery Week 4, 8 for hematology, coagulation, clinical chemistry ♦Urine collected during dosing Weeks 5 and 13 and Recovery Week 8 ♦Blood collected for glucose and insulin analysis Day 1 and Week 11 of dosing from 2 animals/timepoint
Clinical chemistry	
Urinalysis	
Biomarker analysis	Blood collected at scheduled sacrifice – Future analysis to identify circulating biomarkers of bileopancreatic injury
Organ weights	D92, D147
Toxicokinetics	Day 1 and during Week 11 at ~3, 5, 7, 10, and 24h following dosing
Gross pathology	D92, D147

Histopathology	D91, D147 (Control, HD: Standard tissue collection; tissues collected from LD, MD + recovery animals: All lesions, liver, pancreas, lung, spleen, mesenteric lymph node, biliopancreatic duct/duodenal papilla with pancreas)[a]

[a]Note: Biliopancreatic duct segments and duodenal papilla collected for future exploratory investigation.

Parameter	3 mg/kg		10 mg/kg		30 mg/kg	
	M	F	M	F	M	F
Mortality	None					
Clinical observations	UR					
Body weight[a] (W13)	↓10% HD males following dosing period; weights remained ↓7- 9% during the 8-week recovery period					
Food consumption	UR					
Ophthalmoscopy	UR					
Hematology[a]						
Platelets (D58-87)					↑10-13	↑20-24
Fibrinogen (D87)					↑19	↑44
Coagulation	UR					
Clinical chemistry[a]						
Amylase (D87/RD23/RD51)					UR/↑25/UR	↑12/UR/↑12
Lipase	UR					
Glucose (D87)					↑13	
Globulin (D87)					↑14	↑20
A/G ratio (D87)					↓18	↓24
Cholesterol ((D58-87)						↑18
TSH (M:D10-87 dosing + recov)[b] (F: D58-87)			↑61-192		↑40-123	↑50-73
T3 D10-87)					↑18-26	
T4 (D42-58)		↑13-24				↑30-35
Urinalysis	UR					
Organ weights – absolute[a] (D91)						
Liver					↑15	↑19
Gross pathology (D91)	Biliopancreatic duct: enlarged in HDM and HDF attributable to luminal dilatation, mucosal hyperplasia, and chronic mural inflammation which persisted following recovery					
Histopathology (D91, 147)	See histopathology tables					
Toxicokinetics	See toxicokinetics table					

[a] Percent compared to concurrent control
[b] TSH analyzed in HDM only during recovery; TSH continued to be ↑114% following recovery compared to concurrent controls
Abbreviations: UR = unremarkable, M = males, F = females, RD = recovery day

Effects on fibrinogen and platelets in HD males and females were reflective of inflammatory changes observed histologically in the biliopancreatic/hepatic ducts and duodenum. LDK378-related changes in thyroid hormone concentrations were not accompanied by organ weight or histologic changes following 13 weeks of dosing; the cause of LDK378-related changes on thyroid hormones is undetermined. No thyroid-related adverse events have been reported with LDK378 in the clinic. Thyroid indices were incorporated into the study protocol as a result of

thyroid weight changes and microscopic changes in the thyroid of monkeys administered LDK378 for 4 weeks (Study #0970612).

Table 47: Histopathology Results (Terminal necropsy:D91) – LDK378 administration in rats for 13 weeks

Organ/finding	3 mg/kg (N)		10 mg/kg (N)		30 mg/kg (N)	
	M	F	M	F	M	F
Duodenum (N)	9	10	9	6[a]	9	9
/degeneration, necrosis (minimal)	2	1	1			4
/hyperplasia (minimal-moderate)					8	5
/dilatation (minimal-moderate)					8	5
/inflammation (minimal-slight)					3	7
/vacuolation (minimal-slight)	2		4	4	8	7
Liver (N)	10	10	10	10	10	10
/bile duct vacuolation (min-moderate)			8		10	10
Mesenteric lymph node	10	10	10	10	10	10
/increased macrophage aggregates (minimal-slight)					8	6
Lung	10	10	10	10	10	10
/macrophage aggregates, alveolus (minimal)					5	6

[a] No explanation was provided for the limited number of animals examined.

Table 48: Incidence of biliopancreatic duct histopathology at terminal necropsy (D91) in male rats

Finding/Section (N=8-10/dose)	3 mg/kg			10 mg/kg			30 mg/kg		
	A	D	E	A	D	E	A	D	E
Chronic inflammation (min-moderate)	2	0	1	1	2	1	9	10	10
Degeneration/necrosis (min-moderate)	2	4	4	2	2	2	9	10	10
Erosion/ulcer (min-slight)	5	2	2	4	4	1	8	6	2
Hyperplasia (min-moderate)	0	1	4	0	0	1	8	8	9
Duct dilatation (min-moderate)	0	1	4	0	0	1	8	8	9
Vacuolation (min-marked)	1	1	0	9	10	7	9	10	10

Note duct section location: Section A: adjacent to liver; section D: adjacent to duodenum; section E: longitudinal section adjacent to duodenum.

Table 49: Incidence of biliopancreatic duct histopathology at terminal necropsy (D91) in female rats

Finding/Section (N=7-10/dose)	3 mg/kg			10 mg/kg			30 mg/kg		
	A	D	E	A	D	E	A	D	E
Chronic inflammation (min-moderate)	1	2	1	6	1	1	10	9	90
Degeneration/necrosis (min-moderate)	2	1	3	0	0	2	7	4	10

Finding/Section (N=7-10/dose)	3 mg/kg			10 mg/kg			30 mg/kg		
	A	D	E	A	D	E	A	D	E
Erosion/ulcer (min-slight)	4	3	0	9	8	3	6	4	40
Hyperplasia (min-moderate)	0	0	0	0	0	2	3	5	4
Duct dilatation (min-moderate)	0	0	0	0	0	2	3	5	4
Vacuolation (min-marked)	0	0	0	8	10	9	10	10	10

Note duct section location: Section A: adjacent to liver; section D: adjacent to duodenum; section E: longitudinal section adjacent to duodenum.

Note: Erosion and ulceration of bilopancreatic duct was more prominent at the MD compared to the HD in females.

Table 50: Recovery Necropsy – LDK378 administration in rats for 13 weeks

Organ/finding	control (N)		30 mg/kg (N)	
	M (5)	F (6)	M (6)	F (5)
Duodenum/degeneration/necrosis (min)	0	0	1	0
/vacuolation (min)	0	0	0	1
Mesenteric lymph node/↑macrophage aggregates (min-slight)	0	1	4	2

Table 51: Incidence of biliopancreatic duct histopathology following recovery in males and females

Finding/Section (N=6/sex)	30 mg/kg - Males			30 mg/kg - Females		
	A	D	E	A	D	E
Chronic inflammation (min-moderate)	5	2	5	3	3	5
Degeneration/necrosis (min-slight)	3	3	4	4	3	4
Erosion/ulcer (min-slight)	1	1	1	3	4	4
Hyperplasia (min-moderate)	0	0	5	0	0	2
Duct dilatation (min-moderate)	0	0	5	0	0	2
Vacuolation (min-slight - males; min-moderate -females)	6	5	5	6	4	6

Note duct section location: Section A: adjacent to liver; section D: adjacent to duodenum; section E: longitudinal section adjacent to duodenum.

Table 52: Toxicokinetics following administration of LDK378 at Days 1 and 73

Dose/Gender/Day			C_{max} (ng/ml)	AUC_{24} (hr.ng/ml)	Normalized C_{max} (ng/ml)	Normalized AUC_{24} (hr.ng/ml)
3 mg/kg	M	D1	91.3	1330	30.4	445
		D73	126	2010	42	670
	F	D1	67.1	955	22.4	318
		D73	115	1720	38.3	574
10 mg/kg	M	D1	490	7270	49	727
		D73	633	10200	63.3	1020
	F	D1	382	6160	38.2	616
		D73	720	9790	72	979
30 mg/kg	M	D1	1720	26900	57.3	898
		D73	1710	29600	57	986
	F	D1	1470	22100	49	735
		D73	1970	31100	65.7	1040

Note: Plasma samples from 5 control males were found to be contaminated with test material on D73. The Applicant states that this contamination appears to be carryover from a previously dosed HD male; it appears that the plasma from a treated HD animal contaminated the control samples.

The Applicant documented T_{max} between 5-10 hours postdose; timepoints between 10 and 24 hours were not studied. LDK378 exposure increased with dose in a slightly over-proportional manner. Gender differences were slight with male exposure increased on D1 (see table below). Slight but consistent drug accumulation was observed at D73 following repeat dosing, and appears to be marginally higher in females (see table below).

Table 53: LDK378 exposure comparison between males and females

		LDK378 AUC0-24h		
Dose (mg/kg/day)	Day	Male	Female	ratio Male / Female
3	1	1330	955	1.4
	73	2010	1720	1.2
10	1	7270	6160	1.2
	73	10200	9790	1.0
30	1	26900	22100	1.2
	73	29600	31100	1.0

AUC0-24h in [ng*Hours/mL]

Table 54: LDK378 exposure comparison between dosing Days 1 and 73

		LDK378 AUC0-24h		
Dose (mg/kg/day)	Gender	Day 73	Day 1	ratio Day 73 / Day 1
3	Male	2010	1330	1.5
	Female	1720	955	1.8
10	Male	10200	7270	1.4
	Female	9790	6160	1.6
30	Male	29600	26900	1.1
	Female	31100	22100	1.4

AUC0-24h in [ng*Hours/mL]

(Excerpted from Applicant's submission)

Figure 35 Mean concentrations of LDK378 versus time in rat plasma on D1

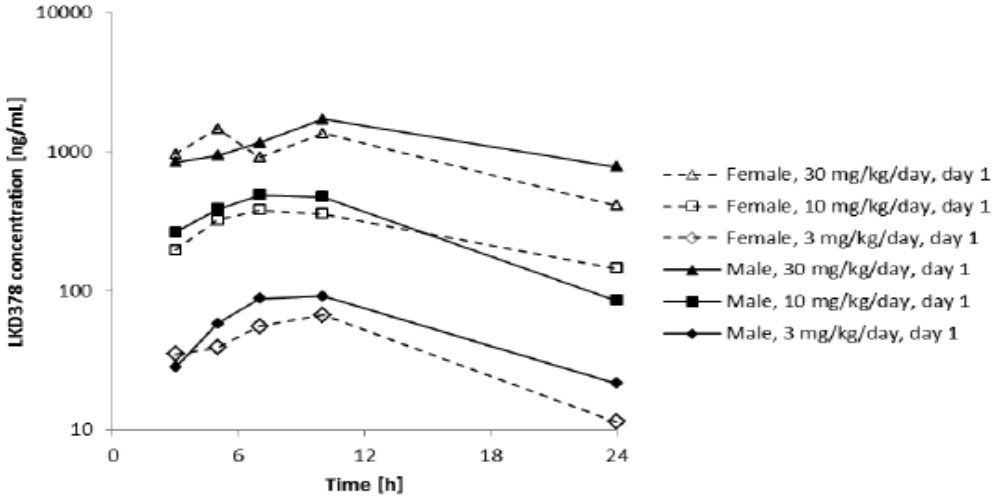
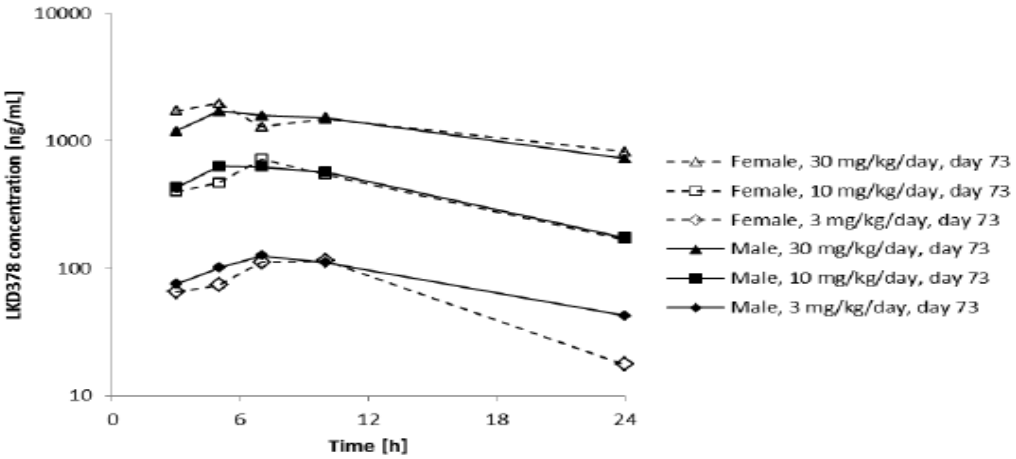


Figure 36 Mean concentrations of LDK378 versus time in rat plasma on D73



(Excerpted from Applicant's submission)

Study title: 4-week oral (gavage) toxicity study in monkeys with a 4-week recovery period

Study no.:	0970612
Study report location:	Electronic submission, M4.2.3.2
Conducting laboratory and location:	Novartis Pharmaceuticals
Date of study initiation:	December 9, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, batch #, and % purity:	LDK378, batch #0850001, purity 98.5%

Methods

Doses:	3, 10, 30 mg/kg/day
Frequency of dosing:	Daily for 4 weeks
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	Formulated in 0.5% (w/v) methylcellulose (400 cPs) in reverse osmosis water
Species/Strain:	Monkey, cynomolgus (2-2.5 years in age; weight: M: 2.2-3.7kg; F: 2.2-2.8 kg)
Number/Sex/Group:	

Group	No. of animals		Dose level	Dose concentration
	M	F		
Control	3	3	0	0
Low	3	3	3	0.6
Mid	3	3	10	2
High	3	3	30	6

Study Summary

This 4-week repeat-dose toxicology study in cynomolgus monkeys was reviewed by Sachia Khasar, Ph.D. in October, 2010. Following 4 weeks of LDK378 administration, the primary target sites included erosion of the epithelial lining and hyperplasia of the duodenum in 5/6 HD monkeys, accompanied by congestion, vacuolation, and macrophage infiltration. Decreased pancreatic zymogen and atrophy, histiocytosis of mesenteric lymph nodes, and lymphoid depletion of the thymus were exhibited at all doses with partial to full recovery. Thyroid gland weights were decreased 30-49% at doses ≥3 mg/kg; colloid depletion and small follicles were observed microscopically in these animals with uncertain association to LDK378.

When cynomolgus monkeys were administered higher doses of 10, 40, and 100 mg/kg LDK378 for 2 weeks in an earlier study (Study #0870126), the 100 mg/kg dose was lethal. Clinical signs of hunched posture, absent/reduced feces, depression hypothermia, dehydration and emesis were exhibited at 100 mg/kg, as well as 40 mg/kg.

Study title: 13-week oral gavage toxicity and toxicokinetic study with LDK378 in cynomolgus monkeys with a 8-week recovery phase

Study no.:	1270165
Study report location:	Electronic submission, M4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 30, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, batch #, and % purity:	LDK378, batch #1251005, purity 100%

Key Study Findings

● Primary target sites include cystic bile duct, hepatic bile duct, common bile duct (inflammation and vacuolation) and duodenum (congestion and hemorrhage) of HD males, as well as vacuolation and inflammation of the duodenum of HD monkeys of both genders.

● Dose related increases in ALT correlates with similar findings in rodents administered LDK378. Non-dose related increases in glucose and insulin correlate with the hyperglycemia observed clinically.
● Increased lipase in males during dosing and following recovery; no changes in lipase in females, or amylase in either male or female monkeys. Increased lipase correlates with findings observed clinically.
● Systemic exposure between individual monkeys was variable.
● In general, LDK378 exposure increased with dose, exposure was slightly higher in males (which may explain the increased toxicity observed in these animals), and drug accumulation was more pronounced with increasing dose on D73.

Methods

Doses: 3, 10, 30 mg/kg/day
Frequency of dosing: Daily for 92 days (13 weeks)
Route of administration: Oral gavage
Dose volume: 5 mL/kg
Formulation/Vehicle: Formulated in 0.5% (w/v) methylcellulose (400 cPs) in reverse osmosis water
Species/Strain: Monkey, cynomolgus
Number/Sex/Group:

Group	No. of animals		Dose level	Dose concentration
	M	F		
Control	6	6	0	0
Low	4	4	3	0.6
Mid	4	4	10	2
High	6	6	30	6

Age: 2-4 years
Weight: M: 2.5-3.3 kg; F: 2.5-3.0 kg
Satellite groups: None
Unique study design: 8 week recovery period in 2 monkeys/control and HD groups. Dose levels were justified by 4 week study performed in cynomolgus monkeys.
Blood samples were collected at scheduled necropsies for future exploratory biomarker analysis.
Tissue collections made using the embedding material OCT may be used for future immunohistochemical analysis and/or laser capture microdissection.
Deviation from study protocol: A large number of protocol deviations were noted, although deviations do not appear to have had a major impact on the study.

Observation	Time of assessment
Mortality	2x/d
Clinical observations	Pretest and ~3 hours postdose during dosing; daily during recovery

Physical examination	Pretest
Body weight	Pretest, weekly thereafter
Food consumption	Daily
Ophthalmoscopy	Pretest and W13 on control and HD animals
EKG	Pretest, during W13 during dosing and during W8 on surviving animals during recovery
Hematology	♦Blood collection at pretest, and during W5, 9 and 13 during dosing, and W4 and 8 during recovery,for hematology and clinical chemistry ♦Blood collection for glucose and insulin analyses on D1 and during W11 of dosing at ~3, 5, 7, 10, and 24 h postdose. Blood collected for amylase and lipase on D3 of dosing. ♦Prior to W5, urine collected prior to blood collection. Beginning W5, blood collected prior to dosing and urine collected following dosing. ♦Blood collected from femoral vein.
Clinical chemistry	
Urinalysis	
Biomarker analysis	Blood collected at scheduled sacrifice – Future analysis to identify circulating biomarkers of bileopancreatic injury
Organ weights	D92, D147
Toxicokinetics	Day 1 and during week 11 at ~3, 5, 7, 10, and 24h following dosing
Gross pathology	D92, D147
Histopathology	♦D91: Standard tissue collection from all animals; D147: macroscopic lesions, duodenal papilla with pancreas, bile duct, pancreatic duct, and liver from surviving control and HD animals ♦Tissue collections for OCT and immunohistochemistry evaluations on days of scheduled sacrifice considered exploratory and not part of current study. Tissues collected for OCT included common bile duct and pancreatic duct segments. Tissues collected for immunohistochemistry included duodenal papilla with pancreas, bile duct and pancreatic duct.

Parameter	3 mg/kg		10 mg/kg		30 mg/kg	
	M	F	M	F	M	F
Mortality	None					
Clinical observations	♦ Emesis: all doses ♦ Liquid feces: HD ♦ Findings of excessive salivation, dehydration, emesis, hypoactivity and swollen abdomen were infrequently observed and were not considered drug related findings					
Body weight[a]	UR					
Food consumption[a]	UR					
Ophthalmoscopy	UR					
EKG	UR					
Hematology[a]	UR					
Coagulation	UR					
Clinical chemistry[a]						
ALT (M: D88; F: D32/88)				↑54/↑31	↑93	↑92/↑72
Glucose (M: D1/73; F: D1/73)	- /↑42		- /↑33		↑37/↑40	↑32/↑28
Insulin (D32/73/88)			- / - /↑2-fold		↑2-3-fold[b]	
Amylase	UR					
Lipase (D88/RD53)	↑80/UR				↑2-fold/↑35	
Urinalysis	UR					
Organ weights – absolute[a]	UR					
Gross pathology	Bile duct discoloration in 1/6 HD males and females					
Histopathology	See histopathology tables					
Toxicokinetics	See toxicokinetics table.					

[a] Percent compared to concurrent control
[b] D1: HDM: ↑80%; D32: HDM ↑268%; D60: HDM ↑202%; D73: HDM ↑206-357%; D88: HDM ↑265%
(Note: MDM ↑196% D73, hour 3 only – insulin not elevated in MDM at hours 5-10 on D73.
Abbreviations: UR = unremarkable, M = males, F = females, RD = recovery day

Table 55: Histopathology (Terminal necropsy:D91) – LDK378 administration in monkeys for 13 weeks

Organ/finding	3 mg/kg (N)		10 mg/kg (N)		30 mg/kg (N)	
	M (4)	F (4)	M (4)	F (4)	M (4)	F (4)
Bile duct, cystic/inflammation (min-slight)					3	
Bile duct, hepatic/inflammation (min-slight)					3	
Duodenum/congestion, hemorrhage (minimal)					2	
/vacuolation (minimal)					3	2
/inflammation(min-slight)					4	2

Table 56: Incidence of common bile duct histopathology at terminal necropsy (D91) in male monkeys

Finding/Section (N=3 – 4/dose)	3 mg/kg			10 mg/kg			30 mg/kg		
	A	D	E	A	D	E	A	D	E
Inflammation (min-slight)							3	2	3
Vacuolation (minimal)							3	3	2

Note duct section location: Section A: section adjacent to liver, D:transverse section adjacent to duodenum; section, E: longitudinal section adjacent to duodenum.

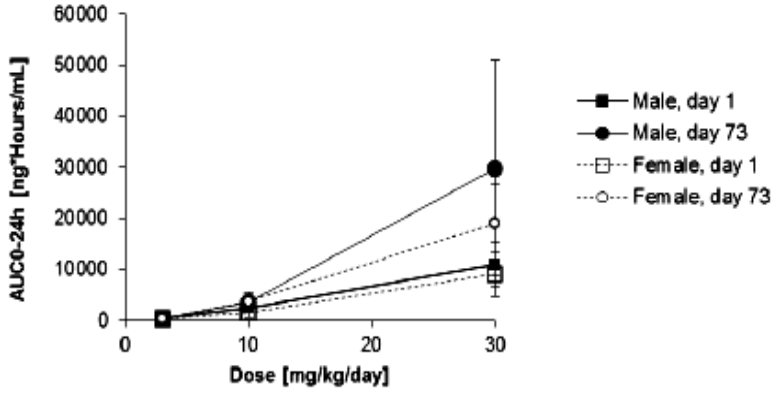
No notable changes were observed in the cystic or hepatic ducts of female monkeys, and no drug-related microscopic findings were observed in tissues closely associated with the bile

ducts, including the pancreas, pancreatic ducts and duodenal papilla, the site where the pancreatic ducts enter the duodenum..

Table 57: Toxicokinetics following administration of LDK378 at Days 1 and 73

Dose/Gender/Day			C_{max} (ng/ml)	AUC_{24} (hr.ng/ml)	Normalized C_{max} (ng/ml)	Normalized AUC_{24} (hr.ng/ml)
3 mg/kg	M	D1	39.3	591	13	197
		D73	35.7	453	11.9	151
	F	D1	27.3	378	9.1	126
		D73	31	363	10.3	121
10 mg/kg	M	D1	178	2550	17.8	255
		D73	260	3700	26	370
	F	D1	107	1650	10.7	165
		D73	271	3740	27.1	374
30 mg/kg	M	D1	636	11000	21.2	366
		D73	1570	29900	52.2	995
	F	D1	547	9110	18.3	304
		73	1180	19000	39.4	635

Figure 37 $AUC_{0\text{-}24h}$ versus dose in monkeys administered LDK378



(Excerpted from the Applicant's submission)

The Applicant documented T_{max} between 3-10 hours postdose for all doses. In general, LDK378 exposure increased with dose in a slightly over-proportional manner (see above figure). Exposure was generally higher in males for all doses (25-30%) which may explain the increased toxicity observed in these animals. Drug accumulation was more pronounced with increasing dose on D73 compared with D1 (see table below). It should be noted that systemic exposure was highly variable between individual monkeys (up to 80%).

Table 58: Exposure comparison between D73 and D1 following dosing with LDK378

		LDK278 AUC0-24h		
Dose (mg/kg/day)	Gender	Day 73	Day 1	ratio Day 73 / Day 1
3	Male	453	591	0.8
	Female	363	378	1.0
10	Male	3700	2550	1.5
	Female	3740	1650	2.3
30	Male	29900	11000	2.7
	Female	19000	9110	2.1

Units: AUC0-24h in [ng*Hours/mL]

(Excerpted from Applicant's submission)

Table 59 Exposure comparison between male and female monkeys dosed with LDK378

		LDK378 AUC0-24h		
Dose (mg/kg/day)	Day	Male	Female	ratio Male / Female
3	1	591	378	1.6
	73	453	363	1.2
10	1	2550	1650	1.5
	73	3700	3740	1.0
30	1	11000	9110	1.2
	73	29900	19000	1.6

Units: AUC0-24h in [ng*Hours/mL]

(Excerpted from Applicant's submission)

Figure 38 Mean concentrations of LDK378 in monkey plasma: D1

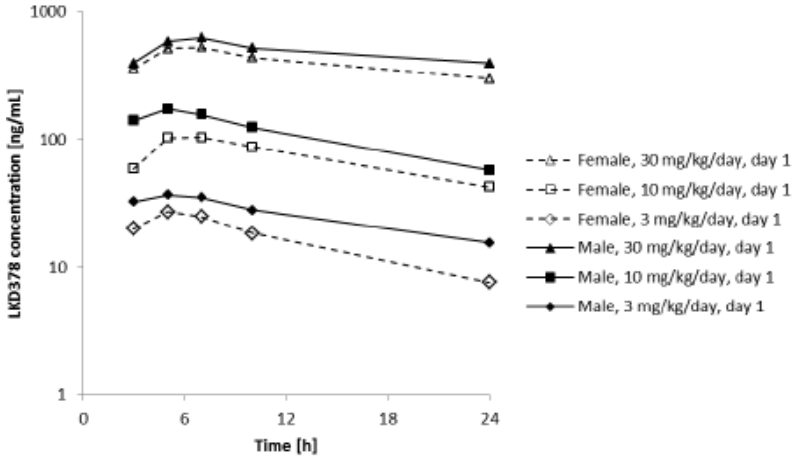
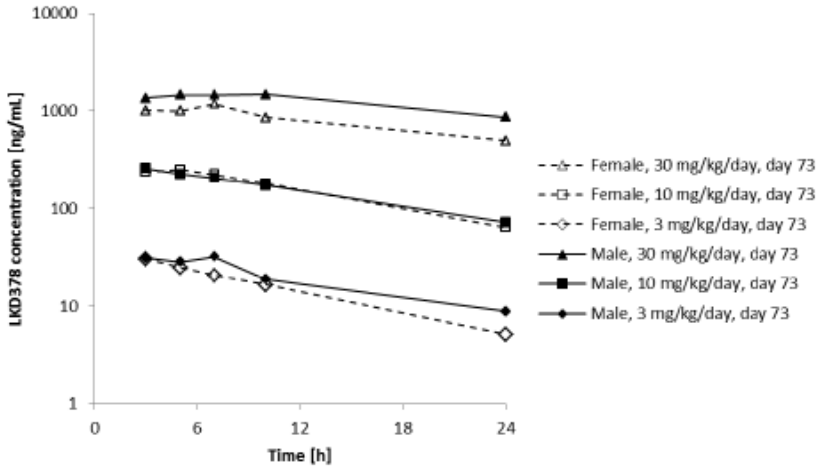


Figure 39: Mean concentrations of LDK378 in monkey plasma: D73



(Excerpted from Applicant's submission)

7 Genetic Toxicology

Study #	Title	Test System	LDK378 Concntration/Doses	Study Findings
0970421 (GLP)	Reverse Mutation in five histidine-requiring strains of *S. typhimurium*	Strains TA1535, TA97, TA98, TA100, TA102 ±S9	0.064, 0.32, 1.6, 8.0, 40, 100, 200, 500 µg/plate depending on toxicity of strain	Negative
0613212 (Non-GLP)	Miniscreen Ames test	TA98, TA100 ±S9	30, 100, 300, 1000 µg/well	Negative with caveat[1]
09760419 (GLP)	Induction of chromosome aberrations in cultured human peripheral blood lymphocytes	Cultured human peripheral blood lymphocytes ±S9	0.10, 0.25, 0.50, 2.0, 4.0, 8.0, 10.0, 12.0, 16,0, 22.0 µg/mL: 3,17h ±S9, 20h -S9	Positive[2]
0714901 (Non-GLP)	Micronucleus test in vitro using cultured human peripheral blood lymphocytes	Cultured human peripheral blood lymphocytes ±S9	♦18.6, 11.4, 7, 4.3, 2.6 µg/mL :44h -S9 ♦18.6, 11.4, 7, 4.3 µg/mL: 3h +S9 ♦16.4, 12.2, 9, 6.6, 4.9 µg/mL: 3h -S9	Negative
0614110 (Non-GLP)	Micronucleus test in vitro using TK6 cells	TK6 cells	3 or 20h -S9 3h +S9 Up to 33µg/mL	Positive after 20 hours

[1]Positive and negative control results were not provided for comparative purposes.
[2] Significant increases in numerical aberrations associated with increased numbers of cells exhibiting polyploidy were observed in LDK378-treated cultures.

Key Study Findings:
● LDK378 was not mutagenic in vitro in the presence or absence of metabolic activation when tested up to cytotoxic concentrations in the bacterial reverse mutation (Ames) assay.

● LDK378 induced significant increases in numerical aberrations in the in vitro cytogenetic assay using human lymphocytes, and micronuclei in the in vitro micronucleus test using TK6 cells.
● LDK378 was not clastogenic in the in vivo mouse micronucleus assay.

The genetic toxicology studies tabulated above were initially reviewed by Sachia Khasar in October 2010; studies were re-reviewed for data comparison. In general, studies reviewed were negative with the exception of the In Vitro Clastogenicity Assay in TK6 cells (Micronucleus Assay; Study No. 0614110) in which LDK378 induced increased numbers of cells containing micronuclei following a 20-hour treatment without metabolic activation, but not after a 3-hour treatment with or without metabolic activation when compared to concurrent controls. It was noted that this positive effect was observed at concentrations exhibiting a higher level of cytotoxicity (see tables below); however, based on the study criteria (noted below), the dose effect response, and the consistent finding exhibited from 3 assays, LDK378 is aneugenic at concentrations ≥2.0 ug/mL. This was a non-GLP study. In addition, significant increases in numerical aberrations associated with increased numbers of polyploidy cells were observed in GLP study # 09760419.

Study Criteria for Study No. 0614110: Micronucleus inducing effect for the tested concentration was considered positive if the frequency of micronucleated cells was:
♦ ≥ 2% and showed at least a doubling of the concurrent solvent control value, or
♦ < 2% and showed at least a 3-fold increase over the concurrent solvent control

Table 60: Micronucleus tests in vitro using TK6 cells

Experiment 1

Treatment (3 hr, +S9)	% Cell growth	% cells with MN	Treatment (20 hr, -S9)	% Cell growth	% cells with MN
S9/DMSO	100.0	0.75	RPMI/DMSO	100.0	0.90
2.0 µg/ml	83.3	0.60	1.0 µg/ml	86.9	1.00
3.9 µg/ml	80.6	0.95	2.0 µg/ml	85.2	1.15
7.8 µg/ml	72.2	0.80	3.9 µg/ml	54.1	**2.35**
15.6 µg/ml	80.6	1.05	7.8 µg/ml	3.3	n.d. (too toxic)
31.3 µg/ml	38.9	n.d. (too toxic)			
CP, 30 µM	58.3	**6.90**	EMS, 160 µM	60.7	**5.00**

Experiment 2

Treatment (3 hr, +S9)	% Cell growth	% cells with MN	Treatment (3 hr, -S9)	% Cell growth	% cells with MN
S9/DMSO	100.0	1.00	RPMI/DMSO	100.0	0.85
7.3 µg/ml	90.0	0.80	5.0 µg/ml	48.8	1.95
10.6 µg/ml	82.5	1.35	7.3 µg/ml	37.2	n.d. (too toxic)
15.5 µg/ml	65.0	1.50			
22.7 µg/ml	57.5	1.50			
33.1 µg/ml	5.0	n.d. (too toxic)			
CP, 30 µM	57.5	**6.40**	EMS, 4 mM	60.5	**5.10**

Experiment 2

Treatment (20 hr, -S9)	% Cell growth	% cells with MN
RPMI/DMSO	100.0	0.90
2.0 µg/ml	81.4	1.65
2.8 µg/ml	52.5	**2.30**
4.1 µg/ml	10.2	n.d. (too toxic)
EMS, 160 µM	50.8	**6.00**

Experiment 3

Treatment (3 hr, +S9)	% Cell growth	% cells with MN	Treatment (3 hr, -S9)	% Cell growth	% cells with MN
S9/DMSO	100.0	1.05	RPMI/DMSO	100.0	1.00
10.0 µg/ml	100.0	0.70	3.4 µg/ml	70.6	1.45
12.9 µg/ml	64.1	0.75	4.4 µg/ml	76.5	1.15
16.7 µg/ml	66.7	1.30	5.7 µg/ml	76.5	1.60*
21.5 µg/ml	43.6	n.d. (too toxic)	7.5 µg/ml	41.2	n.d. (too toxic)
CP, 30 µM	43.6	**4.05**	EMS, 4 mM	52.9	**3.30**

* only 1000 cells analyzed

Experiment 3

Treatment (20 hr, -S9)	% Cell growth	% cells with MN
RPMI/DMSO	100.0	0.55
2.0 µg/ml	83.7	1.50
2.6 µg/ml	67.3	**1.95**
3.4 µg/ml	34.7	n.d. (too toxic)
EMS, 160 µM	51.0	**6.00**

Abbreviations: EMS: ethyl-methanesulphonate; CP: cyclophosphamide
(Excerpted from Applicant's submission)

Study title: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes (not previously reviewed)

Study no: 0870222
Study report location: Electronic submission, M4.2.3.2
Conducting laboratory and location: (b) (4)
Date of study initiation: February 2009
GLP compliance: No
QA statement: No
Drug/ batch #: LDK378, batch #0850001

Key Study Findings

♦ Study incomplete; 2nd assay cancelled (-S9: 20h; +S9: 3+17h) by the Applicant

♦ Formulation analysis was not conducted
♦ Assay 1 indicated an increased frequency of cells with numerical aberrations in LDK378-treated cultures which were associated with increases in polyploidy (see tables below). This finding was also exhibited in Study 0970419.

Results

LDK378 concentrations of 4 to16 µg/mL were associated with an increased frequency of cells with numerical aberrations. Assay 2 was not completed for confirmation of results, and study validity criteria were not provided.

Table 61: Results of chromosomal aberration induction in human blood lymphocytes

3 hour treatment -S-9, 17 hour recovery (3+17), Experiment 1
Donor sex: female

Treatment (µg/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Vehicle	A	101	1	0	0	1	1.0
	B	101	0	1	0	1	1.0
	Total	202	1	1	0	2	1.0
1.000	A	101	0	0	1	1	1.0
	B	102	0	0	2	2	2.0
	Total	203	0	0	3	3	1.5
4.000	A	173	0	0	**73 #**	73	**42.2 #**
	B	202	1	0	**101 #**	102	**50.5 #**
	Total	375	1	0	174	175	46.7
6.000	A	155	0	0	**55 #**	55	**35.5 #**
	B	150	0	0	**50 #**	50	**33.3 #**
	Total	305	0	0	105	105	34.4
NQO, 5.00	A	98	0	0	0	0	0
	B	100	0	0	1	1	1.0
	Total	198	0	0	1	1	0.5

** Total cells examined for numerical aberrations

3 hour treatment +S-9, 17 hour recovery (3+17), Experiment 1
Donor sex: female

Treatment (µg/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Vehicle	A	100	0	0	0	0	0
	B	102	0	0	2	2	2.0
	Total	202	0	0	2	2	1.0
4.000	A	102	0	0	2	2	2.0
	B	109	1	0	8 #	9	8.3 #
	Total	211	1	0	10	11	5.2
8.000	A	200	0	1	99 #	100	50.0 #
	B	201	0	0	101 #	101	50.2 #
	Total	401	0	1	200	201	50.1
12.00	A	160	0	1	59 #	60	37.5 #
	B	106	0	0	41 #	41	38.7 #
	Total	266	0	1	100	101	38.0
16.00	A	80	0	0	13 #	13	16.3 #
	B	118	0	0	18 #	18	15.3 #
	Total	198	0	0	31	31	15.7
CPA, 12.5	A	34	0	0	0	0	0
	B	55	0	0	0	0	0
	Total	89	0	0	0	0	0

** Total cells examined for numerical aberrations

(Excerpted from Applicant's submission)

Study title: Induction of micronuclei in bone marrow of treated rats (not previously reviewed)

Study no:	1370166
Study report location:	Electronic submission, M4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study release:	September, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	LDK378, batch #0850001

Key Study Findings

♦ LDK378 did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of male Wistar rats when treated up to 2000 mg/kg/day.

Methods

Doses in definitive study:	200, 1000, 2000 mg/kg/day (150, 300, 600, 1000, 2000 mg/kg/day: rangefinding study)
Frequency of dosing:	Daily for 2 days
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% (w/v) methylcellulose (400 cps)
Species/Strain:	Male Han Wistar rats
Number/Sex/Group:	2-3 rats/dose (rangefinding);
Satellite groups:	None
Basis of dose selection:	Dose range-finding

Negative control: 0.5% (w/v) methylcellulose (400 cps)
Positive control: 20 mg/kg CPA

Study Validity

6.2 Acceptance criteria

The assay was considered valid if all the following criteria were met:

1. The vehicle control MN PCE data were comparable with the laboratory's historical vehicle control ranges.
2. At least five animals (with the exception of the positive control group) out of each group were available for analysis, and
3. The positive control chemical (CPA) induced a statistically significant increase in the frequency of MN PCE.
4. The high dose was considered to be the MTD, the maximum practicable dose or one that demonstrated cytotoxicity to the target cells.

If required, acceptance under any other criteria are discussed in the results section.

6.3 Evaluation criteria

For valid data, the test item was considered to induce clastogenic / aneugenic damage if:

1. A statistically significant increase in the frequency of MN PCE occurred at one or more dose levels.
2. The incidence and distribution of MN PCE in individual animals at such a point exceeded the laboratory's historical vehicle control data.
3. The group mean MN PCE value at such a point exceeded the 95% calculated confidence interval for the mean historical vehicle control data.
4. A dose-response trend in the proportion of MN PCE was observed.

The test item was considered positive in this assay if all of the above criteria were met.

The test item was considered negative in this assay if none of the above criteria were met.

Results which only partially satisfied the above criteria were dealt with on a case-by-case basis. Evidence of a dose-related effect was considered useful but not essential in the

The data generated in this study confirm that:

1. The incidence and distribution of micronucleated PCE in the vehicle control group were consistent with the laboratory's historical vehicle control data, and
2. at least five animals out of each group (with the exception of the positive control group) were available for analysis, and
3. the positive control chemical (CPA) induced a statistically significant increase in the frequency of micronucleated PCE (Table 7-1 Summary of Micronucleus Data).
4. The high dose was considered to be the maximum recommended dose according to current regulatory guidelines.

The assay data were therefore considered valid.

Results

No clinical signs of toxicity were observed in LD or MD animals. Piloerection and hypoactivity were exhibited in 6 of 6 HD animals 2-4h following the second dose; piloerection continued to be observed until necropsy in 2 HD animals.

Table 62: Results of micronuclei induction in the bone marrow of treated rats

Group / Treatment (mg/kg/day)	PCE scored	Number of MN PCE	% PCE	MN / 2000 PCE	% MN PCE	SD	Heterogeneity		2 x 2 Contingency	
							X^2	S	X^2	S
1M / Vehicle (0)	12000	19	45.37	3.17	0.16	0.07	3.43	NS	-	-
2M / LDK378 (200)	12000	17	48.77	2.83	0.14	0.07	3.83	NS	0.03	NS
3M / LDK378 (1000)	12000	10	39.10	1.67	0.08	0.06	4.40	NS	2.21	NS
4M / LDK378 (2000)	12000	16	45.63	2.67	0.13	0.07	3.50	NS	0.11	NS
5M / CPA (20)	6000	93	31.07	31.00	1.55	0.26	-	-	123.04	P≤0.001

Linear trend: z = -0.678 (NS)

(Excerpted from Applicant's submission)

8 Carcinogenicity

Carcinogenicity studies were not conducted.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Fertility and early embryonic development studies were not conducted.

9.2 Embryonic Fetal Development

Study title: LDK378: An oral gavage study of embryo-fetal development in the rat

Study no.:	1370073/9000189
Study report location:	Electronic submission, M4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 29, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	LDK378, batch #1251005, purity 99.5%

Key Study Findings

♦ LDK378 did not induce embryolethality or fetotoxicity at doses tested.
♦ The incidence of skeletal anomalies were generally low, although several findings were dose related (incomplete ossification of skull and vertebral column, and wavy ribs)
♦ The incidence of a single external anomaly, abnormal hindlimb flexure, at the HD was marginally higher than the concurrent control and test facility historical control.

♦ Maternal toxicity as demonstrated by depressed gestational body weights was observed at mid- and high-doses.

Methods

Doses:	0, 1, 10, 50 mg/kg/dose [dose concentration:0, 0.2, 2 and 10 mg/mL]
Frequency of dosing:	Daily from D6 to D17 postcoitus (pc)
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Formulated in 0.5% (w/v) methylcellulose (400 cPs) in reverse osmosis water
Species/Strain:	Rat/Wistar Hannover (Crl:Wi[Han])
Age/weight:	F: 77-86 days/ 198-253 g
Number/Sex/Group:	24 dams/dose
Satellite groups:	Toxicokinetics: 3 control+5 dams/LDK378 group
Study design:	♦Evaluation of LDK378 on development of embryo and fetus following oral exposure of pregnant female from implantation to closure of hard palate. ♦F_0 generation females were mated with breeder male rats; day of mating confirmation (sperm or copulatory plug = GD (gestation day 0) ♦ Justification of doses based on results of 4-week rat toxicology study and preliminary data from 13-week toxicology rat study.
Deviation from study protocol:	♦No toxicokinetic sampling of HD dam due to condition of bleeding site ♦No fetal examination for single fetus of MD dam (dam #3504) ♦Dams not dosed at same time/day on several occasions

Observation	Time or description of assessment
Mortality	2X/d
Clinical Observations	2X/d on dosing days; 1X/d on non-dosing days
Body weights	GD0, 3, 6, 9, 12, 15, 18 and 21
Food consumption	GD3-6, 6-9, 9-12, 12-15, 15-18, and 18-20

Table 63: Necropsy schedule of main and toxicokinetic rats (Embryo-fetal development)

Group no.	No. of animals	Scheduled euthanasia day pc	Necropsy procedures				Histology	Histopathology
			Ovarian/ uterine examination	Necropsy	Tissue collection	Organ weights		
1	3[a]	17	Pregnancy Status	-	-	-	-	-
2	5[a]							
3	5[a]							
4	5[a]							
Unscheduled Deaths[a]			Pregnancy Status	Limited	-	-	-	-
1	24[b]	21	Full Exam	X	X	Gravid uterus	-	-
2	24[b]							
3	24[b]							
4	24[b]							

X = Procedure conducted; - = Not applicable
[a] Females assigned to the toxicokinetic study
[b] Females assigned to the main study

♦ Limited necropsy of 2 toxicokinetic (tk) animals (1LD, 1MD) found dead prior to scheduled sacrifice [D16/17 pc]; pregnancy status recorded. Results documented below.
Necropsies were not performed on surviving toxicokinetics animals.
♦ Main study animals survived until scheduled sacrifice; histopathology was performed.

(Excerpted from Applicant's submission)

Toxicokinetics	Maternal blood collection: D16 at 0.5, 1, 3, 7, and 24h postdose Fetal blood collection: D17 at 3h postdose of tk dams
Day of C-section	21 days pc
Ovarian and uterine examination	Scheduled termination: ♦Corpora lutea ♦Implantation sites ♦Placenta abnormalities, size and shape ♦Live/dead fetuses ♦Early/late resorptions
Organ weights	Scheduled termination: gravid uterus
Fetal examination	♦Abnormalities classified as malformations or variations ♦External abnormalities photographed at discretion of study director ♦Gender of fetus recorded ♦Body weight recorded ♦50% of fetuses examined for visceral abnormalities ♦50% of fetuses examined for skeletal abnormalities

Table 64: Results exhibited following maternal dosing (Embryo-fetal development in rats)

Observations Gender	F		
Dose (mg/kg)	1	10	50
Mortality	1*	1*	
Clinical observations	Decedent F: labored breathing, weakness, skin pallor, decreased muscle tone LD/HD: alopecia		

*LDF death attributable to jugular venipuncture; MDF death attributable to gavage error – Deaths considered accidental and not related to LDK378 administration

Table 65: Summary of mating and fertility in females (N= 24 /group) (Embryo-fetal development in rats)

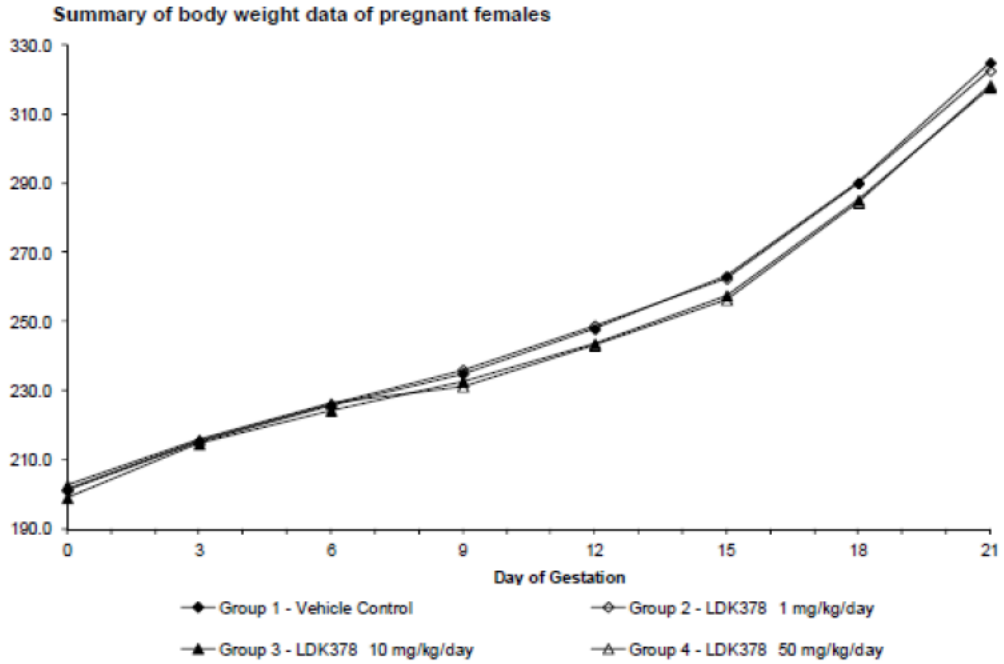
Dams (mg/kg)	Control	1	10	50
Gestational body weights[a]	UR			
Corrected BW gains[a] (D6-21 pc)		UR	↓14	↓36
Gestational food consumption[a]	UR			
# pregnant females	24 (100%)	24 (100%)	24 (100%)	24 (100%)
# aborted or total resorption of litter	0	0	0	0
Mean # corpora lutea	13.1	12.8	12.8/13.0[b]	12.4
Mean # implantations	12.3	11.6	11.4/11.7[b]	11.8
# pregnant at C-section	24	24	24	24
Dams with viable fetuses	24	24	24	24
Dams with no viable fetuses	-	-	-	-
Mean % pre-implantation loss	6.5	8.6	12/10.4[b]	5.1
Mean # live births	11.6	11.0	10.6/11.0[b]	11.4
Mean # total resorptions Early resorptions Late resorptions	0.7 0.7	0.6 0.5 0.1	0.6/0.7[b] 0.6/0.7[b]	0.3 0.3
Mean # dead fetuses	0	0	0	0
Mean % post-implantation loss	5.5	5.6	9.4/5.4[b]	2.9
Gravid uterine weight (g)	82.1	79.2	78.6	81.2
Mean fetal body weight (g) Males Females	 5.4 5.1	 5.5 5.2	 5.5 5.1	 5.4 5.2
Fetal sex ratios (% males)	51.4	45.3	46.3	49.6

[a] Percent compared to concurrent controls (corrected BW gain = % difference of treated from controls – statistical significance is based on actual data at HD)

[b] Including/excluding animal identified as pregnant by ammonium sulfide [Single MDF (#3515) was determined to be pregnant by ammonium sulfide staining of the uterus with 4 implantation sites]. The reason for this separation in identification was not documented by the study laboratory).

Abbreviations: UR = unremarkable

Figure 40 Summary of body weight data of pregnant female rats (Embryo-fetal development)



(Excerpted from Applicant's submission)

Table 66: Fetal anomalies, malformations and variations (Embryo-fetal development in rats)

Dose (mg/kg)	control	1	10	50
External anomalies -Total affected fetuses (litters); presented as %				
# Fetuses examined	278	263	253	274
# Litters evaluated	24	24	23	24
Hindlimbs – abnormal flexture				0.7 (8.3)
Lower jaw absent (agnathia)			0.4 (4.3)[a]	
Tongue absent (aglossia)			0.4 (4.3)[a]	
Cleft palate (WT)			0.8 (4.3)[a]	
Skeletal anomalies - Total affected fetuses (litters); presented as %				
# Fetuses examined	138	130	124	135
# Litters evaluated	24	24	23	24
Skull; Incomplete ossification of parietal bone	10.0 (33.3)	6.9 (16.7)	11.3 (26.1)	13.3 (41.7)
Incomplete ossification of interparietal bone	18.8 (41.7)	13.1 (37.5)	21.8 (39.1)	22.2 (50.0)
Incomplete ossification of frontal bone			0.8 (4.3)	
Incomplete ossification of zygomatic temporal			0.8 (4.3)	
Incomplete ossification of zygomatic process	0.7 (4.2)	1.5 (8.3)		
Vertebral column; Extra presacral vertebrae	2.9 (8.3)	1.5 (4.2)		3.7 (8.3)

Dose (mg/kg)	control	1	10	50
Ossification center on 1st lumbar or 14th thoracic vertebrae	26.8 (79.2)	26.9 (83.3)	31.5 (82.6)	36.3 (91.7)
Extra sternebra(e)				0.7 (4.2)
Sternebrae 1 to 4 unossified/ incomplete ossification/semi-bipartite/bipartite)			08 (4.3)	0.7 (4.2)
Ribs; wavy rib(s)		2.3 (8.3)		3.0 (12.5)
Rudimentary 14th rib(s)	12.3 (41.7)	21.5 (54.2)	19.4 (56.5)	16.3 (62.5)
Ossification center(s) on 7th cervical vertebra	2.2 (8.3)	3.1 (16.7)	1.6 (4.3)	2.2 (12.5)
Visceral anomalies - Total affected fetuses (litters); presented as %				
# Fetuses examined	140	133	130	139
# Litters evaluated	24	24	23	24
Comparative incidence of visceral anomalies in LDK378 fetuses ≤ incidence in control fetuses				

[a] Single animal; single litter
Note: Incidence of findings changed marginally depending on technique of examination (e.g. use of Wilson technique).

Table 67: Historical control range of findings for test facility (Embryo-fetal development in rats)

Malformation/variation	Fetal incidence (%)	Litter incidence (%)
Agnathia	0 - 0.5	0 - 4.8
Aglossia	0 - 0.5	0 - 4.8
Cleft palate	0 - 1.4	0 - 4.8
Abnormal flexure of hindlimbs	0 - 0.4	0 - 4.4

The incidence of abnormal flexure of hindlimbs exhibited in HD fetuses was marginally higher than the test facility historical control incidence for that external anomaly. Other external anomalies exhibited in test fetuses were within the historical control range (see table). The incidence of visceral anomalies in test fetuses was ≤ incidence in concurrent control fetuses. The incidence of skeletal anomalies in test fetuses was similar to control incidence or were noted at a low incidence, although several findings were dose related (incomplete ossification of skull and vertebral column, and wavy ribs. The incidence of these findings was not significant; the comparative incidence of skeletal anomalies for the test facility was not provided.

Table 68: Toxicokinetic parameters of dams administered LDK378

Dose (mg/kg)	Cmax (ng.mL)	Cmax/dose	$AUC_{0\text{-}24h}$ (ng*hr/mL)	$AUC_{0\text{-}24h}$ /dose
1	9.8	9.8	142	142
10	202	20.2	2940	294
50	831	16.6	14900	298

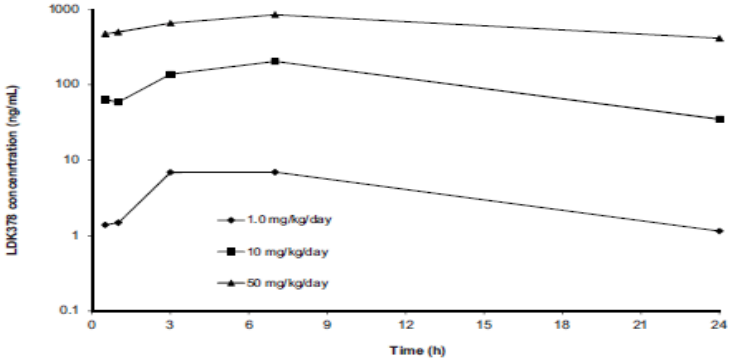
Plasma samples taken D16 pc; N=5/LDK378 groups; N=3/control group

Table 69: Fetal plasma concentrations at D17

Dose (mg/kg)	Fetal plasma concentrations (3h) (ng/mL)
1	0
10	9.23
50	61.2

In general, LDK378 exposure increased with increasing dose with some noted variability. The individual LDK378 concentration ratios were up to 20 times higher in maternal plasma on D16 pc compared to fetal plasma concentrations on D17 pc.

Figure 41 Mean concentrations (ng/mL) of LDK378 in maternal plasma on D16 pc



(Excerpted from Applicant's submission)

Study title: LDK378: An oral gavage dose range finding study of embryo-fetal development in the rabbit
Study no: 1370071

Study summary:
Pregnant rabbits were administered LDK378 doses of 5, 25, 35, 50 and 100 mg/kg/day from GD 7-20. The highest dose of 100 mg/kg was terminated due to excessive maternal toxicity. Depressed maternal body weights and food consumption were observed at 35 mg/kg, with abortions noted at 35 and 50 mg/kg. The 50 mg/kg dose was embryolethal and fetotoxic.

Study title: LDK378: An oral gavage study of embryo-fetal development in the rabbit

Study no.:	1370072/9000190
Study report location:	Electronic submission, M4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 27, 2013
GLP compliance:	Yes [Exception to GLP: characterization and stability testing of LDK378 at a GMP laboratory]

QA statement:	Yes
Drug, lot #, and % purity:	LDK378, batch #1251005, purity 99.5%

Key Study Findings

♦ LDK378 did not induce significant embryolethality or fetotoxicity at doses tested although pre-implantation loss was minimally increased, and gravid uterine weights were marginally depressed at 25 mg/kg.
♦ Number of live births and fetal body weights similar in dosed and control litters
♦ Incidence of skeletal anomalies were generally low, although several findings were dose related or incidence was higher at HD compared to control (incomplete ossification of skull, misshapen skull, vertebral column bipartite or displaced). In addition, incomplete ossification of sternebrae was significant in all dosed groups.
♦ Increased incidence of visceral anomalies observed in a small number of fetuses at all LDK378 dose levels, specifically gallbladder and heart.
♦ Maternal toxicity as demonstrated by mildly depressed gestational body weight and food consumption was observed at high-doses.

Methods

Doses:	0, 2, 10, 25 mg/kg/dose [dose concentration:0, 0.4, 2 and 5 mg/mL]
Frequency of dosing:	Daily from D7 to D20 postcoitum (pc)
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Formulated in 0.5% (w/v) methylcellulose (400 cPs) in reverse osmosis water
Species/Strain:	Rabbit, New Zealand White (Hra[NZW]SPF)
Age/weight:	F: 5-6 months/3.1-3.7kg
Number/Sex/Group:	20 dams/dose
Satellite groups:	Toxicokinetics: 3 control+5 rabbits/LDK378 group
Study design:	♦Evaluation of LDK378 on development of embryo and fetus following oral exposure of pregnant females from implantation to closure of hard palate. ♦F_0 generation females were mated with breeder male rabbits; day of mating was considered to be D0 postcoitum (pc) [Gestation day (gd) 0] ♦Dose selection based on preliminary results of oral gavage dose range-finding study of embryo-fetal development in the rabbit (Study 9000189).
Deviation from study protocol:	♦2 LD females replaced prior to initiation of dosing due to weight loss. ♦Dose reflux of daily dose occurred in control, MD and HD groups This was not considered to have an impact on the study. See table below for occurrence of reflux. ♦Additional minor deviations were noted which were not considered to impact the study including dosing control animals with dosing tube not flushed following dosing of test animals.

Animal no.	Study day (pc)	Treatment group	Comment
1504	10	1	Reflux, no flush given
1512	15	1	Reflux, no flush given
1513	18	1	Reflux
3512	17	3	No flush given
3522	18	3	Reflux
4506	13	4	Reflux
4503	17	4	Reflux

(Excerpted from Applicant's submission)

Observation	Time or description of assessment
Mortality	2X/d
Clinical Observations	2X/d on dosing days (predose, 3h postdose); 1X/d on non-dosing days
Body weights	D0, 5, 7, 10, 14, 17, 21, 24, 29 pc for main animals; D0, 5, 7, 10, 14, and 17 pc for toxicokinetic animals
Food consumption	Daily from D5 pc for main animals

Table 70: Necropsy schedule of main and toxicokinetic animals (Embryo-fetal development in rabbits)

Group no.	Targeted no. of animals	Scheduled euthanasia day pc	Necropsy procedures				Histology	Histopathology
			Ovarian/ uterine examination	Necropsy	Tissue collection	Organ weights		
1	3[a]	20	Pregnancy Status	-	-	-	-	-
2	5[a]						-	-
3	5[a]						-	-
4	5[a]						-	-
1	20	29	Full exam	X	X	X[b]	-	-
2	20						-	-
3	20						-	-
4	20						-	-
Unscheduled deaths			Full exam	X	X	-	-	-

X = Procedure conducted; - = Not applicable.
a Rabbits assigned to the toxicokinetic study.
b Gravid uterus.

(Excerpted from Applicant's submission)

♦ Complete necropsy (carcass, musculoskeletal system, external surfaces, cranial cavity, external surfaces of brain, thoracic, abdominal and pelvic cavities with associated organs and tissues) conducted on main study animals
♦ Study animals euthanized following last blood collection on D29 from inguinal/axillary arteries
♦Single HD main study animal (#4503) euthanized prior to scheduled termination due to gavage error. Pregnancy status and ovarian/uterine contents were recorded.

Toxicokinetics	Maternal blood collection: D7, 13 and19 pc at 0.5, 1, 3, 5, 7 and 24h postdose Fetal blood collection: D20 at 3h postdose of toxicokinetic F
Ovarian and uterine examination	Scheduled termination: ♦Corpora lutea ♦Implantation sites ♦Empty implantation sites ♦Placenta abnormalities, color, size and shape ♦Live/dead fetuses ♦Early/late resorptions
Tissue collection	♦Standard list of tissues collected at scheduled necropsy from 2 gravid control rabbits for future comparison ♦Cervix, gross lesions/masses, ovaries, and uterus collected from any pregnant animal euthanized prior to scheduled sacrifice
Organ weights	Scheduled termination: gravid uterus
Fetal examination	♦Abnormalities classified as malformations or variations ♦External abnormalities ♦Gender of fetus recorded ♦Body weight recorded ♦Visceral abnormalities ♦Skeletal abnormalities

Table 71 Results exhibited following maternal dosing (Embryo-fetal development in rabbits)

Observations Gender	F		
Dose (mg/kg)	2	10	25
Mortality			1*
Clinical observations	UR		

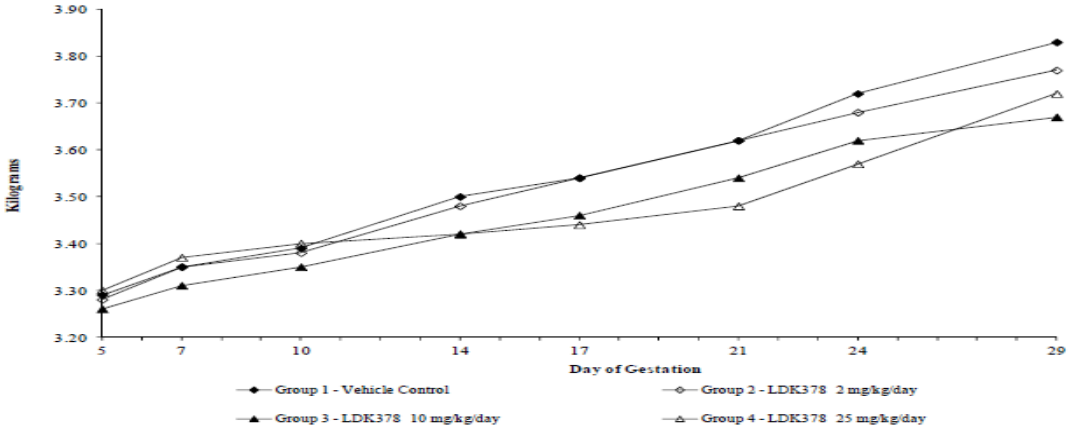
*HDF (animal #4503) sacrificed moribund on D18 due to gavage error, with labored breathing following dose administration on D17. Gross findings included blood in thoracic cavity and discoloration of lungs, thymus and diaphragmatic muscle. Death was considered accidental and were not related to LDK378 administration

Table 72: Summary of mating and fertility in does (N= 24/group) (Embryo-fetal development in rabbits)

Dose (mg/kg)	Control	2	10	25
Gestational body weights[a] (D7-29)	UR; HD depression in BW <10% compared to concurrent controls			
Corrected BW gains[a] (D7-29 pc)	UR			
Gestational food consumption[a]	GD13-15:↓25; recovery by GD25 (similar to concurrent controls)			
# pregnant females	20	20	20	20
# aborted or total resorption of litter	0	0	0	0
Mean # corpora lutea	9.8	9.8	10.6	9.7
Mean # implantations	9.0	8.7	9.6	8.3
# pregnant at C-section	20	20	20	20
Dams with viable fetuses	20	20	20	20
Dams with no viable fetuses	0	0	0	0
Mean % pre-implantation loss	8.0	11.0	10.6	14.2
Mean # live births	8.9	8.5	9.3	8.1
Mean # total resorptions	0.2	0.3	0.4	0.2
Early resorptions	0.1	0.2	0.3	0.1
Late resorptions	0.1	0.1	0.1	0.1
Mean # dead fetuses	0	0	0	0
Mean % post-implantation loss	1.3	3.1	3.6	2.4
Gravid uterine weight (g)	545	512	533	477
Mean fetal body weight (g)				
Males	45.2	44.7	42.4	44.5
Females	44.3	44.5	40.8	42.8
Fetal sex ratios (% males)	51.2	47.3	51.8	47.4

[a] Percent compared to concurrent controls

Table 73: Body weight change of pregnant females



(Excerpted from Applicant's submission)

Table 74: Fetal anomalies, malformations and variations (Embryo-fetal development in rabbits)

Dose (mg/kg)	control	1	10	50
External anomalies -Total affected fetuses (litters); presented as %				
# Fetuses examined	177	170	185	153
# Litters evaluated	20	20	20	19[a]
Eye; microphthalmia		0.6 (5.0)		0.7 (5.3)
eye opacity		0.6 (5.0)		
Abdomen; intestines protruding at umbilicus		0.6 (5.0)		
Tail; threadlike				0.7 (5.3)
Limb; abnormal flexure of forepaw		0.6 (5.0)	0.5 (5.0)	
Skeletal anomalies - Total affected fetuses (litters); presented as %				
# Fetuses examined	177	170	185	153
# Litters evaluated	20	20	20	19
Skull; nasal bone incomplete ossification		0.6 (5.0)	0.5 (5.0)	0.7 (5.3)
parietal bone incomplete ossification				0.7 (5.3)
hyoid bone in complete ossification	1.1 (10.0)	5.3 (40.0)	2.2 (15.0)	5.2 (31.6)
hyoid bone misshapen	1.1 (10.0)	1.2 (10.0)	2.2 (20.0)	3.3 (26.3)
Vertebral column; thoracic vertebral centrum semi-bipartite	4.5 (25.0)	5.9 (25.0)	5.4 (30.0)	8.5 (42.1)
caudal vertebra(e) displaced			0.5 (5.0)	1.3 (5.3)
Sternebra(e); fused	2.8 (15.0)	1.8 (15.0)	1.6 (15.0)	3.3 (21.1)
unossified/incomplete ossification	26 (70.0)	42.9 (95.0)**	51.4 (90.0)***	42.5 (84.2)**
Pubic bone; incomplete ossification		0.6 (5.0)		2.0 (5.3)
unossified				0.7 (5.3)
Limb; reduced number of phalanges in forpaws/pollex /hindpaws		0.6 (5.0)		
Reduced number of phalanges in tarsals		0.6 (5.0)		0.7 (5.3)
Visceral anomalies - Total affected fetuses (litters); presented as %				
# Fetuses examined	177	170	185	153
# Litters evaluated	20	20	20	19
Gallbladder; absent		1.8 (10.0)		
small			0.5 (5.0)	0.7 (5.3)
malpositioned			0.5 (5.0)	
Heart; subclavian retroesophageal			0.5 (5.0)	0.7 (5.3)

[a] No explanation for evaluation of only 19 liters at HD
** Significantly different from control group at p≤0.01
*** Significantly different from control group at p≤0.001

The historical control range of external, skeletal or visceral anomalies for the test facility were not provided.

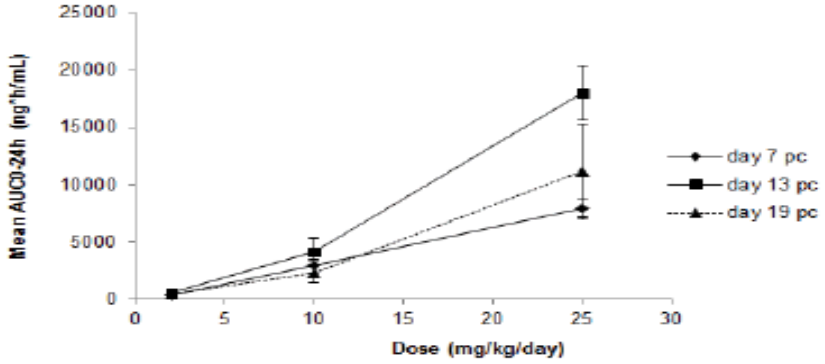
Table 75: Toxicokinetic parameters of does administered LDK378 (Embryo-fetal development in rabbits)

Dose (mg/kg)/day	C_{max} (ng.mL)	C_{max}/dose	AUC_{0-24h} (ng*hr/mL)	AUC_{0-24h} /dose
2/ D7	23.3	11.6	347	174
D13	34.8	17.4	543	272
D19	26.5	13.3	403	202
10/ D7	193	19.3	2890	289
D13	260	26.0	4180	418
D19	149	14.9	2340	233
25/ D7	472	18.9	7870	315
D13	917	36.7	18000	719
D19	582	23.3	11200	448

Plasma samples taken D7, 13 and 19 pc; N=5/LDK378 groups; N=3/control group

Following multiple doses, LDK378 exposure increased with increasing dose. Slight drug accumulation was observed between D7 and D13 only; exposures at D19 were depressed relative to exposures at D13 for all dose levels.

Figure 42 Mean LDK378 exposure in maternal plasma Embryo-fetal development in rabbits)



(Excerpted from Applicant's submission)

Table 76: Fetal plasma concentrations at D20 pc (n=5)

Dose (mg/kg)	Fetal plasma concentrations (3h) (ng/mL)
2	1.58
10	6.5
25	41.2

9.3 Prenatal and Postnatal Development

A prenatal/postnatal development study has not been conducted and will not be required.

10 Special Toxicology Studies

Study title: LDK378: In vitro 3T3 NRU phototoxicity test

Study no.:	0770606
Study report location:	Electronic submission, M4.2.3.7
Conducting laboratory and location:	Safety Profiling and Assessment, Novartis Pharma AG, Basel, Switzerland
Date of study release:	December 20, 2007
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	LDK378, batch # LDK-A1-1, purity 99%

Key Study Findings

♦ LDK378 was phototoxic in vitro at concentrations ≥ 4.4 µg/mL.

Summary and Results:
Balb/c 3T3 fibroblast cells were treated for 1 hour with concentrations of LDK378 ranging from 0.125 to 16 µg/mL prior to irradiation. One set of plates was exposed to 5.0 J/cm2 UV-A for 50 minutes, with comparative plates kept in darkened conditions for the same period. Approximately 20 - 22 hours following irradiation and incubation, cytotoxicity was assessed by the Neutral Red Uptake assay. Chlorpromazine was used as the positive control.

The EC_{50} values with and without irradiation were 0.54 and 4.4 for test 1 and 0.97 and 4.9 µg/mL for test 2, respectively. Photo-Irritation Values (PIF) of 8.1 and 5.1, respectively, indicated a phototoxic potential for LDK378 (defined as a PIF >5.0 according to OECD test guidelines).

The acceptance criteria for the assay were met demonstrating the functionality of the test system. The data were considered to be valid.

Table 77: EC_{50} and PIF results for in vitro phototoxicity potential of LDK378

	EC_{50} (-Irr)	EC_{50} (+Irr)	PIF
1st valid experiment	4.4 µg/mL	0.54 µg/mL	8.1
2nd valid experiment	4.9 µg/mL	0.97 µg/mL	5.1

(Excerpted from Applicant's submission)

Acceptance criteria (Excerpted from Applicant's submission):

The assay met the acceptance criteria if:

1. Positive control Chlorpromazine met the expected range (EC_{50} (+Irr) within 0.1 - 2.0 µg/mL, EC_{50} (–Irr) within 7.0 - 90.0 µg/mL, PIF was ≥ 6).
2. The viability of the irradiated negative control compared to the non-irradiated negative control was at least 80 % (radiation sensitivity).
3. The Neutral Red uptake of non-irradiated cells (NC) led at least to an OD of 0.2 ($OD_{540} \geq 0.2$). This value proved to produce valid results for phototoxicity at the test facility.
4. The mean OD_{540} of the negative controls on the left (NC_L) and the right (NC_R) side of one MTP did not differ by more than 15 % from the mean OD_{540} of all negative controls on that plate (deviation within negative controls).

Study title: LDK378: Assessment of phototoxic potential with the murine Local Lymph Node Assay (UV-LLNA by oral route)

Study no.:	39887 TSS/1270757
Study report location:	Electronic submission, M4.2.3.7
Conducting laboratory and location:	(b) (4)
Date of study release:	June 12, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	LDK378, batch # 1151004, purity not provided
Positive control:	Sparfloxacin (5mg/mL concentration in carboxymethylcellulose vehicle)

Key Study Findings

♦ LDK378 did not have phototoxic potential.

Summary and Results:

The LLNA assay is designed to identify the phototoxic potential of the test material following oral exposure and UVA light, but not the mechanism of phototoxicity. Additional information is assessed from the lymph node activating potential in connection with the presence or absence of ear skin erythema and/or ear weight changes.

Following administration of LDK378 at doses of 10, 30 or 100 mg/kg/day for 3 days, groups of 6 mice were exposed to an UV-A light of 10 J/cm^2 for 165-195 minutes; positive control animals were exposed for 60 to 90 minutes. Blood samples were taken from toxicokinetic satellite groups of 9 mice/group pre-dose, and 0.5, 1, 3, 7 and 24 hours post-dose. Following sacrifice on D4, ears, auricular lymph nodes and an abdominal skin sample were collected and a lymph node suspension was prepared for comparative cell count.

Clinical observations included half-closed eyes and hypoactivity on D2 in 6/6 HD mice, and D3 in 6/6 MD mice following irradiation for 1-2 hours. A single control F exhibited half-closed eyes following irradiation on D2 to D3; hypoactivity was not observed. Neither erythema of ears or tails, nor change in ear weights was observed in LDK378 mice.

There were no clear differences between irradiated and non-irradiated lymph nodes of treated mice. Lymph node weights of MD and HD mice (both irradiated and non-irradiated) were depressed 20-30% when compared to concurrent controls. In addition, a decrease of 25 to 40% was observed in the lymph node cell count of MD and HD mice (irradiated and non-irradiated groups) when compared to concurrent controls. Changes were not significant (see table below). The reduction in lymph node parameters were not considered to be related to an increase in the phototoxic potential of LDK378.

Table 78: Phototoxicity data in mice following administration of LDK378

Treatment group	Dose-level (mg/kg/day)		Ear weight (mg)#	Ear weight ratio	LN weight (mg)#	LN weight ratio	Total cells	LN cells ratio (Vs Vehicle)	LN cells ratio (with Vs without UVA)
1 (vehicle)	0	**Mean**	**22.16**	**1.00**	**5.64**	**1.00**	**1.21E+07**	**1.00**	-
		SD	*1.28*		*1.88*		*4.19E+06*		
2 (SPX without UVA)	100	**Mean**	**21.90**	**0.99**	**4.99**	**0.88**	**1.09E+07**	**0.90**	**2.08**
		SD	*0.54*		*0.67*		*1.61E+06*		
3 (SPX with UVA)	100	**Mean**	**36.26**	**1.64 *¥**	**9.56**	**1.70 *¥**	**2.27E+07**	**1.87 *¥**	
		SD	*3.14*		*1.05*		*3.60E+06*		
4 (Test item without UVA)	10	**Mean**	**21.64**	**0.98**	**5.14**	**0.91**	**1.05E+07**	**0.87**	**1.05**
		SD	*0.89*		*1.34*		*2.65E+06*		
5 (Test item with UVA)	10	**Mean**	**23.07**	**1.04**	**5.03**	**0.89**	**1.11E+07**	**0.91**	
		SD	*0.80*		*0.62*		*1.77E+06*		
6 (Test item without UVA)	30	**Mean**	**20.68**	**0.93**	**4.63**	**0.82**	**9.00E+06**	**0.74**	**0.80**
		SD	*0.57*		*0.40*		*2.15E+06*		
7 (Test item with UVA)	30	**Mean**	**22.06**	**1.00**	**4.17**	**0.74**	**7.23E+06**	**0.60**	
		SD	*0.81*		*0.45*		*1.23E+06*		
8 (Test item without UVA)	100	**Mean**	**21.65**	**0.98**	**4.23**	**0.75**	**8.79E+06**	**0.72**	**0.92**
		SD	*1.12*		*0.44*		*1.53E+06*		
9 (Test item with UVA)	100	**Mean**	**22.58**	**1.02**	**4.00**	**0.71**	**8.07E+06**	**0.66**	
		SD	*1.32*		*0.71*		*1.45E+06*		

* = statistical significance (p value < 0.05) when compared to the vehicle group
¥ = statistical significance (p value < 0.05) when compared to the corresponding non irradiated group
# = weighted by pairs
LN = lymph node
- = not available
vehicle = 0.5% methylcellulose in drinking water
SPX = sparfloxacin
Test item = LDK378

(Excerpted from Applicant's submission)

Toxicokinetics indicated that LDK378 skin concentrations increased with increasing dose and the level of LDK378 in skin compared to plasma at D4 was ~ one order of magnitude higher irrespective of dose or UVA exposure. (see tables below).

Table 79: Toxicokinetics and exposure comparison of mouse tissue and plasma following dosing with LDK378

Table Mean toxicokinetic parameters of LDK378 in mouse plasma on day 3

dose	group	AUC_{0-24h}	±SE	$AUC_{0-24h/Dose}$	C_{max}	±SE	C_{max}/Dose	t_{max}
10	10	2450	313	245	178	22.9	17.8	3.0
30	11	14300	692	477	875	37.9	29.2	3.0
100	12	47200	1700	472	2660	55.7	26.6	3.0

Dose (mg/kg/day), t_{max} (h), C_{max} (ng/mL), SE C_{max} (ng/mL), C_{max}/Dose (ng/mL)/(mg/kg/day), AUC_{0-24h} (ng*h/mL), SE AUC_{0-24h} (ng*h/mL), $AUC_{0-24/Dose}$ ((ng*h/mL)/(mg/kg/day)), n=3.

Table Exposure comparison of mouse tissue and mouse plasma

group	dose (mg/kg/day)	skin mean concentration (ng/g)	plasma mean concentration (ng/mL)	ratio skin mean concentration / plasma mean concentration
4	10	208	14.9	14.0
5	10 (UVA)	118	7.95	14.8
6	30	1650	247	6.68
7	30 (UVA)	2090	302	6.92
8	100	22400	2810	7.97
9	100 (UVA)	20300	2360	8.60

(Excerpted from Applicant's submission)

Validation criteria:

The test is considered valid if the following criteria are reached:

- for the vehicle control group, ear weight, LN weight and cell counts should fall into the historical data range (when available),
- for the positive control irradiated group:
  - statistically significant difference in ear weight, LN weight and LN cell count when compared to the non irradiated positive control and vehicle groups,
  - an ear weight index > 1.05, a LN weight index > 1.2 and a cell index > 1.3,
  - the ear weight, LN weight and cell indices should fall into the historical data range (when available).

Positivity criteria:

The results of the LLNA endpoints were evaluated according to the following criteria. The following thresholds were derived from an analysis of historical data and comparison to published results (Ulrich P *et al.* 2001): ear weight index: 1.05, LN weight: 1.2, LN cell count: 1.3.

1. Values which exceed these thresholds are considered positive:
- if no statistical significance was obtained, but a clear concentration-dependence is visible,
- if a statistically significant increase in one of the parameters (ear weight or LN weight and cellularity, respectively) occurs and a clear concentration-dependence can be derived.

2. Values which are below these thresholds are considered positive:
- if a statistical significance occurs in one of the parameters together with a clear concentration-dependence.

3. Values are considered negative:
- in case of being below the thresholds and without a statistical significance,
- in case of being below the thresholds, with a statistical significance, but without a clear concentration-dependence,
- in case of being above the thresholds, without statistical significance and without a clear concentration-dependence.

For phototoxicity testing, each dose group with UVA light exposure has to be compared with the concurrent non-irradiated dose.

11 Integrated Summary and Safety Evaluation

Pharmacology

The pharmacologic activity of LDK378 (certinib) was examined in a series of in vitro and in vivo assays. Against a panel of 36 recombinant human protein kinases, LDK378 was the most selective for anaplastic lymphoma kinase (ALK), exhibiting an IC_{50} value of 0.15 nM. ALK is a receptor tyrosine kinase of the insulin receptor superfamily, which also includes the InsR, IGF-1R, and ROS1. In the same experiment LDK378 also inhibited InsR and IGF-1R with IC_{50} values of 7 nM and 8 nM, respectively. Thus, LDK378 was approximately 50-fold more selective for ALK than for other members of the insulin receptor superfamily included in the kinase panel. LDK378 was greater than 700-fold more selective for ALK than for the remaining 33 kinases. In in vitro proliferation studies, LDK378 inhibited the in vitro proliferation of Ba/F3 cells stably expressing the NPM-ALK and EML4-ALK fusion proteins with IC_{50} values of 35 nM and 27 nM, respectively. In contrast, LDK378 was relatively inactive against Ba/F3 cells stably expressing the irrelevant control constructs TEL-FES or TEL-FER. LDK378 also inhibited the in vitro proliferation of ALCL Karpas299 cells expressing NPM-ALK, NSCLC NCI-H2228 cells expressing EML4-ALK, and neuroblastoma NB-1 cells with ALK amplification (IC_{50} values of 45 nM, 11 nM, and 24 nM, respectively). LDK378 caused a concentration-dependent decrease in phosphorylated NPM-ALK (IC_{50} = 46 nM) and its downstream mediator STAT3 (IC_{50} = 150 nM) in Karpas299 cells with no change in total ALK and STAT3 protein levels, correlating with its antiproliferative activity. Further, LDK378 sensitivity correlated with the presence of ALK translocations, but not total ALK expression, in a panel of 95 human NSCLC cell lines.

Although LDK378 inhibited recombinant human InsR and IGF-1R in vitro, single pharmacodynamically active doses of LDK378 did not significantly inhibit the IGF-1R/AKT signaling pathway in NIH3T3 xenografts stably expressing human IGF-1R and IGF-2. Further, LDK378 did not result in off-target effects on glucose metabolism or insulin resistance in adult male wild-type C57BL/6J mice. Despite these results, hyperglycemia has been observed both in longer term animal studies and clinically in patients treated with ceritinib, consistent with observations of hyperglycemia in patients treated with IGF-1R inhibitors. The potential activity of LDK378 on these additional members of the ALK family of kinases was further examined in Ba/F3 cells stably transfected with a panel of 38 constitutively activated kinases. LDK378 inhibited proliferation of Ba/F3-TEL-ALK-Q1 cells with an IC_{50} of 56 nM. LDK378 also exhibited anti-proliferative activity against Ba/F3 cells transduced with constitutively activated TEL-ROS1, TEL-IGF-1R, and TEL-InsR (IC_{50} values of 180 nM, 220 nM, and 400 nM, respectively). The inhibitory activity of LDK378 against recombinant human ROS1 was not assessed in previous biochemical assays as, according to the Applicant, a ROS1 biochemical assay was not available. Thus, under the conditions of this assay, LDK378 was only approximately 3- to 7-fold more selective for ALK than the other members of the insulin receptor superfamily.

The in vivo efficacy of LDK378 was examined in rodent models of human NSCLC and ALCL expressing EML4-ALK and NPM-ALK fusions, respectively. Daily dosing with LDK378 for 14 days at dose levels of 25 and 50 mg/kg resulted in dose-dependent inhibition of NSCLC H2228 and ALCL Karpas299 xenograft growth in female SCID mice and nude rats, generally at exposures that are achievable in the clinic at the recommended dose of ceritinib. The activity of LDK378 was also compared to crizotinib using SCID mice implanted with the H2228 NSCLC tumor cell line. Daily dosing for 14 days with 25 and 50 mg/kg LDK378 or 100 mg/kg crizotinib

resulted in H2228 tumor regression in SCID mice; however, treatment with LDK378 resulted in a more sustained anti-tumor response than that seen with crizotinib.

Consistent with the proposed indication of ceritinib for the treatment of patients with NSCLC who have (b) (4) with crizotinib, the activity of LDK378 in in vitro and in vivo models of crizotinib resistance was also evaluated. Compared to crizotinib, LDK378 exhibited stronger anti-proliferative activity against Ba/F3 cell lines transduced with not only wild-type EML4-ALK but also with EML4-ALK expressing secondary mutations in the ALK kinase domain associated with acquired crizotinib resistance (I1171T, L1196M, C1156Y, S1206A, G1269S). Daily dosing with LDK378 resulted in dose-dependent tumor inhibition in H2228 xenograft models of crizotinib resistance, whereas dosing with 100 mg/kg of crizotinib was ineffective, as expected. Daily dosing with 25 mg/kg LDK378 induced statistically significant tumor inhibition in H2228 xenografts bearing an ALK I1171T mutation at exposures approximately 2.5-fold lower than those achieved in humans at the recommended dose (based on AUC); at the 50 mg/kg dose level (exposures approximately equal to those achieved in humans at the recommended dose) LDK378 induced complete tumor regression in this model. Daily dosing with ≥ 50 mg/kg LDK378 resulted in statistically significant tumor inhibition in H2228 xenografts bearing an ALK C1156Y mutation, and 100 mg/kg induced complete tumor regression. LDK378 plasma exposures at 50 and 100 mg/kg were approximately 1.3 and 2.5 fold higher than those achieved in humans at the recommended dose, respectively. LDK378 also exhibited anti-tumor activity in crizotinib-resistant H2228 xenografts whose mechanism of resistance was unknown. Taken together, these data suggest that LDK378 may provide therapeutic benefit for patients whose tumors acquire resistance to crizotinib; higher LDK378 exposures may be necessary to inhibit crizotinib-resistant tumors.

Single dose administration PK/PD studies were conducted with LDK378 in an orthotopic Karpas299 mouse model of human ALCL. In this model LDK378 preferentially distributed to tumor tissue versus plasma. Single doses of ≥ 25 mg/kg LDK378 (corresponding to mean tumor Cmax and $AUC_{0\text{-}120h}$ values of ≥ 37,255 nM and 74,254 h*nM, respectively) resulted in ≥ 70% inhibition of pSTAT3. Since daily dosing with 25 mg/kg LDK378 resulted in significant regression of Karpas299 xenografts in SCID mice, the Applicant estimates than an approximately 70% reduction in tumor levels of pSTAT3 at Y705 may be necessary for tumor regression.

The off-target activity of LDK378 was evaluated using in vitro safety pharmacology profiling panels. The Cmax of ceritinib determined in patients at the recommended dose of 750 mg was ~1.8 μM. LDK378 targets with IC_{50} values < 1.8 μM included the histamine H2 receptor (H_2), VMAT2, opiate kappa receptor, melanocortin 3 receptor (MC_3), melanocortin 4 receptor (MC_4), potassium channel (K_A), adenosine 3 receptor (Ad_3), tachykinin NK1 receptor (NK_1), dopamine 2 receptor (long form) (D_2), and norepinephrine transporter (NET). With the exception of H_2, LDK378 did not inhibit these targets by 50% at clinically-relevant free drug concentrations. In follow-up functional studies LDK378 antagonized Ad_3 (IC_{50} of 8.3 μM), which mediates sustained cardioprotection. LDK378 also antagonized MC_4 (35% at 10 μM), NK_1 (79% at 30 μM), and $Ca2^+$(L)DHP (IC_{50} of 21 μM) and showed agonist activity for D_2 (EC_{50} of 6 μM), NK_1 (35% at 30 μM), and the somatostatin sst2 receptor (147% at 30 μM) at concentrations 118- to 592-fold higher than the approximate free drug concentration achieved in the clinic at the recommended dose. While LDK378 did show inhibition of H2 in a biochemical assay with an IC_{50} of 170 nM, the drug did not exhibit agonist or antagonist activity on H_2 at concentrations up to 10 μM in the follow-up assay. Thus, LDK378 is unlikely to exhibit functional agonism or antagonism of any of these targets at clinically achievable concentrations.

Safety Pharmacology

Both in vitro and in vivo safety pharmacology studies were conducted to assess the effects of LDK378 on cardiovascular, behavioral, general physiological, and respiratory function. LDK378 significantly inhibited hERG channel activity when tested at concentrations of 0.3, 0.4, 1, and 2.4 µM using manual patch-clamp electrophysiology on HEK293 cells transfected with hERG cDNA. The IC_{50} for the inhibitory effect of LDK378 on hERG potassium current was 0.4 µM. In monkeys administered a single dose of LDK378 at dose levels of 10 or 30 mg/kg there was little effect on most cardiovascular parameters, although 10-19 hours following administration of 100 mg/kg, one of four animals exhibited QT/QTc prolongation of 14-44 ms above predose baseline. QT prolongation has been noted clinically. No drug-related effects were observed on blood pressure, heart rate or body temperature. In an earlier non-GLP telemetry study in monkeys, no electrocardiography changes were observed following a single dose of 250 mg/kg LDK378. There were no adverse cardiovascular findings observed in rats or monkeys administered LDK378 for up to 13 weeks in general toxicology studies at exposures equal to or greater than 0.5 or 1.5-fold, respectively, the human exposure by AUC.

In a functional observational battery (FOB) in rats, significant behavioral and physiological changes were not observed following single doses of 100 mg/kg LDK378. In this same study, transient but significantly higher breaths per minute (BPM) (~20%) were observed from 35-60 minutes following single doses of 100 mg/kg LDK378.

Genotoxic and Non-genotoxic impurities

Seven process impurities were assayed by (b) (4) screening and predicted to be genotoxic, but were controlled to acceptable levels for a drug intended for the treatment of patients with cancer. Six of these impurities were monitored in development batches of drug substance and found to be present at levels (b) (4) a level which corresponds to a clinical intake of (b) (4) in patients treated at the recommended dose of ceritinib. Due to its instability, the 7th potential genotoxic impurity, (b) (4) The (b) (4) was not identified as potentially genotoxic according to (b) (4) screening. This (b) (4) was monitored in development batches of drug substance at (b) (4) which corresponds to a clinical intake of (b) (4) based on the recommended clinical dose of 750 mg/day.

Individual non-genotoxic impurities specified in LDK378, impurities (b) (4) were acceptably qualified at the proposed specification limit of NMT (b) (4) in the 4-week rat toxicology study # 0970416. The individual proposed release/stability acceptability criteria for all other specified and unspecified impurities is NMT (b) (4) which is below the ICH Q3B(R2) qualification threshold.

Pharmacokinetics

The Applicant evaluated the in vivo pharmacokinetics of LDK378 following oral and IV administration to monkeys, rats, and mice. An oral dose of LDK378 was moderately absorbed in monkeys and rats, and exhibited a slow rate of absorption in all three species. Oral bioavailability was moderate in rats, monkeys, and mice (~43-58%). According to the Applicant, the absolute bioavailability of LDK378 has not been determined in humans. LDK378 exhibited a relatively long terminal half-life in monkeys and rats following oral (~12-16 hrs) and IV administration (~10-29 hrs), which was less than that observed in humans (~40 hrs). Following IV administration, LDK378 exhibited low to moderate clearance in rats, monkeys, and mice. The

steady-state volume of distribution of LDK378 was high in all three species (6.5-20 L/kg), indicating extensive distribution into tissues.

In keeping with the high calculated volume of distribution, in vivo studies demonstrated that LDK378 was widely distributed to most rat tissues following a single 25 mg/kg oral dose of [^{14}C]LDK378 to LEH pigmented rats. Most LDK378 tissue concentrations were higher than those observed in blood, except for the brain, epididymis, eye, seminal vesicles, spinal cord, and testis (tissue-to-blood ratio based on Cmax < 1). Tissue-to-blood ratios based on AUC_{inf} were ≤ 1 for the brain, eye, spinal cord, and white fat. LDK378 concentrations were still high and/or quantifiable in the Harderian gland, pituitary gland, uveal tract, epididymis, kidney, liver, lung, skin, spleen, and testis 168 hrs post-dose; later time points were not evaluated. LDK378 concentrations were the highest in the colon wall and the small intestine wall, and high tissue-to-blood ratios were observed in the GI tract, liver, and lungs, consistent with observations of GI toxicity, increased ALT and AST, and pulmonary toxicity in the clinic. Notably, there was some distribution of LDK378 to the brain. LDK378 concentrations in the brain peaked at 4 hrs (Cmax = 0.07 nmol/g) but were not distinguishable from the surrounding tissues and/or background by 72 hrs post-dose. The AUC_{inf} and Cmax of LDK378 in the brain were approximately 15% and 7% of that in blood, respectively. In a separate study, the $AUC_{(0\text{-}7h)}$ and Cmax of LDK378 in the brain following a single oral dose of 20 mg/kg LDK378 to mice were 12% and 13% of that in plasma, respectively. Together these data indicate that LDK378 is able to cross the blood-brain barrier, at least at low levels. Further, LDK378 exhibited high distribution to the pancreas, potentially associated with symptoms of acute pancreatitis observed in the clinic and findings in the animal toxicology studies. LDK378 also showed significant retention to melanin in the uveal tract.

The Applicant evaluated [^{14}C]LDK378 distribution to blood cells, serum, and plasma proteins in the rat, dog, monkey and human. [^{14}C]LDK378 distributed slightly more to red blood cells than to plasma in vitro. The blood:plasma concentration ratio of [^{14}C]LDK378 was highest in the monkey (2.59), followed by the rat (1.72), dog (1.38), and human (1.35). LDK378 exhibited strong plasma protein binding (94.7-98.5%) in all tested species. The anticoagulant heparin did not significantly affect [^{14}C]LDK378 protein binding. 97.2% of LDK378 was bound to human plasma protein, resulting in a free drug concentration of approximately 50.7 nM in humans at the recommended dose of LDK378. Of the 36 recombinant human kinases tested, LDK378 only inhibited ALK, InsR, and IGF-1R at free drug concentrations achievable in the clinic at the recommended dose of LDK378. LDK378-induced inhibition of ROS1 was only assessed in cellular assays; however, LDK378-mediated inhibition of ROS1 dependent cell proliferation occurred at lower concentrations than that of InsR or IGF-1R indicating that ROS1 is another potentially relevant target of the drug in the clinic.

The Applicant also evaluated the in vitro and in vivo metabolism of LDK378. Following a single IV or oral dose of [^{14}C]LDK378 to monkeys or rats (intact and bile duct-cannulated), unchanged LDK378 was the major circulating component in plasma regardless of the route of administration, accounting for ~84-100% of the total drug-related AUC. Eight metabolites were identified in monkey plasma, but they accounted for <4% of the radioactivity AUC following IV and oral dosing. Unchanged LDK378 was also the major component in feces, accounting for ~60-80% of the administered oral dose and ~55-83% of the administered IV dose in intact rats and monkeys. In addition, unchanged LDK378 was the major component in the feces and bile of bile duct-cannulated rats. Total metabolites identified in rat and monkey feces and bile were present at <9% and <11% of the administered oral and IV doses, respectively. The primary biotransformation reactions of LDK378 observed in these studies included mono-oxygenation and O- and S-dealkylation; secondary biotransformation reactions of the primary

biotransformation products included additional di-oxygenation, dehydrogenation, glucuronidation, and sulfation. Further, a thiol conjugate of O-dealkylated LDK378 was also observed.

The in vitro metabolism of [^{14}C]LDK378 was evaluated using rat, monkey, and human hepatocytes. Monkey hepatocytes metabolized LDK378 the most, followed by human hepatocytes; no significant metabolism was observed in rat hepatocytes. Unchanged LDK378 accounted for 32% and 62% of radioactivity in monkey hepatocytes following 24 hrs of incubation with 2.5 and 12.5 µM [^{14}C]LDK378, respectively. Unchanged LDK378 accounted for the majority of radioactivity in human hepatocytes (81.4 and 90.8% at 2.5 and 12.5 µM, respectively). Only four metabolites were identified in human hepatocytes (M27.5/M27.6, M32.9, M33.4, and M37.3). The mean contribution to the radioactivity in the sample was ≤ 9% for each metabolite, and all of them were also found in monkey hepatocytes. M27.5 (hydroxylated LDK378; ≤9%) and M37.3 (loss of sulfone moiety; ≤2%) were identified in human hepatocytes but not observed in the in vivo animal studies or in human plasma/feces. In general similar biotransformation reactions were observed in vitro and in vivo, except for the loss of the sulfonylpropyl group from LDK378 to form M37.3 in human and monkey hepatocytes.

The potential for radiolabelled LDK378 to bind covalently to protein when incubated with cryopreserved rat and human liver microsomes or hepatocytes was also assessed. After incubation with liver microsomes in the presence of NADPH, the levels of non-extractable LDK378 in rat and human microsomes were ~34- and 12.4-fold lower, respectively, than that of a reference compound. Apparent covalent binding of LDK378 appeared to be relatively similar between rats and humans in the presence of NADPH. Overall, LDK378 had a low potential for covalent binding in rat and human microsomes. After a four hour incubation with rat or human hepatocytes, the levels of non-extractable LDK378 increased with time, but remained ~1.8- or 1.5-fold lower than that of the reference compound. The Applicant stated that these results only provide an indication of potential covalent binding, not definitive proof.

The Applicant also evaluated in vivo excretion of LDK378. Following a single IV or oral dose of [^{14}C]LDK378 to monkeys and rats (intact and bile duct-cannulated), excretion of radioactivity was primarily through the fecal route. Fecal excretion accounted for ~90-100% of the radioactivity dose in monkeys and intact rats, whereas urinary excretion was minor (<1%). These nonclinical results were consistent with findings in the clinic, where 91% of the radioactivity dose in humans was eliminated in the feces, and only 1.3% was eliminated in the urine. Following IV administration of [^{14}C]LDK378 to bile-duct cannulated rats, excretion into the urine, feces, and bile was <1%, 29.8%, and 65.4% of the radioactivity dose, suggesting that fecal excretion was the result of both biliary and GI excretion. Following oral administration of [^{14}C]LDK378 to bile-duct cannulated rats, excretion into the urine, feces, and bile was 1%, 65%, and 24.3% of the radioactivity dose. Thus, hepatic clearance through biliary excretion is an important route of elimination for LDK378 in both humans and animals.

General Toxicology

Target organs of LDK378-mediated toxicity were identified in rats and monkeys dosed for up to 13 weeks, and included the pancreas, biliopancreatic ducts, bile ducts, gastrointestinal tract and liver. Target organs and dose limiting toxicities were generally consistent for shorter and longer periods of dosing.

LDK378 was administered to rats at dose levels of 7.5, 25, and 75/50 mg/kg for 4 weeks and 3, 10 and 30 mg/kg for 13 weeks. Four- and 13-week studies in monkeys were both conducted at

dose levels of 3, 10 and 30 mg/kg LDK378. Marked pancreatic atrophy and inflammation was observed following 4 weeks of LDK378 administration in monkeys and rats at dose levels of 30 and 75/50 mg/kg, respectively (0.15 and 1.5-fold, respectively, the human exposure by AUC at the recommended dose). Amylase and lipase were not measured at 4 weeks, but following 13 weeks of treatment, amylase was elevated 12-25% in HD rats following dosing and during recovery; no changes in lipase were observed in rats. In male monkeys, lipase was elevated up to 2-fold compared to controls during dosing and remained elevated 35% following recovery at the HD; this is consistent with the higher drug exposures observed in males. Changes in lipase were unremarkable in female monkeys, and there were no changes in amylase in either gender. In humans, increased lipase has been reported. Consistent with clinical findings of hyperglycemia, insulin levels were increased 2-3-fold in monkeys treated for 13 weeks.

Erosion, degeneration/necrosis, inflammation, hyperplasia, dilatation, and vacuolation of the biliopancreatic duct and inflammation and dilatation of the bile duct were observed following 4 and 13 weeks of LDK378 administration in rats at exposures ≥ 5% of human exposure by AUC at the recommended dose; the same findings were observed following up to 8 weeks of recovery at a similar incidence. Bile duct hemorrhage and inflammation (cystic bile duct, hepatic bile duct, and common bile duct) were also exhibited in monkeys dosed for 13 weeks at exposures equal to or greater than 50% the human exposure by AUC. Necrosis, hyperplasia, and hemorrhage of the duodenum was observed at 13 weeks in monkeys at 50% the clinical exposure, and in rats at an exposure similar to that observed clinically. Findings in both species were generally more pronounced in males, which is consistent with the observed higher drug exposures. In both species, the incidence and severity of findings was increased at the high dose levels, consistent with the increase in LDK378 exposure with increases in dose level; drug accumulation was observed following 13 weeks of repeat dosing.

In general, bile duct findings were more pronounced in rats compared to monkeys which may be due to the higher comparative plasma exposures in rats, the physiological differences in bile duct physiology between the species, and the differences in drug metabolism between species. A higher degree of LDK378 metabolism occurred in monkeys, with a greater percentage of parent compound observed in the bile of rats and humans.

LDK378-related hepatotoxic changes were generally reflective of treatment-related systemic toxicity in rats and monkeys and characterized by significant elevations in liver enzymes; similar increases have been noted clinically. In 4-week studies in rats, AST and ALT parameters were elevated 2-4 fold compared to controls at exposures that approximate clinical exposures at the recommended dose of 750 mg. Transaminase elevation has been observed in patients administered ceritinib.

Additional target sites for LDK378 in rats included the lungs, with phospholipidosis following 4 weeks of administration at the high dose of 75/50 mg/kg (exposure ~1.5-fold the clinical exposure at the recommended dose), and lung macrophage aggregates following 13 weeks of administration at 30 mg/kg (exposure similar to that observed clinically at the recommended dose). These findings correlate with pneumonitis and interstitial lung disease observed clinically.

Thyroid indices (TSH, T3, and T4) were increased in rats following 13 weeks of LDK378 administration at exposures ≥ 5% of human exposure, although these findings were not accompanied by organ weight or histologic changes. Thyroid weight changes were observed in monkeys administered LDK378 for 4 weeks.

Genetic Toxicology

The parent drug LDK378 was not mutagenic *in vitro* in the bacterial reverse mutation (Ames) assay but induced numerical aberrations (aneugenic) in the *in vitro* cytogenetic assay using human lymphocytes, and micronuclei in the *in vitro* micronucleus test using TK6 cells. LDK378 was not clastogenic in the *in vivo* mouse micronucleus assay.

Carcinogenicity

Carcinogenicity studies with LDK378 have not been conducted.

Reproductive and Developmental Toxicology

Fertility/early embryonic development studies were not conducted with LDK378. There were no adverse effects on male or female reproductive organs in general toxicology studies conducted in monkeys and rats at exposures equal to or greater than 0.5 and 1.5-fold, respectively, the human exposure by AUC.

Administration of LDK378 at dose levels of 1, 10, or 50 mg/kg to pregnant rats during the period of organogenesis resulted in dose-related skeletal anomalies at the high dose level (less than 50% the human exposure by AUC at the recommended dose). Findings included delayed ossifications and skeletal variations. LDK378 did not induce embryolethality or fetotoxicity at doses tested. Concentrations of LDK378 in fetal plasma were low, less than 10% of the maternal Cmax.

In rabbits, administration of LDK378 at dose levels above 35 mg/kg resulted in clear signs of maternal toxicity and abortion. In pregnant rabbits administered LDK378 daily during organogenesis at dose levels of 2, 10, or 25 mg/kg, dose-related skeletal and visceral anomalies, including incomplete ossification, absent or malpositioned gallbladder and retroesophageal subclavian cardiac artery were observed at doses ≥ 10mg/kg/day (approximately 13% of the human exposure by AUC at the recommended dose). Pregnancy category D was recommended. The half-life of LDK378 is ~41 hours at the recommended clinical dose; it is advised that females of reproductive potential use effective contraception during treatment with ceritinib and for up to 2 weeks following cessation of treatment.

Special Toxicology

LDK378 was found to be phototoxic *in vitro* in Balb/c 3T3 fibroblast cells, and showed association with melanin in distribution studies, but was not considered to have phototoxic potential following administration of mice when treated with LDK378 up to 100 mg/kg for 3 days.

12 Appendix/Attachments

None

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

/s/

MARGARET E BROWER
03/25/2014

EMILY M FOX
03/25/2014

WHITNEY S HELMS
03/25/2014

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 205,755 **Applicant: Novartis** **Stamp Date: December 24, 2013**

Drug Name: LDK378 **NDA Type: Breakthrough/ Priority**

On **initial** overview of the NDA 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		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
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.)			Potential impurity issues have been identified by P/T and should be reviewed by CMC
11	Has the applicant addressed any abuse potential issues in the submission?			Not Applicable
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not Applicable

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

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

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

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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

/s/

MARGARET E BROWER
01/21/2014

WHITNEY S HELMS
01/21/2014