CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

022405Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Vandetanib

Date: March 25, 2011
To: File for NDA 22405
From: John K. Leighton, PhD, DABT
Associate Director for Pharmacology/Toxicology
Office of Oncology Drug Products

I have examined pharmacology/toxicology supporting review and labeling provided by Drs. Gehrke and Dorsam and supervisory memorandum provided by Dr. Verbois. I concur with their conclusions that vandetanib may be approved for the proposed indication with the carcinogenicity studies to be provided as described in the Action Letter.

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/s/

JOHN K LEIGHTON
03/25/2011

MEMORANDUM

Date: March 25, 2011
From: S. Leigh Verbois, Ph.D.
Supervisory Pharmacologist
Division of Drug Oncology Products
To: File for NDA #022405
Vandetinib
Re: Approvability of Pharmacology and Toxicology

Non-clinical studies that investigated the pharmacology and toxicology of vandetinib provided to support NDA 022405 for the treatment of patients with unresectable locally advanced or metastatic medullary thyroid cancer were reviewed in detail by Brenda Gehrke, Ph.D., and Robert Dorsam, Ph.D. The supporting information included studies of orally administered vandetinib that investigated the drug's pharmacology, toxicokinetics and ADME, safety pharmacology, general toxicology (rat and dog), and genetic toxicity (*in vivo* and *in vitro*). Reproductive and developmental toxicology studies were conducted in rats to assess the effects of vandetanib on fertility, embryofetal development, and pre- and post-natal development. The studies cited in the review consist primarily of original research conducted by the applicant.

The pharmacology studies submitted to the NDA demonstrate that vandetinib is a tyrosine kinase inhibitor which binds multiple receptor tyrosine kinases including VEGF, EGF, RET, BRK, TIE2, and members of EPH and SRC family of kinases. These kinases were inhibited at concentration lower than levels than that achieved in the plasma clinically. Based on this, the pharmacological classification of vandetinib is a kinase inhibitor. Drug induced toxicity, including gastrointestinal, cardiovascular, toxicity, skin, spleen, and teeth and bone (in rats) toxicity were observed non-clinically. These findings were well characterized non-clinically. Cardiovascular toxicity was highly discussed throughout the review process and manifested both in toxicology studies as well as safety pharmacology studies as prolongation of QT interval.

Vandetinib was not mutagenic or clastogenic when tested in the Ames assay or the *in vitro* cytogenetic assay using human lymphocytes and an *in vivo* rat micronucleus assay, respectively. Although carcinogencity studies have not been initiated to date, evidence of masses was detected in the repeat dose rat study in multiple organs. Masses were detected at both clinical observation and gross pathology. Given the proposed patient indication which has an extended life expectancy, studies are required to assess the risk of carcinogenicity.

Vandetinib may impair fertility in males and females. In a fertility study in male rats, vandetanib had no effect on copulation or fertility rate at doses approximately 0.03 to 0.38 times the AUC in patients with cancer at the recommended human dose of 300 mg/day. However, there was a slight decrease in the number of live embryos at 20

mg/kg/day (120 mg/m^2) and an increase in preimplantation loss at $\geq$5 mg/kg/day (30 mg/m^2). In a female fertility study, there was a trend towards increased estrus cycle irregularity, a slight reduction in pregnancy incidence and an increase in implantation loss. In a repeat-dose toxicity study in rats, there was a decrease in the number of corpora lutea in the ovaries of rats administered 75 mg/kg/day vandetanib (450 mg/m^2; approximately 1.8 times the AUC in patients with cancer at the recommended human dose) for 1 month.

Vandetanib is embryotoxic, fetotoxic, and teratogenic to rats, at exposures equivalent to or lower than those expected at the recommended human dose of 300 mg/day. When vandetanib was administered to female rats prior to mating and through the first week of pregnancy, there were increases in pre-implantation loss, post-implantation loss and early embryolethality resulting in a significant reduction in the number of live embryos. This dose administered to rats during organogenesis, caused an increase in post-implantation loss including embryofetal death. Vandetanib caused total litter loss when administered at a dose of 25 mg/kg/day (150 mg/m^2/day) during organogenesis until expected parturition. When administered during organogenesis, vandetanib doses of greater than 1 mg/kg/day (greater than 0.03 the C_{max} in patients with cancer at the recommended human dose), caused malformations of the heart vessels and delayed ossification of the skull, vertebrae and sternum, indicating delayed fetal development. A no effect level for these malformations was not identified in this study.

In a rat pre- and post-natal development study, at doses producing maternal toxicity (1 and 10 mg/kg/day; 6 and 60 mg/m^2/day) during gestation and/or lactation, vandetanib, decreased pup survival, and/or reduced post-natal pup growth. Reduced post-natal pup growth was associated with a delay in physical development. In the pre- and post-natal development study, it was determined that vandetinib was excreted in rat milk and found in the plasma of pups. Vandetinib was found in the plasma of pups pre-dosing and eight hours post-dosing indicating the persistence of vandetinib in plasma of dams, persistant excretion into breast milk or residence in breast milk or slow excretion of pups. Given the long half life of the drug it likely the first and last. Because the potential benefit from the use of the vandetinib in pregnant women in this patient population may outweigh the potential risk to the developing fetus, Pregnancy Category D is recommended for this patient population.

Recommendations: I concur with Drs. Gehrke's and Dorsam's conclusion that pharmacology and toxicology data support the approval of NDA 022405 for vandetinib. There are no outstanding nonclinical issues related to the approval of vandetinib for the proposed indication.

The following information should be conveyed in the approval letter regarding the PMRs:

1. To evaluate the potential for a serious risk of carcinogenicity, it is necessary to assess the potential for carcinogenicity by conducting a long-term (2 year) rodent carcinogenicity study in the rat. Submit the carcinogenicity protocol for a Special Protocol Assessment prior to initiating the study. Notify the Agency in writing at least 30

days prior to submission of the study that a carcinogenicity protocol will be arriving. Submit the carcinogenicity protocol and questions regarding the protocol with sufficient time prior to the anticipated initiation of the study to allow for meaningful discourse with the Agency and resolution of any issues before study initiation. Clearly mark in bold black letters as a REQUEST FOR SPECIAL PROTOCOL ASSESSMENT. Once the study has been completed, submit the final study report and a prior approval labeling supplement containing proposed labeling to update package inserts to reflect study findings.

2. To evaluate the potential for a serious risk of carcinogenicity, it is necessary to assess the potential for carcinogenicity by conducting a rodent carcinogenicity study in the mouse. Submit the carcinogenicity protocol for a Special Protocol Assessment prior to initiating the study. Notify the Agency in writing at least 30 days prior to submission of the study that a carcinogenicity protocol will be arriving. Submit the carcinogenicity protocol and questions regarding the protocol with sufficient time prior to the anticipated initiation of the study to allow for meaningful discourse with the Agency and resolution of any issues before study initiation. Clearly mark in bold black letters as a REQUEST FOR SPECIAL PROTOCOL ASSESSMENT. Once the study has been completed, submit the final study report and a prior approval labeling supplement containing proposed labeling to update package inserts to reflect study findings.

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/s/

SANDI L VERBOIS
03/25/2011

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	022405
Supporting document/s:	0000
Applicant's letter date:	July 7, 2010
CDER stamp date:	July 7, 2010
Product:	Vandetanib
Indication:	Unresectable locally advanced or metastatic medullary thyroid cancer
Applicant:	IPR Pharmaceuticals INC C/O AstraZeneca Pharmaceuticals LP
Review Division:	Division of Oncology Drug Products (HFD-150)
Reviewers:	Brenda J. Gehrke, Ph.D.
Supervisor/Team Leader:	S. Leigh Verbois, Ph.D. and Robert T. Dorsam, Ph.D. (Acting team leader)
Division Director:	Robert Justice, M.D.
Project Manager:	Lisa Skarupa

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Any information or data necessary for approval of NDA 022405 that IPR Pharmaceuticals INC C/O AstraZeneca Pharmaceuticals LP 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 022405.

Labeling Review for vandetanib tablets (NDA 020405):

The Sponsor Proposes:	We recommend:	Justification:
Highlights:		
Indications and Usage (b) (4)	**Indications and Usage** Vandetanib is a kinase inhibitor indicated for the treatment of symptomatic or progressive medullary thyroid cancer in patients with unresectable locally advanced or metastatic disease. Use of vandetanib in patients with indolent, asymptomatic or slowly progressing disease should be carefully considered because of the treatment related risks of vandetanib.	(b) (4)
Warnings and Precautions (b) (4)	**Warnings and Precautions** Vandetanib can cause fetal harm when administered to a pregnant woman. Women should be advised to avoid pregnancy while receiving vandetanib and for four months following treatment (5.14, 8.1).	
5. Warnings and Precautions		
5.9 Use in Pregnancy **Pregnancy Category D** (b) (4)	**5.14 Use in Pregnancy** Vandetanib can cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies in pregnant women using vandetanib. In nonclinical studies in rats, vandetanib was embryotoxic, fetotoxic, and teratogenic, at exposures equivalent to or lower than those expected at the recommended human dose of 300 mg/day. As expected from its pharmacological actions, vandetanib has shown significant effects on all stages of female	

The Sponsor Proposes:	We recommend:	Justification:
(b) (4)	reproduction in rats. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant during treatment with vandetanib. Women should be advised that they must use effective contraception to prevent pregnancy during treatment and for at least four months following the last dose of vandetanib [see *Use in Specific Populations, (8.1)*].	(b) (4)
	8. Use in Specific Populations	
	8.1 Pregnancy Pregnancy Category D *[see Warnings and Precautions (5.14)]*. Vandetanib can cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies of vandetanib in pregnant women. Vandetanib is embryotoxic, fetotoxic, and teratogenic to rats, at exposures equivalent to or lower than those expected at the recommended human dose of 300 mg/day. When vandetanib was administered to female rats prior to mating and through the first week of pregnancy, there were increases in pre-implantation loss and post-implantation loss resulting in a significant reduction in the number of live embryos. This dose administered to rats during organogenesis, caused an increase in post-implantation loss including embryofetal death. Vandetanib caused total litter loss when administered at a dose of 25 mg/kg/day	

The Sponsor Proposes:	We recommend:	Justification:
(b) (4)	during organogenesis until expected parturition. When administered during organogenesis, vandetanib doses of 1, 10 and 25 mg/kg/day (approximately 0.03, 0.4, and 1.0 times respectively, the C_{max} in patients with cancer at the recommended human dose) caused malformations of the heart vessels and delayed ossification of the skull, vertebrae and sternum, indicating delayed fetal development. A no effect level for these malformations was not identified in this study. In a rat pre- and post-natal development study, at doses producing maternal toxicity (1 and 10 mg/kg/day) during gestation and/or lactation, vandetanib decreased pup survival and/or reduced post-natal pup growth. Reduced post-natal pup growth was associated with a delay in physical development. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid pregnancy while taking vandetanib and for at least four months following the last dose of vandetanib.	(b) (4)
(b) (4)	**8.3 Nursing Mothers** In nonclinical studies, vandetanib was excreted in rat milk and found in plasma of pups following dosing to lactating rats. Vandetanib transfer in breast milk resulted in relatively constant exposure in pups due to the long half-life of the drug. It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from vandetanib, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into	(b) (4)

The Sponsor Proposes:	We recommend:	Justification:
	account the importance of the drug to the mother.	
	12. Clinical Pharmacology	
(b) (4)	**12.1 Mechanism of Action** Vandetanib is a tyrosine kinase inhibitor. *In vitro* studies have shown that vandetanib inhibits the activity of tyrosine kinases including members of the epidermal growth factor receptor (EGFR) family, vascular endothelial cell growth factor (VEGF) receptors, rearranged during transfection (RET), protein tyrosine kinase 6 (BRK), TIE2, members of the EPH receptors kinase family, and members of the Src family of tyrosine kinases. Vandetanib inhibits endothelial cell migration, proliferation, survival and new blood vessel formation in *in vitro* models of angiogenesis. Vandetanib inhibits EGFR-dependent cell survival *in vitro*. In addition, vandetanib inhibits epidermal growth factor (EGF)-stimulated receptor tyrosine kinase phosphorylation in tumor cells and endothelial cells and VEGF-stimulated tyrosine kinase phosphorylation in endothelial cells. *In vivo* vandetanib administration reduced tumor cell-induced angiogenesis, tumor vessel permeability, and inhibited tumor growth and metastasis in mouse models of cancer. There is no evidence of a relationship between RET mutations and efficacy with vandetanib.	(b) (4)

The Sponsor Proposes:	We recommend:	Justification:
		(b) (4)
(b) (4)	Deleted section.	
13. Nonclinical Toxicology		
13.1 Carcinogenesis, Mutagenesis, Impairment	**13.1 Carcinogenesis, Mutagenesis, Impairment**	

The Sponsor Proposes:	We recommend:	Justification:
of Fertility (b) (4)	**of Fertility** Carcinogenicity studies have not been conducted with vandetanib. Vandetanib was not mutagenic *in vitro* in the bacterial reverse mutation (Ames) assay and was not clastogenic in both the *in vitro* cytogenetic assay using human lymphocytes or in the *in vivo* rat micronucleus assay. Based on nonclinical findings, male and female fertility may be impaired by treatment with vandetanib. In a fertility study in male rats, vandetanib had no effect on copulation or fertility rate when undosed females were mated with males administered 1, 5, or 20 mg/kg/day of vandetanib (approximately 0.03, 0.22, or 0.40 times, respectively, the AUC in patients with cancer at the recommended human dose of 300 mg/day). There was a slight decrease in the number of live embryos at 20 mg/kg/day and an increase in preimplantation loss at ≥5 mg/kg/day. In a female fertility study, there was a trend towards increased estrus cycle irregularity, a slight reduction in pregnancy incidence and an increase in implantation loss. In a repeat-dose toxicity study in rats, there was a decrease in the number of corpora lutea in the ovaries of rats administered 75 mg/kg/day vandetanib (approximately 1.8 times the AUC in patients with cancer at the recommended human dose) for 1 month.	(b) (4)
13.2 Animal Pharmacology and/or Toxicology (b) (4)	**13.2 Animal Pharmacology and/or Toxicology** In an animal model of wound-healing, mice dosed with vandetanib had reduced skin-breaking strength compared with controls. This suggests that vandetanib slows but does not prevent wound	(b) (4)

The Sponsor Proposes:	We recommend:	Justification:
(b) (4)	healing. The appropriate interval between discontinuation of vandetanib and subsequent elective surgery required to avoid the risks of impaired wound healing has not been determined. Nodular masses were observed in a 6-month toxicology study in rats during treatment with ≥5 mg/kg/day vandetanib (approximately 0.22 or 0.40 times, respectively, the AUC in patients with cancer at the recommended human dose of 300 mg/day). Masses were palpable during clinical assessments as early as week 13, were observed in multiple organs, and were associated with hemorrhagic or inflammatory findings.	(b) (4)
15. References		
None	**15. References** 1. NIOSH Alert: Preventing occupational exposures to antineoplastic and other hazardous drugs in healthcare settings. 2004. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2004-165. 2. OSHA Technical Manual, TED 1-0.15A, Section VI: Chapter 2. Controlling Occupational Exposure to Hazardous Drugs. OSHA, 1999. http://www.osha.gov/dts/osta/otm/otm_vi/otm_vi_2 html 3. American Society of Health-System	

The Sponsor Proposes:	We recommend:	Justification:
	Pharmacists. ASHP Guidelines on Handling Hazardous Drugs: Am J Health-Syst Pharm. (2006) 63:1172-1193. 4. Polovich, M., White, J. M., & Kelleher, L. O. (eds.) 2005. Chemotherapy and biotherapy guidelines and recommendations for practice (2nd. ed.) Pittsburgh, PA: Oncology Nursing Society.	
16. How Supplied/Storage and Handling		
16.1 Storage and Handling (b) (4)	**16.1 Storage and Handling** Vandetanib tablets should be stored at 25°C (77°F); excursions permitted to 15°C – 30°C (59°F – 86°F) [See USP controlled room temperature]. Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published.[1-4] Vandetanib tablets should not be crushed. Direct contact of crushed tablets with the skin or mucous membranes should be avoided. If such contact occurs, wash thoroughly as outlined in the references. Personnel should avoid exposure to crushed tablets.	(b) (4)
17. Patient Counseling Information		
17.3 Pregnancy and Nursing (b) (4)	**17.6 Pregnancy and Nursing** Patients of childbearing potential must be told to use effective contraception during therapy and for at least four months following their last dose of vandetanib. Breast-feeding mothers are advised to discontinue nursing while receiving vandetanib therapy.	(b) (4)

The Sponsor Proposes:	We recommend:	Justification:
	17.7 Drug Handling Vandetanib tablets should not be crushed. Direct contact of crushed tablets with the skin or mucous membranes should be avoided. (b) (4)	(b) (4)

Note: The trade name Zictifa was rejected. At this time another trade name, (b) (4), is being considered. Since a trade name may not be accepted before drug approval, vandetanib may be the marketed name at the time of approval and has been used as the trade name in the label.

Reference:

McCarty, M.F., Wey, J., Stoeltzing, O., Liu, W., Fan, F., Bucana, C., Mansfield, P.F., Ryan, A.J., & Ellis, L.M. (2004). ZD6474, a vascular endothelial growth factor receptor tyrosine kinase inhibitor with additional activity against epidermal growth factor receptor tyrosine kinase, inhibits orthotopic growth and angiogenesis of gastric cancer. *Mol Cancer Ther, 3*(9): 1041-1048.

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/s/

BRENDA J GEHRKE
03/17/2011

ROBERT T DORSAM
03/17/2011

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	022405
Supporting document/s:	0000 and 0006
Applicant's letter date:	July 7, 2010
CDER stamp date:	July 7, 2010
Product:	Vandetanib
Indication:	Unresectable locally advanced or metastatic medullary thyroid cancer
Applicant:	IPR Pharmaceuticals INC C/O Astrazeneca Pharmaceuticals LP
Review Division:	Division of Oncology Drug Products (HFD-150)
Reviewers:	Brenda J. Gehrke, Ph.D., Robert T. Dorsam, Ph.D.
Supervisor/Team Leader:	S. Leigh Verbois, Ph.D.
Division Director:	Robert Justice, M.D.
Project Manager:	Lisa Skarupa

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Any information or data necessary for approval of NDA 022405 that IPR Pharmaceuticals INC C/O Astrazeneca Pharmaceuticals LP 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 022405.

TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

NDA 022405 has been submitted as a full New Drug Application (NDA) for vandetanib for the indication of the treatment of patients with unresectable locally advanced or metastatic medullary thyroid cancer. Vandetanib is a new molecular entity that is a kinase inhibitor with activity at multiple kinases including vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), and RET kinase. The proposed clinical dose of 300 mg is administered orally as a tablet once daily. Nonclinical pharmacology, pharmacokinetic, and toxicology studies have been submitted to support the approval of vandetanib for the proposed indication.

Brief Discussion of Nonclinical Findings

Vandetanib and its major metabolites are kinase inhibitors with both *in vitro* and *in vivo* activity at multiple kinases. Vascular endothelial growth factor receptors (VEGFR), epidermal growth factor receptor (EGFR), and RET kinase are inhibited by vandetanib. Other kinases are inhibited as well including BRK, TIE2, and members of the EPH kinase and Src family tyrosine kinase families, and may be responsible for the pharmacologic activity and toxicity of the drug. The IC_{50} values for many kinases where lower than 1.8 μM, the C_{max} value in the pivotal clinical study (Study 58), therefore the inhibition of other kinases may contribute to the toxicity of the drug. Based on this data, the pharmacological classification of vandetanib is a kinase inhibitor. *In vivo* effects of vandetanib were demonstrated in numerous studies using angiogenesis assays and in human tumor xenograft models in nude mice. Treatment with vandetanib showed a dose dependent inhibition of VEGF-induced angiogenesis and inhibition of VEGFR2 phosphorylation and pEGFR staining in xenograft models. These findings provide some evidence that vandetanib has *in vivo* activity against VEGFR and EGFR.

In an animal model of wound healing, wound breaking strength was dose-dependently decreased in mice treated with vandetanib compared to controls. This suggests that vandetanib slows but does not prevent wound healing. No wound healing complications have been reported in patients, therefore, this non-clinical finding is being reported in the package insert.

Pharmacokinetics of vandetanib were studied in mice, rats, and dogs, the non-clinical species tested for toxicity, and for both intravenous and oral administration. In the pivotal clinical study (Study 58) in patients treated with 300 mg daily, the C_{max} at steady state (Day 56) was 857 ng/mL (1.80 μM) and the AUC_{ss} was 19829 ng·h/mL (41.6 μM·h). Vandetanib was well absorbed in the rat and dog. Oral bioavailability of >90% was achieved in the rat at 5 mg/kg (30 mg/m^2). Although a higher dose of 30 mg/kg (180 mg/m^2) increased exposure, slightly lower bioavailability (72-78%) was observed. Following an oral dose of 20 mg/kg to dogs, bioavailability was 56%. Absorption was long with T_{max} typically 2-8 hours in the rat and dog. Exposure increased with an increase in dose for both males and females. Increases in exposure were dose proportional at lower doses, but less than proportional at higher doses. Exposure (AUC) of vandetanib was higher in females than males in both rats and dogs. The half-life of vandetanib is relatively long and ranged from 15-55.2 hours in rats and dogs, with no dose-related pattern. This half-life in animals is significantly different than the plasma half-life of 19 days in humans observed in the clinic. Based on the large difference in pharmacokinetics

between humans and animals, the animal models for pharmacology are not highly predictive of the time course of pharmacologic activity of the drug, and should not be relied upon for predicting activity of alternative regimens in the clinical setting.

Distribution studies in animals show that high concentrations of vandetanib were observed in the gastrointestinal tract, lungs, liver, and kidneys, which are all target organs of toxicity. This finding suggests that vandetanib produces toxicity in organs that contain have high concentrations of the drug. Disposition studies show that with oral administration, vandetanib is primarily eliminated in the feces (55-85%), with some excretion in the urine (5-10%). The two major metabolites of vandetanib are the N-desmethyl and N-oxide forms. The N-desmethyl metabolite has shown similar pharmacological activity to vandetanib. Vandetanib has been shown to affect the activity of human liver P450 isozymes, and clearly inhibits CYP2D6 activity.

Both *in vivo* and *in vitro* safety pharmacology studies were conducted to assess the effects of vandetanib on neurological, cardiovascular, pulmonary, renal, and gastrointestinal functioning. In a functional battery assessing neurological function in rats, oral administration of vandetanib (1200 and 6000 mg/m^2) lowered body weight gain, slowed pupil response, and reduced activity in open field assessments. Decreased landing foot splay, reduced grip strength, and piloerection were also observed at 6000 mg/m^2. Based on these findings observed in the rat, vandetanib appears to impair aspects of autonomic and neuromuscular function. The effects of vandetanib on pulmonary, renal, and gastrointestinal function were evaluated in *in vivo* rat studies. A high dose of vandetanib (6000 mg/m^2) increased peak inspiratory flow and reduced inspiratory time. In a diuretic test in rats, a dose of 50 mg/kg (300 mg/m^2) vandetanib reduced sodium, potassium, and chloride ions and increased total protein content in the urine. Vandetanib (240, 1200, and 6000 mg/m^2) also dose dependently inhibited both intestinal transit and gastric emptying in rats. These results indicate that vandetanib is both toxic to the kidneys and impairs gastrointestinal function, and are consistent with repeat-dose toxicology findings of renal toxicity in the rat and gastrointestinal toxicity in the rat and dog observed in the repeat-dose toxicology studies.

Vandetanib impairs cardiac function in non-clinical studies. The effects of vandetanib on cardiovascular functioning were assessed using multiple *in vitro* and *in vivo* and studies including hERG channel and Purkinje fiber assays and telemetry studies in rats and anesthetized dogs. The potential for vandetanib or its N-desmethyl or N-oxide metabolites to delay repolarization and prolong of the QT interval was evaluated in hERG-expressing HEK cells. Vandetanib inhibited the hERG channel with an $-p[IC]_{50}$ of 6.4 ± 0.1, which equates to 0.4 µM or 190 ng/mL. The inhibition produced by the N-desmethyl and N-oxide metabolites was 3- and 10-fold less than vandetanib, respectively. Co-administration of vandetanib and ondansetron at their respective IC_{50} values inhibited the hERG channel more than either compound alone, but was sub-additive. *In vivo* telemetry studies show that vandetanib increases blood pressure and QTcV interval. Mean systolic and diastolic blood pressures were dose-dependently increased with single oral doses of vandetanib (75 or 300 mg/m^2) in rats. Hypertension was a common adverse event (>20%) observed clinically with vandetanib. Changes in blood pressure with vandetanib may be linked to effects of VEGF inhibition on vasculature. In anesthetized dogs, intravenous vandetanib caused both QTcV prolongation at doses $\geq$ 40 mg/m^2 and dose-dependent increases in T wave amplitude and polarity at doses $\geq$ 134 mg/m^2. Based on these results, vandetanib produces QT prolongation in anesthetized dogs at doses below the proposed clinical dose of 300 mg (185 mg/m^2). QT prolongation was observed at this clinical dose with a

mean increase in QTc interval of 35 ms and an increase >60 ms in 35.5% of the patients in the pivotal clinical trial (Study 58). Two cases of torsade de pointes have been observed with vandetanib. Sudden death has also been observed and may be associated with QT prolongation. QT prolongation is a major safety concern with vandetanib and is being addressed through a boxed warning in the package insert and additional safety measures.

Chronic repeat-dose toxicity studies were conducted in both rats and dogs in order to fully characterize vandetanib-induced toxicities. In a six-month oral study, male and female Wistar derived rats were administered vandetanib (0, 1, 5, or 20/10 mg/kg/day; 0, 6, 30, or 120/60 mg/m^2/day) daily for 26 weeks with a 13 week recovery period. In a 9 month oral study, male and female Beagle dogs were administered vandetanib (0, 1, 5, or 20/15 mg/kg/day; 0, 20, 100, or 400/300 mg/m^2/day) daily for 40 weeks with a 13 week recovery period. The highest dose was reduced in both studies due toxicity including adverse effects on body weight gain in both species and mortality in the rat. Probable causes of mortality in the rat include toxicities of the lung (pleuritis, abcess, foreign body pneumonia, and alveolar congestion and/or edema), esophagitis in the esophagus, pancreatitis in the pancreas, and cholangitis in the bile duct. Respiratory toxicities were observed clinically with vandetanib including respiratory failure, respiratory arrest, and aspiration pneumonia resulting in death and interstial lung disease. In the rat, teeth abnormalities (broken, missing, loose, or discolored) and histopathology findings of dental dysplasia and adjacent tissue abscess were observed in the high dose group. Teeth abnormalities have been observed in other studies including the one month repeat-dose rat study and the female fertility study in rats. These findings are consistent with other kinase inhibitors in rats. Masses were also observed in multiple organs and were palpable during clinical assessments, primarily in high dose rats but were also noted in the mid dose. Histopathology was not conducted on the masses. Given the patient population, this information should be reflected in the package insert, Increases in WBC and neutrophils and histopathology findings of inflammatory cell infiltration in multiple organs indicate inflammation. Renal toxicity was observed in rats based on histopathology changes in the kidney, and increases in urea and creatinine in the blood. Pericarditis and ventricular myocardial fibrosis of the heart were observed in the high dose groups and are evidence of cardiotoxicity in the rat. Cardiotoxicity has also been shown in dogs in the safety pharmacology studies and clinically in humans . Other target organs in the rat were adrenal gland, mesenteric lymph node, skin, spleen, and thymus. While some skin toxicity was observed in the rat, skin toxicity is a concern clinically with rash observed in 78.8% of patients treated with vandetanib in Phase 3 studies. Stevens-Johnson syndrome was also observed in patients treated with vandetanib. Gastrointestinal toxicity was the major toxicity in dogs and included abnormal feces and gross pathology and histopathology findings in the intestine and stomach. Abnormal feces was the dose limiting toxicity in dogs, and as a result of the dose reduction other possible toxicities like liver toxicity were not observed. Gastrointestinal toxicities were observed clinically as adverse events including diarrhea, colitis, nausea, and abdominal pain. Although the pharmacokinetics of vandetanib are significantly different between rats and dogs and humans, the animal safety pharmacology and toxicology studies were predictive of the toxicities observed in the clinical studies including hypertension, QT prolongation, respiratory toxicity, and gastrointestinal toxicity.

Vandetanib was tested for mutagenicity in an *in vitro* reverse mutation (Ames) assay and tested for clastogenicity in an *in vitro* cytogenetic assay using human lymphocytes and an *in vivo* rat micronucleus assay. At the doses tested in these valid studies, with and without metabolic

activation, vandetanib was not mutagenic or clastogenic. Carcinogenicity studies have not been conducted, however, based on the subset of patients for which vandetanib is currently indicated, carcinogenicity studies are required for vandetanib and will be a post marketing requirement (PMR).

Reproductive and developmental toxicology studies were conducted in rats to assess the effects of vandetanib on fertility, embryofetal development, and pre- and post-natal development. In the male fertility study, male treatment with vandetanib had no effect on copulation or fertility rate, however, in females mated with males treated with vandetanib, there was a slight decrease in the number of implants and live embryos at 120 mg/m^2/day and an increase in pre-implantation loss at $\geq$30 mg/m^2/day. Additionally, two females mated with males treated with 120 mg/m^2/day vandetanib had total resorptions. In the female fertility study, vandetanib was administered prior to mating and through gestational day 6. There was increased incidence of irregular cycle patterns at $\geq$ 60 mg/m^2, and decreased the pregnancy incidence, increases in pre-implantation loss, post-implantation loss, and early embryolethality at 150 mg/m^2, resulting in a significant reduction in the number of live embryos. Results of embryo-fetal development studies, showed that vandetanib is embryotoxic, fetotoxic, and teratogenic to rats at exposures equivalent to or lower than those expected at the recommended dose of 300 mg/day based on C_{max} values. Post-implantation loss was increased and the number of live fetuses was decreased in females treated with 150 mg/m^2/day vandetanib during organogenisis. Fetal weight and mean placental weight were decreased with treatment of vandetanib at both 60 and 150 mg/m^2/day. Treatment with vandetanib caused multiple heart vessel abnormalities at 6, 60, and 150 mg/m^2/day. Therefore, this study did not identify a no effect level (NOEL) for teratogenicity. Vandetanib also reduced ossification of skull bones, vertebrae and sternebrae indicating delayed fetal development. These findings occurred in the absence of maternal toxicity, and are consistent with the pharmacologic effects of vandetanib including the inhibition of VEGF-induced angiogenesis.

In the pre- and post-natal development studies in the rat, females were dosed from gestational Day 6 and during lactation until Day 8 or Day 21 *post-partum*. In one study, vandetanib (150 mg/m^2/day) caused total litter loss; all of these dams were found to have undergone total resorptions early in gestation, indicating that 150 mg/m^2/day was embryotoxic in this study. Toxicokinetics measured in dams and pups on Day 8 of lactation indicated the transfer of vandetanib from the dams to the pups by milk secretion. Transfer through milk resulted in relative constant exposure as indicated by predose exposure in pups. Predose exposure in the pups was approximately equivalent to pup exposure 8 hours after dosing to the dams. This is likely due to the long half-life of the drug. These results are consistent with those of a radiolabeled excretion study indicating that vandetanib and trace amounts of the N-desmethyl and N-oxide metabolites of vandetanib were excreted in milk with peak concentrations observed at 8 hours after dosing. In the pivotal study, there was a decrease in number of live pups per litter at birth and a decrease in pup survival at Day 4 *post-partum* with treatment of 6 and 60 mg/m^2/day. Both doses reduced post-natal pup growth, which was associated with a delay in physical development at 60 mg/m^2/day. No effects of vandetanib were observed in the behavioral tests, mating performance, fertility, and caesarian (uterus content) for the F_1 generation. The reproductive and developmental toxicology studies suggest that administration of vandetanib may impair fertility in women and pose a risk for fetal toxicity. These findings are consistent with a product which has been shown to impair blood vessel formation. Pregnancy category D is recommended.

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 vandetanib in the treatment of unresectable locally advanced or metastatic medullary thyroid cancer.

1.3.2 Additional Non Clinical Recommendations

Based on the results of the clinical trial used to support marketing (Study 58), carcinogenicity studies are required for vandetanib. Results indicate that the median time of exposure to vandetanib was ~90 weeks. Patients with medullary thyroid cancer will be chronically administered the drug for long durations. Additionally, it is estimated that at least 50% of the patients will be living 5 years or longer after first being exposed to vandetanib. In general, carcinogenicity is a safety concern with drug chronic exposure. Vandetanib is a kinase inhibitor and other kinase inhibitors have demonstrated carcinogenicity in non-clinical carcinogenicity studies. There is a concern that chronic exposure to vandetanib could cause additional cancers in patients with medullary thyroid cancer treated with vandetanib given the toxicology study findings of masses in multiple organs. Based on these results, two rodent carcinogenicity studies, a long-term (2 year) rat study and a mouse study, need to be conducted to assess the potential for vandetanib to cause carcinogenicity.

1.3.3 Labeling

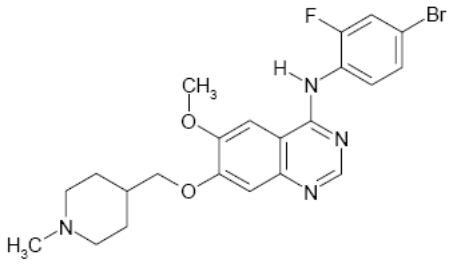
A Separate labeling review will be provided.

2 Drug Information

2.1 Drug

CAS Registry Number	443913-73-3
Generic Name	Vandetanib
Code Name	ZD6474, M382561, AZ11749412
Chemical Name	*N*-(4-bromo-2-fluorophenyl)-6-methoxy-7- [(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine
Molecular Formula/Molecular Weight	$C_{22}H_{24}BRFN_4O_2$/475.36 g/mol

Structure or Biochemical Description



Pharmacologic Class Kinase inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs: IND 60042; NDA (b) (4)

2.3 Drug Formulation

Vandetanib is available as 100 mg and 300 mg tablets containing (b) (4) w/w vandetanib. The 100 mg strength is a round, biconvex, white, film-coated tablet with a diameter of approximately 8.5 mm. 'Z100' is impressed on one side and the other side is plain. The 300 mg strength is an oval-shaped, biconvex, white, film coated tablet with a length of approximately 16.0 mm and a width of approximately 8.5 mm. 'Z300' is impressed on one side and the other side is plain. The quantitative composition of vandetanib 100 and 300 mg tablets is presented in the table below (excerpted from sponsor's submission).

Ingredients	100 mg		300 mg	
	Amount per tablet		Amount per tablet	
	Amount (mg)	Amount (% of tablet core)	Amount (mg)	Amount (% of tablet core)
Tablet core				
Vandetanib	100.0	(b) (4)	300.0	(b) (4)
Dibasic calcium phosphate dihydrate[a]	(b) (4)			
Microcrystalline cellulose				
Crospovidone				
Povidone				
Magnesium stearate				
(b) (4)				
Core tablet weight (mg)				
Tablet coating				
Hypromellose 2910[c, d]				
Polyethylene glycol 300[c, e]				
Titanium dioxide[c]				
(b) (4)				
Nominal coated tablet weight (mg)				

[a] An alternative name is calcium hydrogen phosphate dihydrate.
(b) (4)
[c] Coating constituents may be applied using a proprietary mixture, eg, (b) (4) Relative ratios will remain constant.
[d] An alternative name is hydroxypropyl methylcellulose.
[e] An alternative name is Macrogol.
[f] Total tablet weight gain after coating should be (b) (4)
NA Not applicable.

2.4 Comments on Novel Excipients

The CMC reviewer of the drug product contacted us with a concern about the amount of polyethylene glycol (PEG) in the film coated tablet. In the proposed formulation for vandetanib, (b) (4) Information on polyethylene glycol from the Handbook of Pharmaceutical Excipients indicates that the properties and data for PEG 300 and PEG 400 are

similar including the oral LD_{50} values in the guinea pig (PEG 300: 19.6 g/kg; PEG 400: 15.7 g/kg) and rabbit (PEG 300: 17.3 g/kg; PEG 400: 26.8 g/kg). While the proposed dose of PEG 300 (3-9 mg) is higher than what is currently in approved oral formulations (b) (4), it is much lower than the maximum daily dose of PEG 400 (5886.8 mg) in approved oral formulations. Based on this information, the maximum daily PEG 300 dose of (b) (4) appears to be safe and is not a pharmacology/toxicology concern.

2.5 Comments on Impurities/Degradants of Concern

The proposed specification for the impurity (b) (4) is NMT (b) (4), which is above the ICH qualification threshold of 0.15%. With a clinical dose of 300 mg (185 mg/m^2), the dose of (b) (4) for this proposed specification is (b) (4). The nonclinical batches used in the 6 month rat and 9 month dog toxicology studies contained (b) (4). Below is a table with the (b) (4) doses for the toxicology studies calculated using a range of (b) (4) in non-clinical batches. The doses of (b) (4) were (b) (4), therefore, the proposed specification of NMT (b) (4) for (b) (4) is qualifed.

Dose based on BSA (mg/m^2)

Impurity: (b) (4)					
Clinical use or toxicology Study	Batch/Lot	Route	Species	%	Dose (mg/m^2)
NDA (b) (4) Proposed specification		Oral	Human	(b) (4)	
TPR2939 (6 month rat)	#C268/1	Oral	Rat		
TPD1043 (9 month dog)	#C268/1	Oral	Dog		

2.6 Proposed Clinical Population and Dosing Regimen

Patients with unresectable locally advanced or metastatic medullary thyroid cancer.

Vandetanib (300 mg tablet) is to be administered orally once daily.

2.7 Regulatory Background

Vandetanib has been developed as an anti-cancer drug by Astrazeneca Pharmaceuticals LP and clinical trials have been conducted since 2000 under IND 60042. Orphan drug designation for vandetanib in the treatment of medullary thyroid carcinoma, (b) (4) On December 22, 2005, vandetanib was granted a Fast Track designation for medullary thyroid carcinoma. (b) (4) The current NDA, 022405, was submitted on July 7, 2010.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacology

Study#	Title
01-report	ZD6474 pharmacology internal report: Inhibition of Flt-1 kinase activity
02-report	ZD6474 pharmacology internal report: Effect of M382558 and M447882 on growth factor stimulated HUVEC proliferation
03-report	ZD6474 pharmacology internal report: Selectivity profile of M382558 and M447882 in recombinant enzyme assays
04-report	ZD6474 pharmacology internal report: Characterisation and quantification of an endothelial cell tube formation assay
05-report	ZD6474 pharmacology internal report: Chronic and acute effects of ZD6474, a VEGF receptor tyrosine kinase inhibitor, on established human tumour xenografts
06-report	ZD6474 pharmacology internal report: VEGF receptor tyrosine kinase inhibitors as potential anti-tumour agents
07-report	ZD6474 pharmacology report: Impact of tumour VEGF expression level on the *in vivo* efficacy of vandetanib (Zactima; ZD6474)
08-report	ZD6474 pharmacology internal report: ZD6474 inhibits both pVEGFR-2 and pEGFR at clinically achievable levels in pre-clinical models
09-report	ZD6474 pharmacology internal report: Inhibition of EGFR and VEGFR signaling in an oncogenic K-ras mouse model of lung cancer
1243	IC_{50} profiling of 7 compounds using RET protein kinase
2501	IC_{50} profiling of 5 compounds using 3 protein kinases
3179	IC_{50} profiling of AZD6474 using 96 protein kinases in two independent experiments
17702	IC_{50} profiling of AZ11749412-003 (ZD6474) using 96 protein kinases
ASZ128ABC	ZD6474 pharmacology external report: IC_{50} profiling ZD6474 using wild-type and mutated EGFR and RET protein kinases
AZJK4	Effect of AZ11749412 on VEGF165-induced angiogenesis of matrigel plugs in athymic nude mice
PRT021381	Antitumour activity study of ZD6126 and ZD6474 in combination with Taxotere® in nude mice bearing subcutaneous human MX-1 breast tumours

Secondary Pharmacology

Study#	Title
0393SY	AZD6474: Selectivity screening in radioligand binding and enzyme assays *in vitro*
0585SY	ZD6474: Selectivity screening in radioligand binding and enzyme assays *in vitro*

Safety Pharmacology

Study #	Title
TSM1124	Zeneca ZM382,561 – Multi-observation test in the mouse.
1228SR	ZD6474: Functional observational battery in the Han Wistar Rat following single oral administration
TSZ36	Study report for the effect of ZD6474 on hERG potassium channel
0048SZ	Study report for the effect of M382558 and M447882 on hERG potassium channel
0102SZ	ZD6474 and ondansetron: The effect on hERG potassium channel
TSD1293	Evaluation of effect on cardiac action potential in isolated canine Purkinje fibres
Internal report #10: Telemetry Study	ZD6474 pharmacology internal report: telemetry study: The effect of ZD6474 on systolic and diastolic blood pressure in rats
0276SD	ZD6474: hemodynamic effects in anesthetized, beagle dogs - Report amendment
0257SD	ZD6474 and ondansetron: QTc investigations in anesthetized, beagle dogs (pilot study) - Report amendment

0258SD	ZD6474 and ondansetron: QTc investigations in anesthetized, beagle dogs - Report amendment
20060012PCR1220SR	ZD6474: Evaluation of effect on respiration in the unrestrained conscious rat following single oral administration
1290SR	ZD6474: Gastric emptying and intestinal motility in the rat following single, oral administration
TSR2922	Zeneca ZM382,561. Diuretic test

Pharmacokinetics

Study #	Title
KPV003	Validation of a High Performance Liquid Chromatography-Mass Spectrometry (HPLCMS-MS) Method for the Determination of Zeneca ZD6474 Concentrations in Rat and Dog Plasma
KPV033	Validation of an Improved High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS-MS) Method for the Determination of AstraZeneca ZD6474 Concentrations in Dog and Rat Plasma
KPV039	Validation of an LC-MS/MS method for the measurement of ondansetron in dog plasma
KK1059	The long term stability of ZD6474 and its metabolites N-oxide-ZD6474 and N-desmethyl-ZD6474 in rat, dog and mouse plasma for a period of 12 months at –20°C and –70°C
KPV061	Method validation study for the Analysis of (b) (4) and ZD6474 in rat plasma by LC-MS/MS
KPV064	Method validation study for the for the analysis of (b) (4) and ZD6474 in dog plasma by LC-MS/MS
KPV065	Method validation study for the for the analysis of (b) (4) and ZD6474 in mouse plasma by LC-MS/MS
KPV076	Method validation study for the Analysis of (b) (4) and ZD6474 in animal tissue by LC-MS/MS
KPV097	Partial Method Validation Study for the Analysis of (b) (4) and ZD6474 in Mouse Plasma by LC-MS/MS
KKR007	Zeneca ZD6474 : the distribution of radioactivity in the blood after oral and intravenous administration of [14^{c}]-Zeneca ZD6474 to rats
KPR056	The pharmacokinetics of ZD6474, N-desmethyl and N-oxide metabolites following single oral and intravenous doses of ZD6474 in male and female rats
KMR080	The tissue distribution of [14^{c}]-AstraZeneca ZD6474 in male rats - Report amendment 1
KKD005	Zeneca ZD6474 : the disposition of [14^{c}]-ZD6474 in the dog following oral and intravenous administration.
KPD057	The pharmacokinetics of ZD6474 and N-desmethyl and N-oxide metabolites following single oral and intravenous doses of ZD6474 to male dogs - Report amendment 1
02-ASTR-UK.PO1R1	Bi-directional CACO-2 permeability of ZD6474
KMM063	AZD6474: the tissue distribution of [14^{c}]-AstraZeneca ZD6474 in nude mice bearing Lovo xenografts - Report amendment 1
KMR014	AstraZeneca ZD6474 : the tissue distribution of [14^{c}]-AstraZeneca ZD6474 in the male pigmented rat and albino rat following single oral administration at 5 mg/kg (quantitative whole body phosphor imaging)
KMN070	Determination of the involvement of human transport proteins MDR1, BCRP and MRP1 in the transport of ZD6474
KMN096	Determination of the potential of ZD6474 to inhibit transport via the human multidrug resistance 1 protein (MDR1), human breast cancer resistance protein (BCRP) and human multidrug resistance protein 1 (MRP1)
KMX083	ZD6474 – *in vitro* assessment of the involvement of the human transport protein OCT2 (SLC22A2) in the transport of ZD6474
KMM068	AstraZeneca ZD6474. The disposition of [14^{c}]-AstraZeneca ZD6474 in the mouse
KMR006	Zeneca ZD6474 : the disposition of [14^{c}]-Zeneca ZD6474 in the rat

KMR038	AstraZeneca ZD6474. The disposition of [14^{c}]-AstraZeneca ZD6474 in the rat
KMR013	AstraZeneca ZD6474 : investigation of biliary secretion and enterohepatic recirculation of [14^{c}]-AstraZeneca ZD6474 in the bile of cannulated male rats
KMD037	AstraZeneca ZD6474. The disposition of [14^{c}]-AstraZeneca ZD6474 in female dogs
KMN012	Comparison of the metabolism of [14^{c}]-AstraZeneca ZD6474 in the rat and dog
KMX095	*In vitro* assessment of human liver cytochrome p450 inhibition potential of ZD6474 and the N-desmethyl metabolite of ZD6474
KMN094	The analysis of ZD6474, (b) (4) in human urine samples by HPLC-MS/MS
KMX020	ZD6474 : investigation of the potential inhibitory effect of AstraZeneca ZD6474 on the metabolism of cytochrome p450 (cyp) model substrates
KMR071	ZD6474. The excretion of drug-related material in the milk following oral administration of [14^{c}]-ZD6474 to lactating rats

Note: Studies KKR007, KKD005, and KMR006 were also reviewed under IND 60042

Repeat-Dose Toxicology

Study#	Title
TPR2939	Zeneca ZD6474: Six month oral toxicity study in rats
TPD1043	Zeneca ZD6474: Nine month oral toxicity study in dogs

Genetic Toxicology

Study#	Title
TMV777	Zeneca ZD6474: Bacterial mutation assay in *S. typhimurium* and *E.coli*
TYX103	Zeneca ZD6474: *In vitro* cytogenetic study using cultured human lymphocytes
TQR2942	Zeneca ZD6474: Micronucleus test in the rat: Oral administration

Note: These studies were also reviewed under IND 60042

Reproductive Toxicology

Study#	Title
TGR3138	Zeneca ZD6474: Fertility study in male rats: Oral administration
TGR2940	Zeneca ZD6474: Oral fertility and early embryonic development study in the female rat
TRR3073	Zeneca ZD6474: Teratology sighting study in rats: Oral administration
TTR2938	Zeneca ZD6474: Oral embryofetal development study in the rat
AA29682 (1107WR)	ZD6474 (Zactima): Dose range-finding pre- and post-natal development study by the oral route (gavage) in the rat
AA32728 (1251WR)	ZD6474 (Zactima): Pre- and post-natal development study by the oral route (gavage) in the rat

Special Toxicology

Study#	Title
TKU9	Cytotoxicity assay *in vitro* with BALB/C3T3 cells: Neutral red (NR) assay with ZD6474 at simultaneous irradiation with artificial sunlight

3.2 Studies Not Reviewed

Primary Pharmacology

Study#	Title
17701	IC_{50} profiling of AZ10027436-040 (Iressa) using 96 protein kinases

Pharmacokinetics

Study #	Title
KML002	The Synthesis of N-(4-Bromo-2-Fluoro[14C]Phenyl)-6-Methoxy- 7-[(1-Methyl-4-Piperidinyl)Methoxy]- 4-Quinazolinamine
KML009	The Synthesis of N-(4-Bromo-2-Fluoro[14C]Phenyl)-6-Methoxy- 7-[(1-Methyl-4-Piperidinyl)Methoxy]- 4- Quinazolinamine
KML045	The Synthesis of N-(4-Bromo-2-Fluoro[14C]Phenyl)-6-Methoxy- 7-[(1-Methyl-4-Piperidinyl)Methoxy]- 4-Quinazolinamine
KML093	Synthesis of Isotopomers of ZD6474
	Potential for interaction with CYP2D6 using the drug-drug interaction simulation software SimCYP
	FM03 genotyping analysis in ZD6474 studies D4200C00002, D4200C00006
KMX021	Evaluation of [14^c]ZD6474 metabolism and identification of major metabolites in human liver microsomes and cryopreserved human hepatocytes
KMX038	D6474 kmx038. Determination of the human microsomal p450 isozymes involved in the metabolism of AstraZeneca zd6474
KMX046	D6474 KMX046. Investigation of the human enzyme systems involved in the metabolism of AstraZeneca ZD6474 to its N-oxide metabolite
KMX054	An *in vitro* study to examine the effect of ZD6474 on human hepatic microsomal cytochrome p450 activity
KMX067	Evaluation of induction potential of cytochrome p450 isoforms by ZD6474 in cultured human hepatocytes
KMN091	Metabolism of ZD6474 by uridine glucuronosyl transferases (UGT)
PHE9224WMB5	An investigation to compare the oral bioavailability in fasted dogs of ZD6474 phase 1 (2 x 200 mg) tablets and the proposed phase 2 (1 x 400 mg) tablets
PHE9224WMB6	An investigation to compare the oral bioavailability in fasted dogs of ZD6474 phase 2 (300 mg) tablets and the proposed commercial (300 mg) tablets

Repeat-Dose Toxicology

Study#	Title
0567AR	ZD6474: 10 day intravenous toxicity study in the rat
0266AR	ZD6474: Two week intravenous toxicity study in the rat
0142AD	ZD6474: 10 day intravenous toxicity study in the dog
0143AD	ZD6474: Two week intravenous toxicity study in the dog

Special Toxicology

Study#	Title
1953BV	AZ13102257: Genetic toxicity evaluation using a limited bacterial reverse mutation test
(b) (4) 8664	ZD4190 (b) (4) Bacterial mutation hazard assessment assay in *S. typhimurium* and *E. coli* (ZMP screen)
(b) (4) SM1003	ZD4190 (b) (4) Mouse bone marrow micronucleus test
1338BV	ZD6474 (b) (4) Genetic toxicity evaluation using a bacterial reverse mutation test
1339BV	(b) (4) Genetic toxicity evaluation using a bacterial reverse mutation test

3.3 Previous Reviews Referenced

IND 60042, pharmacology/toxicology review #1 (Wendelyn Schmidt, Ph.D.)

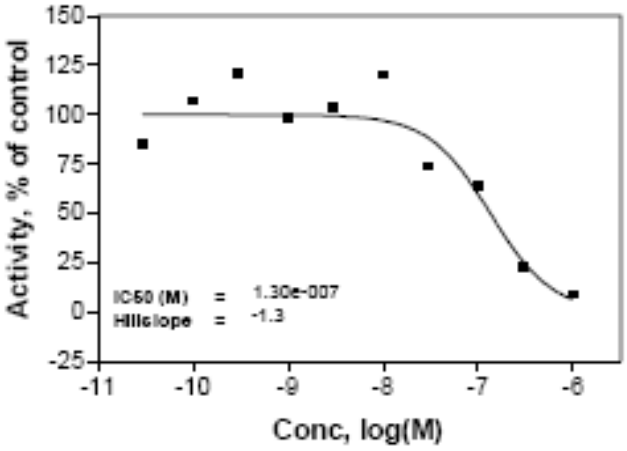
4 Pharmacology

4.1 Primary Pharmacology

Study title: IC_{50} profiling of 7 compounds using RET protein kinase
Study no.: 1243
Study report location: M4.2.1.1

The IC_{50} profile for RET protein kinase was determined for 7 compounds from AstraZeneca, including vandetanib (ZD6474; M382561), using a 96-well plate assay format. IC_{50} values were measured by testing 10 concentrations of each compound ranging from 3×10^{-11} M to 1×10^{-6} M (n=1). RET protein kinase was expressed in Sf9 insect cells as human recombinant GST-fusion protein by means of the baculovirus expression system, and the purity and identity of RET protein kinase was checked by SDS/PAGE/silver staining and by western blot analysis with specific antibodies. IC_{50} profiles for each compound were based on the residual activities (in %) for each of the 10 concentrations and calculated using Prism 3.03 for Windows. The IC_{50} value for inhibition of RET protein kinase for vandetanib was 1.30×10^{-7} M. The concentration curve used to determine the IC_{50} value is shown in the figure below.

Concentration curve for RET activity for M382561
(excerpted from sponsor's submission)



Study title: IC_{50} profiling of 5 compounds using 3 protein kinases
Study no.: 2501
Study report location: M4.2.1.1

The IC_{50} profile for 3 protein kinases (EGF-R, RET, and VEGF-R2) was determined for 5 compounds from AstraZeneca, including vandetanib (ZD6474; AZ11749412), using a 96-well plate assay format. IC_{50} values were measured by testing 10 concentrations of each compound ranging from 3×10^{-9} M to 1×10^{-4} M (n=2). EGF-R, RET, and VEGF-R2 protein kinases were expressed in Sf9 insect cells as human recombinant GST-fusion proteins or His-tagged proteins by means of the baculovirus expression system. The purity of each protein kinase was checked by SDS/PAGE/silver staining and the identity of each protein kinase was verified by western blot analysis with kinase specific antibodies or by mass spectroscopy. The residual activities (in %)

for each of the 10 concentrations and the IC_{50} values were calculated for each kinase and compound using Quattro Workflow V2.0.2.2. The following IC_{50} values were determined for vandetanib: EGF-R (3.59×10^{-8} M), RET (1.17×10^{-7} M), and VEGF-R2 (1.84×10^{-8} M).

Study title: **IC_{50} profiling of AZ11749412-003 (ZD6474) using 96 protein kinases**
Study no.: 17702
Study report location: M4.2.1.1

The IC_{50} profile of ZD6474 was determined using 96 protein kinases in a 96-well plate assay format. IC_{50} values were measured by testing 12 concentrations of ZD6474 ranging from 3×10^{-10} M to 1×10^{-4} M (n=1) in each kinase assay. All protein kinases were expressed in Sf9 insect cells as human recombinant GST-fusion proteins or His-tagged proteins by means of the baculovirus expression system and the identity of the protein kinases was checked by western blot analysis with specific antibodies or by mass spectroscopy. IC_{50} profiles for each kinase were based on the residual activities (in %) for each of the 12 concentrations and calculated using Prism 4.03 for Windows. The following table (excerpted from sponsor's submission) lists the 96 protein kinases used for the determination of inhibitory profiles and the IC_{50} values.

Kinase	IC_{50} (M)	Kinase	IC_{50} (M)	Kinase	IC_{50} (M)
ABL1	1,30E-06	FAK	>1,00E-04	PIM1 (h)	n.v.
AKT1	>1,00E-04	FGF-R1	5,80E-07	PIM2 (h)	n.v.
AKT2	>1,00E-04	FGF-R3	7,50E-07	PKC-alpha	>1,00E-04
AKT3	>1,00E-04	FGF-R4	5,80E-06	PKC-beta1	>1,00E-04
ARK5	>1,00E-04	FGR	1,10E-06	PKC-beta2	>1,00E-04
Aurora-A	2,20E-05	FLT3	1,50E-05	PKC-delta	>1,00E-04
Aurora-B	1,40E-05	GSK3-beta	>1,00E-04	PKC-epsilon	>1,00E-04
Aurora-C	9,20E-05	IGF1-R	>1,00E-04	PKC-eta	>1,00E-04
BRK	3,60E-08	IKK-beta	>1,00E-04	PKC-gamma	>1,00E-04
CDK1/CycB	>1,00E-04	IKK-epsilon	>1,00E-04	PKC-mu	>1,00E-04
CDK2/CycA	>1,00E-04	INS-R	>1,00E-04	PKC-theta	>1,00E-04
CDK2/CycE	>1,00E-04	IRAK4	5,00E-07	PKC-zeta	>1,00E-04
CDK3/CycE	>1,00E-04	ITK	2,60E-05	PLK1 (h)	>1,00E-04
CDK4/CycD1	>1,00E-04	JAK2	>1,00E-04	PRK1 (h)	>1,00E-04
CDK5/p35NCK	>1,00E-04	JAK3	>1,00E-04	RET (h)	1,00E-07
CDK6/CycD1	>1,00E-04	JNK3	>1,00E-04	ROCK2	7,60E-05
CDK7/CycH/Mat1	>1,00E-04	KIT	1,40E-06	S6K (h)	2,90E-05
CHK1	>1,00E-04	LCK	3,70E-07	SGK1	>1,00E-04
CK2	>1,00E-04	LYN	4,00E-07	SGK3	>1,00E-04
COT	>1,00E-04	MET	1,00E-04	SNK (h)	>1,00E-04
CSK	1,60E-06	MST4	5,50E-05	SRC (h)	4,20E-07
DAPK1	>1,00E-04	MUSK	4,50E-06	SYK (h)	8,00E-05
EGF-R	4,30E-08	NEK2	>1,00E-04	TIE2 (h)	5,20E-07
EPHA1	1,70E-07	NEK6	>1,00E-04	TSF1 (h)	>1,00E-04
EPHA2	3,10E-07	NLK	5,70E-05	TSK2	>1,00E-04
EPHA4	3,70E-07	PAK1	>1,00E-04	VEGF-R1 (h)	1,50E-07
EPHB1	4,30E-07	PAK2	>1,00E-04	VEGF-R2 (h)	3,80E-08
EPHB2	1,10E-07	PAK4	>1,00E-04	VEGF-R3 (h)	2,60E-07
EPHB3	1,30E-05	PBK	>1,00E-04	VRK1	>1,00E-04
EPHB4	3,20E-07	PDGFR-alpha	1,10E-06	WEE1 (h)	6,50E-05
ERBB2	3,90E-07	PDGFR-beta	5,30E-06	YES	2,40E-07
ERBB4	7,00E-07	PDK1	>1,00E-04	ZAP70	>1,00E-04

n.v.= non valid, IC_{50} values for PIM1 and PIM2 were not determined due to invalid high controls

IC_{50} values ranged from $>10^{-4}$ M to 3.6×10^{-8} M, with the strongest inhibitions for the BRK (IC_{50}: 36 nM), VEGF-R2 (IC_{50}: 38 nM), and EGF-R (IC_{50}: 43 nM).

Study title: **IC_{50} profiling of AZD6474 using 96 protein kinases in two independent experiments**
Study no.: 3179
Study report location: M4.2.1.1

The IC_{50} profile of ZD6474 was again determined using 96 protein kinases in a 96-well plate assay format. IC_{50} values were measured by testing 12 concentrations of ZD6474 ranging from 3×10^{-10} M to 1×10^{-4} M in two independent experiments (2 x n=1) in each kinase assay. All protein kinases were expressed in Sf9 insect cells as human recombinant GST-fusion proteins or His-tagged proteins by means of the baculovirus expression system and the identity of the protein kinases was checked by western blot analysis with specific antibodies or by mass spectroscopy. IC_{50} profiles for each kinase were based on the residual activities (in %) for each of the 12 concentrations and calculated using Prism 4.03 for Windows. The 96 protein kinases used for the determination of inhibitory profiles and the IC_{50} values for the two separate experiments are listed in the table below (excerpted from sponsor's submission). In this study, IC_{50} values ranged from 4.7×10^{-8} M to $>10^{-4}$ M and in general, were similar between the two independent experiments for each kinase. Once again, ZD6474 showed the strongest inhibition for VEGF-R2 (IC_{50} values: 5.30×10^{-8} and 4.70×10^{-8}), EGF-R (IC_{50} values: 9.5×10^{-8} and 9.30×10^{-8}), and BRK (IC_{50} values: 1.10×10^{-7} and 7.70×10^{-8}).

No.	Kinase	IC50 (M) 1st exp.	IC50 (M) 2nd exp.
1	ABL1	1.60E-06	1.80E-06
2	AKT1	>1E-04	>1E-04
3	AKT2	>1E-04	>1E-04
4	AKT3	>1E-04	>1E-04
5	ARK5	>1E-04	>1E-04
6	Aurora-A	4.80E-05	5.90E-05
7	Aurora-B	1.40E-05	2.60E-05
8	Aurora-C	6.30E-05	9.40E-05
9	BRK	1.10E-07	7.70E-08
10	CDK1/CycB1	>1E-04	>1E-04
11	CDK2/CycA	>1E-04	>1E-04
12	CDK2/CycE	>1E-04	>1E-04
13	CDK3/CycE	>1E-04	>1E-04
14	CDK4/CycD1	>1E-04	>1E-04
15	CDK5/p35NCK	>1E-04	>1E-04
16	CDK6/CycD1	>1E-04	>1E-04
17	CDK7/CycH/Mat1	>1E-04	>1E-04
18	CHK1	>1E-04	>1E-04
19	CK2-alpha1	>1E-04	>1E-04
20	COT	>1E-04	>1E-04
21	CSK	2.50E-06	3.20E-06
22	DAPK1	>1E-04	>1E-04
23	EGF-R	9.50E-08	9.30E-08
24	EPHA1	2.70E-07	2.70E-07
25	EPHA2	8.50E-07	7.50E-07
26	EPHA4	5.10E-07	4.40E-07
27	EPHB1	1.50E-06	1.50E-06
28	EPHB2	2.20E-07	1.80E-07
29	EPHB3	2.80E-05	3.10E-05
30	EPHB4	8.60E-07	8.10E-07
31	ERBB2	2.70E-06	1.90E-06
32	ERBB4	1.30E-06	1.80E-06
33	FAK fl	>1E-04	7.50E-05
34	FGF-R1	1.10E-06	1.90E-06
35	FGF-R3	2.60E-06	2.20E-06
36	FGF-R4	2.90E-05	2.10E-05
37	FGR	3.10E-06	2.60E-06
38	FLT3	2.60E-05	3.40E-05
39	GSK3-beta	>1E-04	>1E-04
40	IGF1-R	>1E-04	>1E-04
41	IKK-beta	>1E-04	>1E-04
42	IKK-epsilon	>1E-04	>1E-04
43	INS-R	>1E-04	>1E-04
44	IRAK4	1.20E-06	9.10E-07
45	ITK	3.90E-05	4.20E-05
46	JAK2	>1E-04	>1E-04
47	JAK3	>1E-04	>1E-04
48	JNK3	>1E-04	>1E-04
49	KIT	3.90E-06	7.30E-06
50	LCK	2.00E-07	2.60E-07
51	LYN	1.70E-06	1.90E-06
52	MET	>1E-04	>1E-04
53	MST4	>1E-04	8.70E-05
54	MUSK	1.40E-05	1.10E-05
55	NEK2	>1E-04	>1E-04
56	NEK6	>1E-04	>1E-04
57	NLK	4.00E-05	4.00E-05
58	PAK1	>1E-04	>1E-04
59	PAK2	>1E-04	>1E-04
60	PAK4	>1E-04	>1E-04
61	PBK	>1E-04	>1E-04
62	PDGFR-alpha	2.30E-06	2.10E-06
63	PDGFR-beta	5.50E-06	8.00E-06
64	PDK1	>1E-04	>1E-04
65	PIM1	>1E-04	>1E-04

66	PIM2	>1E-04	>1E-04
67	PKC-alpha	5.50E-05	>1E-04
68	PKC-beta1	>1E-04	>1E-04
69	PKC-beta2	>1E-04	>1E-04
70	PKC-delta	>1E-04	>1E-04
71	PKC-epsilon	>1E-04	>1E-04
72	PKC-eta	>1E-04	>1E-04
73	PKC-gamma	>1E-04	>1E-04
74	PKC-mu	>1E-04	>1E-04
75	PKC-theta	>1E-04	>1E-04
76	PKC-zeta	>1E-04	>1E-04
77	PLK1	>1E-04	>1E-04
78	PRK1	>1E-04	>1E-04
79	RET	4.50E-07	5.20E-07
80	ROCK2	>1E-04	>1E-04
81	S6K	4.90E-05	4.80E-05
82	SGK1	>1E-04	>1E-04
83	SGK3	>1E-04	>1E-04
84	SNK	>1E-04	>1E-04
85	SRC	4.50E-07	1.50E-06
86	SYK	>1E-04	>1E-04
87	TIE2	3.90E-07	3.70E-07
88	TSF1	>1E-04	>1E-04
89	TSK2	>1E-04	>1E-04
90	VEGF-R1	3.20E-07	3.20E-07
91	VEGF-R2	5.30E-08	4.70E-08
92	VEGF-R3	2.60E-07	3.20E-07
93	VRK1	>1E-04	>1E-04
94	WEE1	>1E-04	>1E-04
95	YES	4.70E-07	6.70E-07
96	ZAP70	>1E-04	>1E-04

Study title: **ZD6474 pharmacology external report: IC_{50} profiling ZD6474 using wild-type and mutated EGFR and RET protein kinases**
Study no.: ASZ128ABC
Study report location: M4.2.1.1

This study was conducted to assess the inhibitory activity of ZD6474 against a panel of recombinant non-mutated (wild-type) and mutated EGFR and RET kinases provided by [redacted] (b) (4) Inhibition of mutated and non-mutated EGFR and RET protein kinase activity by ZD6474 was determined in a 96-well assay format according to the Kinase Profiler Assay protocol established by [redacted] (b) (4). The kinases tested are listed in the following table.

Protein Kinases evaluated (excerpted from sponsor's submission)

Abbreviations	Species	Kinase sequence	Database accession number	Expression sytem	Tag
EGFR	Human	696 - end	(b) (4)	Insect	(b) (4)
EGFR (L858R)	Human	696-end; L858R		Insect	
EGFR (L861Q)	Human	696-end; L861Q		Insect	
EGFR (T790M)	Human	696-end; T790M		Insect	
EGFR (T790M, L858R)	Human	696-end; T790M; L858R		Insect	
Ret	Human	658-end		Insect	
Ret (V804L)	Human	658-end; V804L		Insect	
Ret (V804M)	Human	658-end; V804M		Insect	

Kinase activity assays were carried out in wells in the presence of different (3-fold) dilutions of ZD6474 ranging from 0.001 to 10 µM. Control (n=4) and samples with varying doses of test article (n=2) were expressed as % of control activity and the mean plotted versus the concentration of ZD6474. IC_{50} values were estimated from the graphs for each of the three independent assays and are presented in the table below. An L858R mutation in EGFR had an increase in sensitivity to ZD6474 compared to wild-type EGFR, however, the T790M mutation in EGFR was less sensitive to ZD6474 than wild-type EGFR both in the absence and presence of the L858R mutation. The kinase activity of RET V804M and V804L mutations were not potently inhibited by ZD6474.

Estimated IC_{50} values for inhibition of mutated and non-mutated human EGFR and RET kinase activity by ZD6474
(excerpted from sponsor's submission)

Compound	Kinase	IC_{50} (nM) Part A	IC_{50} (nM) Part B	IC_{50} (nM) Part C
AZD6474	EGFR(h)	29	23	21
AZD6474	EGFR(L858R)(h)	7	3	5
AZD6474	EGFR(T790M)(h)	108	195	279
AZD6474	EGFR(T790M,L858R)(h)	213	190	241
AZD6474	Ret(h)	50	20	34
AZD6474	Ret (V804L)(h)	4,060	2,853	4,006
AZD6474	Ret(V804M)(h)	>10,000	>10,000	>10,000

(h) = human

Study title: **ZD6474 pharmacology internal report: Inhibition of Flt-1 kinase activity**
Study no.: 01-report
Study report location: M4.2.1.1

Previously, the inhibition of Flt-1 kinase by ZD6474 was evaluated using an in-house kinase reagent preparation and the IC_{50} value was 1.6 ± 0.4 µM. This study was conducted to assess the activity of ZD6474 against isolated Flt kinase using recombinant (b) (4) Flt-1 kinase. The inhibition of Flt-1 kinase activity by ZD6474 was determined by conducting a dose response curve for recombinant (b) (4) Flt-1 kinase in a 96-well assay format. An unspecified range of concentrations of ZD6474 and positive and negative controls were used in the assay. The theoretical IC_{50} value for the plate was calculated using mean control values and IC_{50} values for enzyme inhibition by ZD6474 were interpolated using Microcal Origin 6.0 software by nonlinear regression. Three separate assays were conducted. The mean IC_{50} value for inhibition of (b) (4) Flt-1 kinase by ZD6474 was 0.285 ± 0.034µM. These data suggest that the potency of inhibition of Flt-1 kinase depends on the source of recombinant enzyme and that ZD6474 may have the potential to inhibit Flt-1 kinase signaling at submicromolar concentrations.

Study title: **ZD6474 pharmacology internal report: Effect of M382558 and M447882 on growth factor stimulated HUVEC proliferation**
Study no.: 02-report
Study report location: M4.2.1.1

Two metabolites of ZD6474 have been identified in samples from rats and dogs, the N-oxide (M447882) and N-desmethyl (M382558) metabolites. The objective of this study was to examine the inhibitory activity of each metabolite in various growth factor stimulated human umbilical vein endothelial cell (HUVEC) proliferation assays and compare activity to ZD6474. HUVEC proliferation in the presence and absence of growth factors was evaluated using [^{3}H]thymidine incorporation. HUVECs isolated from umbilical cords were plated in 96-well plates (1000 cells/well) and incubated with ZD6474, the N-desmethyl metabolite of ZD6474, or the N-oxide metabolite of ZD6474 and growth factors vascular endothelial growth factor (VEGF; 3 ng/mL) or epidermal growth factor (EGF; 3 ng/mL) or basic fibroblast growth factor (bFGF; 0.3 ng/mL) for 4 days. IC_{50} values were interpolated by nonlinear regression using Microcal Origin software. The mean IC_{50} values for the inhibition of HUVEC proliferation stimulated by VEGF, EGF, or bFGF for ZD6474, the N-desmethyl metabolite of ZD6474, and the N-oxide metabolite of ZD6474 are presented in the table below. The N-desmethyl metabolite of ZD6474 demonstrated very similar inhibitory activity to ZD6474 for inhibition of HUVEC proliferation induced by VEGF, EGF, and bFGF. The N-oxide metabolite of ZD6474 had relatively weak activity in these HUVEC assays compared to ZD6474 and the N-desmethyl metabolite of ZD6474.

Compound	Mean IC_{50} µM (± S.E.)			
	VEGF-stimulated	EGF-stimulated	bFGF-stimulated	N
ZD6474	0.06 ± 0.02	0.17 ± 0.03	0.8 ± 0.07	5-6
N-desmethyl metabolite	0.02 ± 0.01	0.12 ± 0.03	0.95 ± 0.32	5
N-oxide metabolite	3.1 ± 1.9	8.8 ± 1.2	>10	6

Study title: **ZD6474 pharmacology internal report: Selectivity profile of M382558 and M447882 in recombinant enzyme assays**
Study no.: 03-report
Study report location: M4.2.1.1

The objective of this study was to examine the activity of the N-desmethyl and N-oxide metabolites of ZD6474 in various kinase assays to the determine inhibition of two VEGF receptor tyrosine kinases (Kinase insert domain-containing receptor (KDR) and fms-like tyrosine kinase-1 (Flt-1)), the EGF receptor (epidermal growth factor receptor (EGFR)), and the fibroblast growth factor receptor-1 (FGFR1), and compare activity to ZD6474. Recombinant cytoplasmic domains of KDR, Flt-1, EGFR, and FGFR1 were used to determine the ability of ZD6474 and the N-desmethyl and N-oxide metabolites of ZD6474 to the inhibit receptor tyrosine kinase activity. The kinase activity associated with these receptors was determined using an enzyme-linked immunoabsorbent assay (ELISA). ZD6474, the N-desmethyl metabolite of ZD6474, or the N-oxide metabolite of ZD6474 was incubated with enzyme, 10 mM $MnCl_2$, and 2 µM ATP in 96-well microtitre plates. Phosphorylated tyrosine was then detected by sequential incubation with a mouse IgG anti-phosphotyrosine 4G10 antibody, horseradish peroxidase-linked sheep anti-mouse immunoglobulin antibody, and ABTS substrate. Microcal

Origin software was used to interpolate IC_{50} values by nonlinear regression. The mean IC_{50} values for the inhibition of KDR, Flt-1, EGFR, and FGFR1 activity for ZD6474, the N-desmethyl metabolite of ZD6474, and the N-oxide metabolite of ZD6474 are presented in the table below. The N-desmethyl metabolite of ZD6474 demonstrated similar inhibitory activity to ZD6474 for inhibition of KDR, Flt-1, and EGFR tyrosine kinases. The N-oxide metabolite of ZD6474 was less potent than ZD6474 and the N-desmethyl metabolite for KDR and FGFR1 tyrosine kinases, but had similar inhibitory activity to ZD6474 for Flt-1 and EGFR tyrosine kinases.

Compound	Mean IC_{50} µM (± S.E.)				
	KDR	Flt-1	EGFR	FGFR1	No. of tests
ZD6474	0.04 ± 0.01	1.6 ± 0.4	0.5 ± 0.1	3.6 ± 0.9	4-6
N-desmethyl metabolite	0.07 ± 0.04	0.90 ± 0.44	0.17 ± 0.1	6.2 ± 0.25	3
N-oxide metabolite	0.20 ± 0.10	1.92 ± 0.47	0.46 ± 0.04	10.2 ± 4.94	2

Study title: **ZD6474 pharmacology internal report: Characterization and quantification of an endothelial cell tube formation assay**
Study no.: 04-report
Study report location: M4.2.1.1

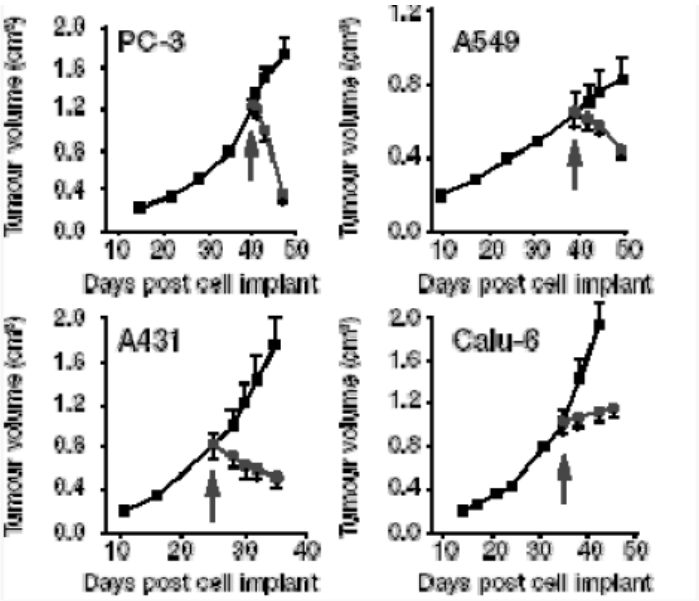
Studies with an *in vitro* endothelial cell tube formation assay were conducted to quantify tubule growth from whole wells using image analysis and to analyze levels of VEGF, bFGF, and interleukin 8 (IL-8) during angiogenesis. The effect of ZD6474 on tubule growth and morphology was also examined in this assay. Studies were conducted using the 24 well (b) (4) consisting of a sterile-well microtitre plate with each well containing a mixture of Human Umbilical Vein Endothelial Cells (HUVECs) and human fibroblasts in the earliest stage of development. The AngioKit was maintained in basal medium containing b-FGF or basal medium in the absence of b-FGF. In this assay, untreated control cultures develop a branching network of tubules after 14 days of culture. Inhibitors or stimulators were introduced from Days 1-11 as media was replenished every 2-3 days and aspirated media was harvested for analysis of VEGF, b-FGF, and IL-8 using ELISA. To quantify tubule growth a whole-well image analysis methodology was developed using a Zeiss KS400 3.0 image analyzer. Tubule formation was examined at Day 11 following fixing and staining of tubules for CD31. Total area of tubule growth, total tubule length, and total number of branch points were measured. Results showed that in the presence of exogenous b-FGF (~700 pg/mL), endogenous VEGF and IL-8 levels measured from harvested media increased over time to a plateau at Day 7, while b-FGF was depleted and remained at lower levels from Day 4. In the absence of exogenous b-FGF there was a 93% reduction in IL-8, a 35% reduction in VEGF, and a 100% reduction in b-FGF levels at Day 11. ZD6474 has been shown to inhibit KDR activity (IC_{50}= 40 nM) and VEGF-stimulated proliferation of HUVEC (IC_{50}= 60 nM). In this assay, ZD6474 at concentrations of 10-500 nM reduced all parameters measured by Day 11. The IC_{50} values of ZD6474 for total tubule area, total vessel length, and number of branch points were 92.70 nM, 60.97 nM, and 33.23 nM respectively. The inhibitory effect of ZD6474 on tubule total vessel length was comparable to the IC_{50} values generated against KDR activity and VEGF-

stimulated proliferation in HUVEC, while the inhibitory effect on branching appeared to be more pronounced.

Study title: **ZD6474 pharmacology internal report: Chronic and acute effects of ZD6474, a VEGF receptor tyrosine kinase inhibitor, on established human tumour xenografts**
Study no.: 05-report
Study report location: M4.2.1.1

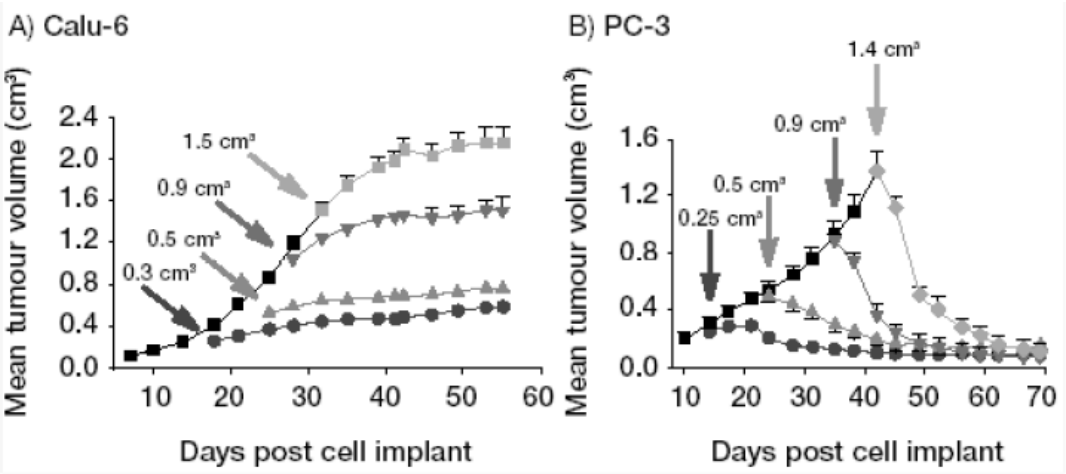
This study examined the effects of ZD6474 on the growth of large established human tumor xenografts in nude mice. Mice with large, established human tumors (PC-3 from prostate, A549 from lung, A431, and Calu-6 from lung) with volumes greater than 0.65 cm^3 were treated daily with vehicle or 100 mg/kg/day ZD6474 orally for the duration of the experiment. To determine the effect of ZD6474 on different tumor volumes, mice bearing Calu-6 or PC-3 tumors were selected for treatment at various time points as tumor growth advanced in additional experiments. The figures below show the effects of ZD6474 on tumor growth of human tumor xenografts. Once daily treatment with ZD6474 inhibited growth in large PC-3, A549, A431, and Calu-6 tumors and induced regression in PC-3, A549, and A431 tumors (Graph 1). Treatment with ZD6474 also inhibited the growth of Calu-6 human lung and PC-3 human prostate cancer xenografts of various sizes (Graph 2).

Graph 1: Effect of ZD6474 on tumor volume of well-established large human tumor xenografts
(excerpted from sponsor's submission)



Arrows indicate start of treatment
Animals randomised to continue to receive vehicle alone (control) or ZD6474 (100 mg/kg/day)
■ denotes vehicle dosed group; ● denotes ZD6474 100 mg/kg/day dosed group
Data points represent mean volume of 5 (PC-3) or 9-10 (A549, A431, Calu-6) tumours ± standard error

Graph 2: Effect of tumor volume on the anti-tumor effect of ZD6474 in well-established human tumor xenografts
(excerpted from sponsor's submission)



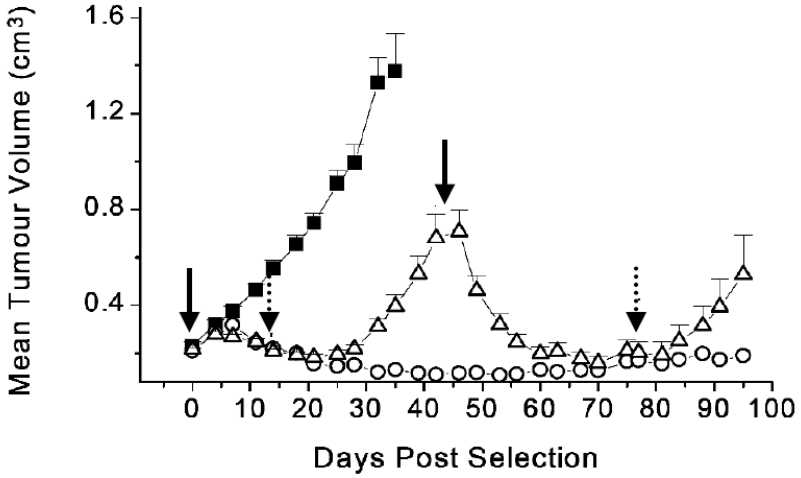
Arrows indicate start of ZD6474 treatment
Data points represent means for 7-10 mice ± standard error

Study title: **ZD6474 pharmacology internal report: VEGF receptor tyrosine kinase inhibitors as potential anti-tumour agents**
Study no.: 06-report
Study report location: M4.2.1.1

This study investigated the effects of ZD6474 treatment, treatment withdrawal, and ZD6474 re-treatment on established PC3 human prostate xenografts in mice. PC-3 tumor xenografts were established in the hind flank of female Swiss athymic (*nu/nu* genotype) mice by subcutaneous injection of 1 x 10^6 cells in 100 µl of 50:50 Matrigel and serum-free media. Treatment with ZD6474 began when tumors reached a volume of 0.15-0.34 cm^3. Mice were dosed orally once daily with ZD6474 (50 mg/kg/day) or vehicle at a volume of 0.1 mL/10 g body weight. The mean tumor volumes for intermittent and continuous dosing of ZD6474 in the PC-3 xenograft model are shown in the figure below. Daily treatment with 50 mg/kg/day of ZD6474 inhibited tumor growth in mice bearing PC-3 xenografts and inhibition of tumor growth was maintained with continuous treatment of ZD6474. Re-growth occurred when the treatment was withdrawn in the intermittent treatment group; however, re-treatment with ZD6474 in the intermittent group inhibited tumor growth once again. This indicates that tumors remain responsive to ZD6474 after a period of withdrawal.

Intermittent and continuous dosing of ZD6474 in a PC-3 human prostate tumor xenograft model

(excerpted from Pharmacology written summary)



Open circles= continuous treatment with ZD6474 on Days 0-95
Open triangles= intermittent treatment with ZD6474 on Days 0-14 and Days 42-77
Solid squares= vehicle-treated control group

Study title: **ZD6474 pharmacology internal report: Impact of tumour VEGF expression level on the *in vivo* efficacy of vandetanib (Zactima; ZD6474)**
Study no.: 07-report
Study report location: M4.2.1.1

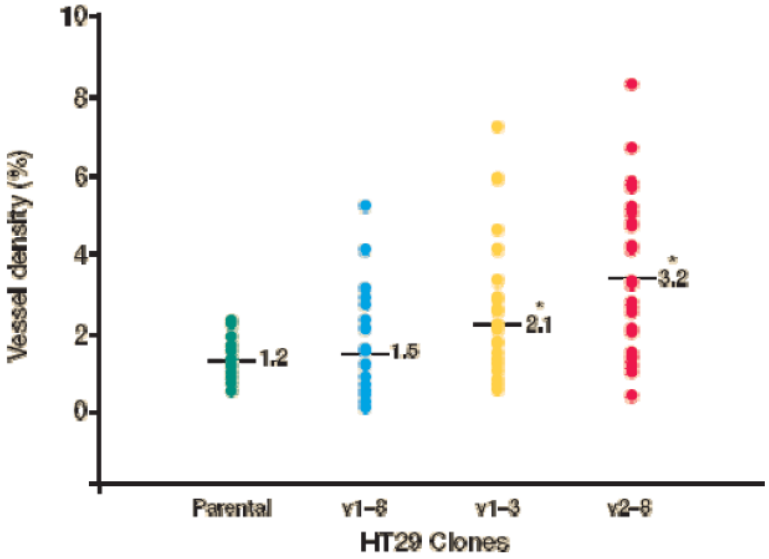
Studies were conducted with ZD6474 to examine how a tumor's inherent level of VEGF expression influences its response to an inhibitor of VEGF signaling. To examine VEGF clones and expression, human colorectal carcinoma (HT29) cells were infected with a recombinant adeno-associated virus vector containing the $VEGF_{165}$ gene and a neomycin resistance gene for selection. Cells (2×10^6 in 2 mL) were plated in 60 mm dishes and following a 24-hour incubation period, the cell culture medium was collected and VEGF expression was assayed by ELISA. Cells clones that expressed varying levels of $VEGF_{165}$ were selected.

In another study, tumors were established with 4 HT29 cell lines expressing different levels of $VEGF_{165}$, by injecting 10^6 parental and clonal cells (v1-8, v1-3, and v2-8) into a single hind limb of athymic nude NCR/nu-nu) mice. The number of days for established tumors (~200 mm^3) to increase in volume by 5 times was recorded. Results indicated that tumors arising from the mid- and high-level VEGF-expressing clones were established more readily and subsequently grew significantly faster than tumors derived from parental HT29 cells. Frozen and formalin-fixed tumor sections were prepared from parental and clonal HT29 tumors (500 mm^3, 3 tumors/group, sizematched) and stained with rat anti-mouse CD31 or hematoxylin and eosin to determine tumor vessel density or degree of necrosis respectively. High VEGF expression levels led to an increase in HT29 tumor vessel density and a decrease in tumor necrotic fraction compared to parental tumors.

The effect of ZD6474 on HT29 tumor angiogenesis was determined in an *in vivo* intradermal assay. Nude mice were treated orally with vandetanib (50 mg/kg/day) for 4 days before

receiving intradermal inoculation with parental or clonal $VEGF_{165}$ HT29 cells (1×10^5). After 3 days, the mice were sacrificed and the skin flap containing the inoculation site was excised. The number of blood vessels intersecting each inoculate was counted. The percentages of vessel density for each group are shown in the figure below.

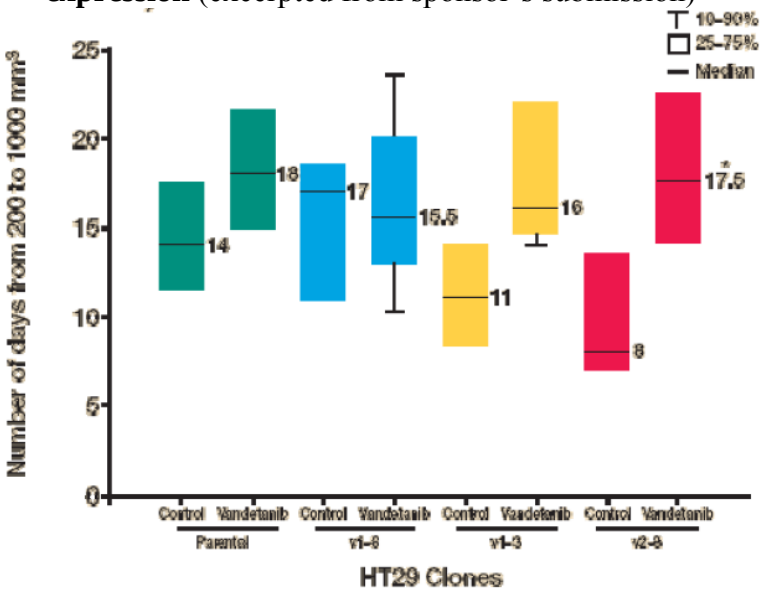
Tumor angiogenesis in parental and clonal HT29 tumors
(excerpted from sponsor's submission)



* = p<0.05 vs parental tumours (Wilcoxon rank sum test)

To determine if ZD6474 treatment would inhibit tumor xenograft growth, mice bearing parental and clonal HT29 tumors (200 mm^3) were treated orally 5 days a week for 2 weeks with vehicle or ZD6474 (25 mg/kg/day). The time required to grow to 5 times the starting volume was recorded. The number of days to grow from 200 to 1000 mm^3 for each group is shown in the figure below. ZD6474 inhibited tumor growth of tumors from parental and cell clones expressing varying levels of $VEGF_{165}$. High VEGF-expressing tumors (v2-8) showed significantly greater inhibition of tumor growth with ZD6474 compared to control than parental tumors.

The effect of ZD6474 on the growth of HT29 tumors with different levels of $VEGF_{165}$ expression (excerpted from sponsor's submission)



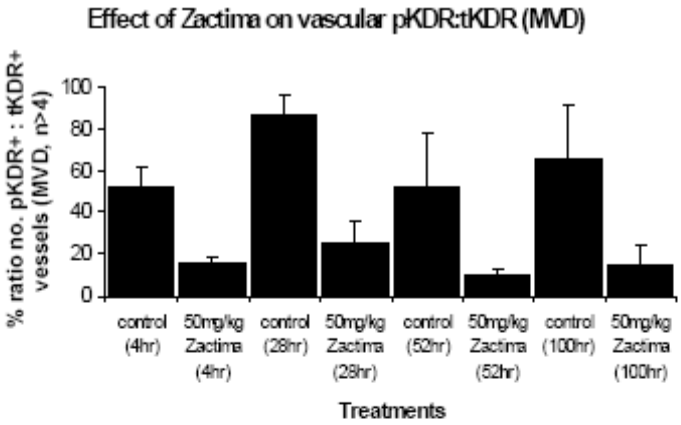
= p<0.05 vs parental tumours (Wilcoxon rank sum test)

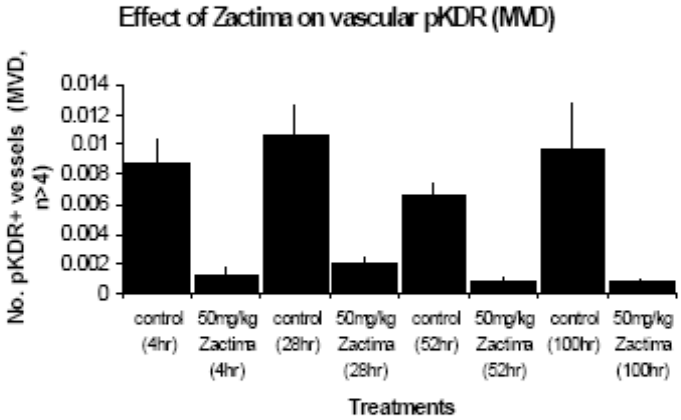
Study title: **ZD6474 pharmacology internal report: ZD6474 inhibits both pVEGFR-2 and pEGFR at clinically achievable levels in pre-clinical models**
Study no.: 08-report
Study report location: M4.2.1.1

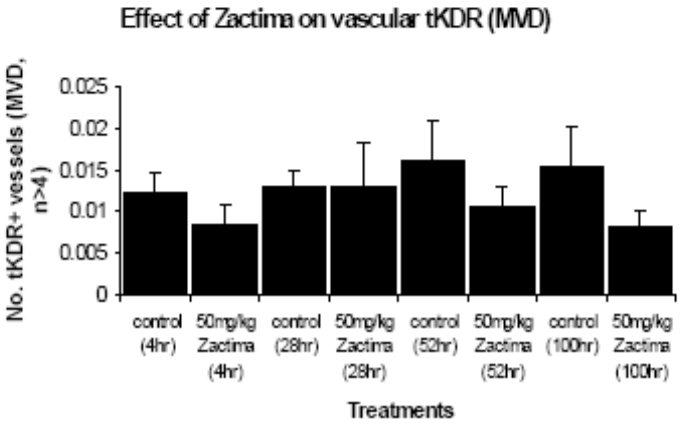
This study assessed the effects of ZD6474 on the expression of pVEGFR-2 and pEGFR levels in paraffin-embedded sections of human lung or colon tumor xenografts. Calu-6 (lung) and LoVo (colon adenocarcinoma) human tumor xenografts (1 cm^3) from mice treated with vehicle or 12.5 and 50 mg/kg ZD6474 for 1, 2, 3, and 5 doses (4, 28, 52, and 100 hours after the first dose, time 0, respectively) were cut into 3 pieces and placed in buffered formalin and embedded in wax. The effect of ZD6474 on the expression of pVEGFR-2 was determined in the Calu-6 model and the effect on pEGFR levels were investigated in the LoVo xenograft model. Tissue samples were stained for pVEGFR-2 or pEGFR by immunohistochemistry.

For the analysis of VEGFR-2 phosphorylation, an analysis threshold was set for both pVEGFR-2 and tVEGFR-2 (phosphorylated and total VEGFR-2) and applied to all treated and control xenograft sections within the study. Viable tissue at the periphery of each tumor (500 µM in from the tumor edge) was selected and analyzed. Data was generated on the number of both pVEGFR-2 and tVEGFR-2 positive vascular structures. Percentage of pVEGFR-2: tVEGFR-2 vascular ratio at the tumor periphery was calculated using the formula: 100 x ((number of pVEGFR-2 positive vascular structures)/ (total area analyzed in μm^2)/(number of tVEGFR-2 positive vascular structures/total area analyzed in μm^2)). The results for VEGFR2 phosphorylation in the Calu-6 xenograft model are shown in the figure below. A dose of 50 mg/kg ZD6474 inhibited VEGFR2 phosphorylation in the Calu-6 lung xenograft model following 1, 2, 3, and 5 doses (4, 28, 52, and 100 hours after the first dose, respectively).

Effects of ZD6474 on phosphorylation of VEGFR-2 in the Calu-6 human lung xenograft model (excerpted from sponsor's submission)

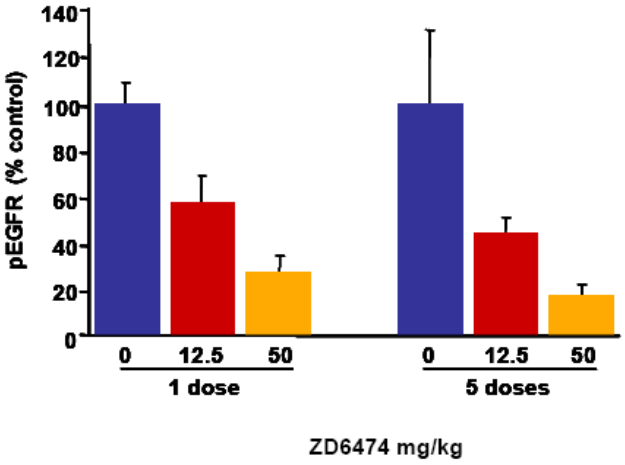






For pEGFR expression, the results were expressed as percentage region brown, which is the amount of pEGFR staining per region examined. ZD6474 inhibited pEGRF staining in the LoVo human colon tumor xenograft model at 50 mg/kg and the decrease was observed after 1 dose and was maintained throughout the study. The pEGFR as a percent of control for treatment with vehicle, 12.5 mg/kg ZD6474, and 50 mg/kg ZD6474 following 1 and 5 doses is shown in the figure below.

Effects of ZD6474 on pEGRF expression in LoVo human colon tumor xenografts
(excerpted from sponsor's submission)



Study title: **ZD6474 pharmacology internal report: Inhibition of EGFR and VEGFR signaling in an oncogenic K-ras mouse model of lung cancer**
Study no.: 09-report
Study report location: M4.2.1.1

This study was conducted to determine the effects of gefitinib and ZD6474 in a conditional oncogenic K-ras-dependent murine model of lung cancer. Male and female LSL-K-ras G12D mice were inoculated with 2.5 x 10^4 PFU recombinant AdCre or media alone (negative control). Inoculations into the lung were performed in MEM media containing 9.8 mM $CaCl_2$ using intratracheal administration. Eight weeks after the administration of AdCre, mice were treated with vehicle, gefitinib (100 mg/kg) or ZD6474 (50 mg/kg) once daily by oral gavage for 8 weeks. Lung tissue was collected for histopathology analysis 16 weeks after AdCre inoculation. The majority of lung samples were represented by 8-11 sections stained with H&E and examined by a pathologist. For each slide, lesion frequency was counted and lesions classified into four different categories according to distinct histological features: atypical adenomatous hyperplasia (AAH), epithelial hyperplasia (EH), adenoma (papillary, solid, or mixed) and adenocarcinoma. Within 2 weeks of AdCre infection Lox-K-ras mice developed AAH of the lung that appeared to give rise to small adenomas by 6 weeks. Epithelial hyperplasia, large adenomas and adenocarcinoma were detected 12 weeks after AdCre infection, along with extensive regions of AAH by 16 weeks. The effects of ZD6474 on lung lesion occurrence in the LSL-K-ras G12D cancer model are shown in the figure below. Treatment with 50 mg/kg ZD6474 reduced the occurrence of all lesion types in Lox-K-ras mice including a 51% decrease in AAH lesions and an 86% reduction in adenomas.

Effects of ZD6474 on lung lesion occurrence in the LSL-K-ras G12D cancer model
(excerpted from sponsor's submission)

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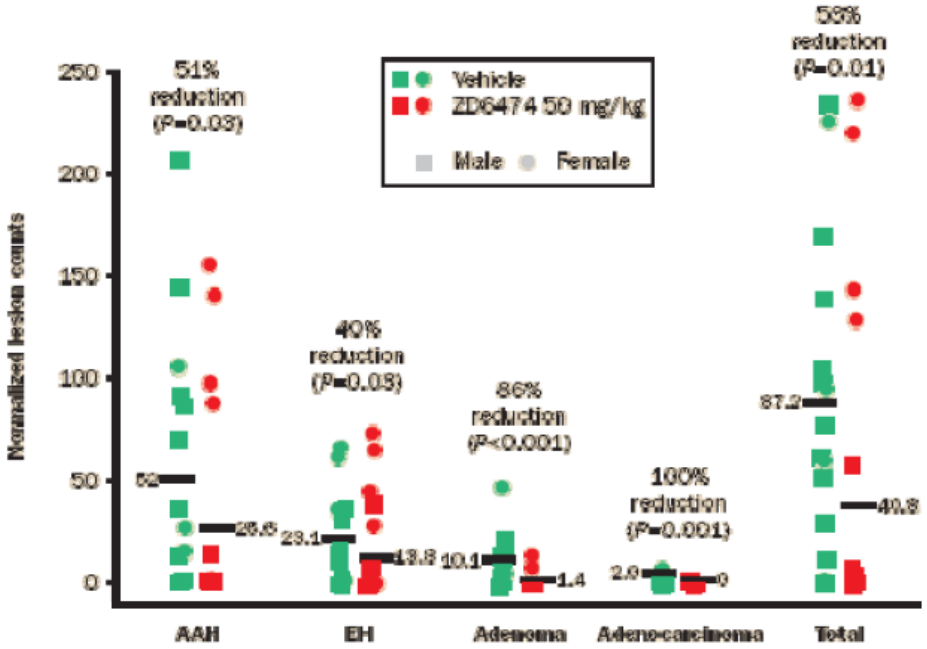
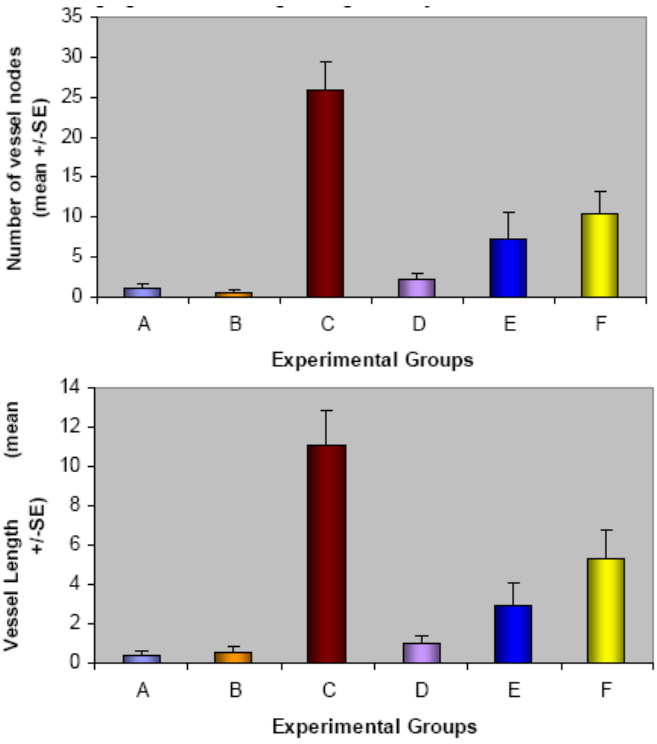


Study title: **Effect of AZ11749412 on VEGF165-induced angiogenesis of matrigel plugs in athymic nude mice**
Study no.: AZJK-4
Study report location: M4.2.1.1

The effect of AZ11749412 on VEGF165-induced angiogenesis, was studied with matrigel plugs in athymic nude mice. Female athymic nude mice were injected subcutaneously with control preparations of matrigel or matrigel containing VEGF165 (150 ng/mL). Mice receiving the matrigel containing VEGF165 were administered vehicle or 12.5, 25, or 50 mg/kg AZ11749412 by oral gavage daily. VEGF165 induced vascularization, and mice treated with AZ11749412 showed reduced vascularization. The figures below show the effects of AZ11749412 on VEGF165-induced angiogenesis, which included a decrease in the number of vessel nodes and vessel length compared to vehicle-treat mice. Therefore, treatment with AZ11749412 showed a dose dependent inhibition of VEGF-induced angiogenesis.

Effects of AZ11749412 on VEGF165-induced angiogenesis of matrigel plugs in athymic nude mice (excerpted from sponsor's submission)

Groups: A) Matrigel alone, vehicle
B) Matrigel + heparin (20 U/mL), vehicle
C) Matrigel + heparin (20 U/mL) + VEGF165 (150 ng/mL), vehicle
D) Matrigel + heparin (20 U/mL) + VEGF165 (150 ng/mL), 50 mg/kg AZ11749412
E) Matrigel + heparin (20 U/mL) + VEGF165 (150 ng/mL), 25 mg/kg AZ11749412
F) Matrigel + heparin (20 U/mL) + VEGF165 (150 ng/mL), 12.5 mg/kg AZ11749412



Study title: **Antitumour activity study of ZD6126 and ZD6474 in combination with Taxotere® in nude mice bearing subcutaneous human MX-1 breast tumours**
Study no.: PRT021381
Study report location: M4.2.1.1

This study was conducted to determine the antitumor activity of ZD6126 and ZD6474 in combination with docetaxel (Taxotere®, TXT) in nude mice bearing SC MX-1 human breast tumors. Five female nude mice were subcutaneously implanted with thawed fragments of MX-1 tumor 24 hours after whole body irradiation with a γ-source (Co^{60}). When tumor sizes reached 700-1000 mm^3, tumors were surgically excised and smaller tumor fragments (20-30 mg) were implanted subcutaneously in the right flank of 120 female nude mice 24 hours after whole body irradiation with a γ-source (Co^{60}). Mice were randomized to form 7 groups (15 mice/group), including groups treated with ZD6126 (150 mg/kg/inj., i.p.), ZD6474 (25 mg/kg, oral), or docetaxel (10 mg/kg/inj., i.v.) alone, groups treated with either ZD6126 or ZD6474 in

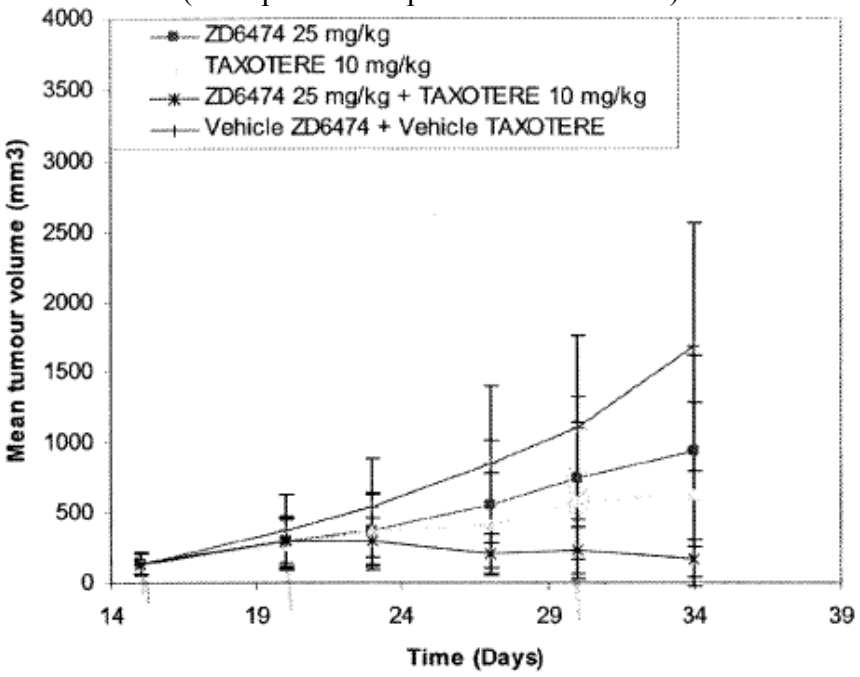
combination with docetaxel, and vehicle groups treated with the vehicles for ZD6126 (PBS with 5% sodium carbonate decahydrate) or ZD6474 (1% polysorbate 80) and the vehicle of docetaxel (polysorbate 80). Drug treatment started on Day 15 when the mean tumor volume reached 134.7±74.4 mm^3. ZD6474 was administered orally once a day for 28 days, and ZD6126 and docetaxel were administered once a week for 4 weeks. For the combination groups, docetaxel was administered 24 hours after ZD6126 administration and 2 hours after ZD6474 administration. The study design is shown in the table below.

Study design
(excerpted from sponsor's submission)

Group	No. animals	Treat.	Route	Dose (mg/kg/inj.)	No. Treat.	Treatment schedule	Combined Treatment	Route	Dose (mg/kg/inj.)	No. Treat.	Interval time between test subt. and TXT adm. (h)	Treat. schedule
1	15	ZD6126	IP	150	4	Q7Dx4	-	-	-	-	-	-
2	15	ZD6474	PO	25	28	Q1Dx28	-	-	-	-	-	-
3	15	TXT	IV	10	4	Q7Dx4	-	-	-	-	-	-
4	15	ZD6126	IP	150	4	Q7Dx4	TXT	IV	10	4	24	Q7Dx4
5	15	ZD6474	PO	25	28	Q1Dx28	TXT	IV	10	4	2	Q7Dx4
6	15	ZD6126 vehicle	IP	-	4	Q7Dx4	TXT vehicle	IV	-	4	24	Q7Dx4
7	15	ZD6474 vehicle	PO	-	28	Q1Dx28	TXT vehicle	IV	-	4	2	Q7Dx4

Treatment with the combination of ZD6474 (25 mg/kg) and docetaxel (10 mg/kg) resulted in significant body weight loss of nude mice bearing SC MX-1 tumors. Body weight was not significantly altered in the other treatment groups. Treatment with docetaxel or ZD6474 alone inhibited tumor growth nude mice bearing SC MX-1 tumors compared to vehicle controls. Mean tumor volumes were slightly lower with treatment of docetaxel alone than ZD6474 alone in this model. The combination of ZD6474 and docetaxel significantly inhibited mean tumor volume compared to vehicle control treatment, with mean tumor volumes lower than treatment with docetaxel or ZD6474 alone. The effects of treatment with ZD6474 alone or in combination with docetaxel on mean tumor volume of nude mice bearing SC MX-1 tumors is shown in the figure below.

Effect of treatment with ZD6474 alone or in combination with docetaxel on mean tumor volume of nude mice bearing SC MX-1 tumors
(excerpted from sponsor's submission)



Study title: Targeted therapy of orthotopic human lung cancer by combined vascular endothelial growth factor and epidermal growth factor receptor signaling blockade
Study no.: Wu et al. 2007, Mol Cancer Ther
Study report location: M4.2.1.1

The effects of ZD6474 on VEGFR2 and EGFR signaling for tumor and endothelial cells *in vitro*, and angiogenesis, vascularization, tumor growth, and metastasis of lung tumors growing orthotopically in mice were investigated in a published study. To characterize the effects of ZD6474 upon VEGFR2 and EGFR activity, MLEC endothelial cells and H441 lung adenocarcinoma cells were pretreated with ZD6474 and briefly stimulated with VEGF or EGF. The western blot analysis is presented in Figure B below and shows that ZD6474 dose-dependently inhibited phosphorylation of VEGFR and EGFR in lung adenocarcinoma cells (H441) and in endothelial cells (MLEC). The direct effect of ZD6474 on migration, invasion, and proliferation of endothelial cells and tumor cells (H441) was studied *in vitro* by treating cells with ZD6474. Results shown in Figure C below indicate that ZD6474 dose-dependently inhibited migration, invasion, and proliferation of HUVEC, HPAEC, MLEC, and H441 cells. It is important to note that these effects on migration and proliferation are simply an effect of ZD6474 since the assay was not conducted with VEGF or EGF stimulation. The effect of ZD6474 on cell survival was investigated MLEC and H441 cells were treated with ZD6474 for 48 hours in the presence or absence of EGF (20 ng/mL) or VEGF165 (20 ng/mL), and apoptosis was determined using the TUNEL assay and cleavage of caspase-3. As shown in Figure D below, ZD6474 (1.25µM) induced apoptosis in MLEC and H441 cells and blocked the protective effects of EGF and VEGF.

Functional analysis of VEGFR2 and EGFR signaling and signaling blockade for human lung cancer and endothelial cells (excerpted from Wu et al. 2007)

The effects of ZD6474 on VEGF- and EGF-induced angiogenesis *in vivo* was investigated in a Gelfoam-agarose sponge assay. To test the effects on microvessel density, gelfoam-agarose sponges containing PBS (control), H441 cell conditioned medium, VEGF (10 ng/mL), or EGF (10 ng/mL) were implanted in mice treated with vehicle or ZD6474 (25 or 50 mg/kg). After 14 days of treatment, sponges were harvested and fixed, and immunohistochemistry with antibodies directed against CD31 was done to determine microvessel density. Results are presented in Figures A and B below and show that treatment with ZD6474 reduced the microvessel density in all implanted sponges (PBS, H441 cell conditioned medium, VEGF, and EGF). To test the effects on vascular permeability, a Miles assay of vascular permeability was conducted in which PBS, H441 cell conditioned medium, VEGF (10 ng/mL), or EGF (10 ng/mL) were injected into the skin of mice treated with ZD6474 for 14 days following intravenous injection of 150 µL of 0.5% Evens blue. Thirty minutes later, the mice were sacrificed, and the skin was removed and photographed. Results are presented in Figures C and D below and show that treatment with ZD6474 decreased vascular permeability induced by H441 cell conditioned medium, VEGF, or EGF.

Effects of ZD6474 on angiogenesis and vascular permeability *in vivo*
(excerpted from Wu et al. 2007)

Study title: **The effects of ZD6474, an inhibitor of VEGF signaling, on cutaneous wound healing in mice**
Study no.: Ko et al. 2005, Journal of Surgical Research
Study report location: M4.2.1.1

The effects of ZD6474 on wound healing in mice was determined by measuring breaking strength in a murine model of cutaneous wound healing in a published study (Ko et al., 2005). Balb/c mice were administered 0, 50 or 100 mg/kg/day ZD6474 by oral gavage once daily starting 7 days before wounding. The wound consisted of two (2 cm) full-thickness horizontal incisions made through the dorsal skin of the mouse through the panniculus carnosus. Treatment of ZD6474 or vehicle continued for a total of 14 or 35 days until 7 or 28 days after wounding respectively, when breaking strength measurements of the wounded skin were performed. Laser Doppler blood flow measurements were also made and microvessel density measurements were performed using computer image analysis of CD31-stained sections. The study design is shown in the figure below.

Results indicated that wound breaking strength was dose-dependently deceased in mice treated with ZD6474 compared to controls at both 7 and 28 days after wounding. The breaking strengths for vehicle and ZD6474-treated mice (50 and 100 mg/kg/day) on Days 7 and 28 after wounding are shown in the figure below. Histological examinations showed that ZD6474-treated mice had a qualitative reduction in the degree of fibrosis and epithelial proliferation at the wound site compared to controls, however, there was no effect of ZD6474 on microvessel density (not shown).

4.2 Secondary Pharmacology

Study title: **AZD6474: Selectivity screening in radioligand binding and enzyme assays *in vitro***
Study no.: 0393SY
Study report location: M4.2.1.2

In vitro radioligand binding and enzyme assays were conducted to assess the pharmacological activity of ZD6474 in a diverse panel of molecular targets including enzymes, receptors, transporters, and ion channels. ZD6474 was initially tested at a single concentration of 1, 10, 30, 100, or 1000 μM in 219 *in vitro* radioligand binding and enzyme assays. Subsequent testing was conducted at four ascending concentrations in $\log_{10}$ intervals to construct concentration-response curves for 21 of these assays, where >50% inhibition was detected when ZD6474 was tested at a single concentration. ZD6474 was also tested at a single concentration of 10 μM in 115 assays and in concentration-response curve with 5 concentrations in half $\log_{10}$ intervals. Data was calculated as % inhibition of activity for enzyme assays or % inhibition of specific binding for radioligand binding assays. For assays in which a concentration-response curve was conducted, IC_{50} values were calculated and K_i and Hill coefficients (n_H) were determined for radioligand binding assays. ZD6474 had significant (defined as >50% inhibition) activity in 59 of the 334 *in vitro* radioligand binding and enzyme assays when tested at a single concentration. The IC_{50} values and K_i and Hill coefficients (n_H) for the 35 of these assays (targets) with concentration response curves are presented in the table below. ZD6474 had an IC_{50} in sub-micromolar

concentrations for adrenergic α_{2A} and α_{2B}, histamine H_1 and H_2, and imidazoline I_2 (central) receptors under the conditions of this assay.

Effect of ZD6474 in *in vitro* radioligand binding and enzyme assays
(excerpted from sponsor's submission)

Target	IC_{50} (μM)	K_i (μM)	n_H
5-HT_{1B}	9.21	8.75	1.1
5-HT_{2A}	2.84	0.813	1.02
5-HT_{2B}	1.87	1.19	0.895
5-HT_{2C}	8.55	4.48	0.912
5-HT_4	9.2	1.53	0.95
Adrenergic α_{1A}	9.6	3.89	1.04
Adrenergic α_{1D}	2.99	1.47	1.11
Adrenergic α_{2A}	0.239	0.0897	0.983
Adrenergic α_{2B}	0.219	0.1	1.03
Adrenergic α_{2C}	4.18	0.607	1.32
Aldose Reductase	33.4	nd	nd
Dopamine D_1	1.51	0.753	0.969
Dopamine D_5	3.54	0.948	0.9
Dopamine Transporter	4.5	3.58	0.902
Fyn	2.21	nd	nd
Histamine H_1	0.142	0.0678	0.979
Histamine H_2	0.733	0.599	0.954
Histamine H_3	7.26	3.23	0.841
Imidazoline I_2, central	0.847	0.565	1.27
L-type Calcium Channel, BZT	4.01	3.56	0.779
L-type Calcium Channel, PHK	5.89	5.72	0.981
Monoamine Transporter	2.33	1.94	0.809
Muscarinic M_1	7.22	1.77	1.26
Muscarinic M_2	9.35	3.93	1.3
Muscarinic M_3	25.3	12.2	1.32
Muscarinic M_4	13.5	2.9	1.23
Muscarinic M_5	11	6.79	1.18
Opiate μ	14.7	5.98	0.763
Opiate κ	5.3	2.12	1.93
PRKCB1 (PKCβ1)	14.7	n.d.	n.d.
Serotonin Transporter	1.51	0.803	0.684

Target	IC_{50} (μM)	K_i (μM)	n_H
Sigma σ_1	10.1	4.23	0.839
Sigma σ_2	5.82	3.58	0.785
Sodium Channel, Site 2	5.12	4.67	1.38
Src	4.58	nd	nd

nd not determined

Study title: **ZD6474: Selectivity screening in radioligand binding and enzyme assays *in vitro***
Study no.: 0585SY
Study report location: M4.2.1.2

In vitro cellular functional assays were conducted to assess the pharmacological activity of ZD6474 at three receptors (histamine H_1, histamine H_2, and adrenergic α_{2c}), as a follow up to significant activity detected in radioligand binding assays in a previous study (0393SY). ZD6474 was tested for both agonism and antagonism of histamine H_1, histamine H_2, and adrenergic α_{2c} receptors in *in vitro* cellular functional assays at 8 concentrations in half-$\log_{10}$ intervals between 0.03 and 100 μM. The data was calculated as % inhibition of reference agonist stimulation for antagonist assays or as % stimulation relative to reference agonist for agonist effect assays. IC_{50} and K_B values were then calculated. Results indicated that ZD6474 inhibited the effect of the reference agonists at all 3 receptors in a concentration-dependent manner. The IC_{50} and K_B values for each receptor are presented in the table below. ZD6474 had no significant agonist effect at histamine H_2 or adrenergic α_{2c} receptors, and had no significant agonist effect at histamine H_1 receptors except at the highest concentration tested. A concentration of 100 μM ZD6474 produced 62% stimulation of histamine H_1, with an EC_{50} value of 93 μM.

Effect of ZD6474 in *in vitro* cellular functional assays: Antagonism
(excerpted from sponsor's submission)

Target	IC_{50} (μM)	K_B (μM)
Histamine H_1	8.8	1.9
Adrenergic α_{2C}	13	0.33
Histamine H_2	33	3.3

4.3 Safety Pharmacology

Neurological effects:

Study Title: ZD6474: Functional Observational Battery in the Han Wistar Rat following Single Oral Administration

Key findings:
Single doses of ZD6474 (0, 40, 200 or 1000 mg/kg) were administered as a single oral dose to male Wistar Han rats and a functional observational battery was assessed 4-hours post-dose. Mid and high-doses (200 and 1000 mg/kg) of ZD6474 resulted in lower body weight gain, slow pupil response and reduced activity levels in open field assessments. Decreased landing foot splay, reduced grip strength and piloerection occurred in the group receiving 1000 mg/kg ZD6474. Rats in each ZD6474-treated group had reduced approach response. ZD6474 appears to have impaired aspects of the autonomic and neuromuscular function under the conditions of this experiment.

Study number: 1228SR
Study report location: M4.2.1.3
Conducting laboratory and location: Safety Assessment UK, Astra Zeneca Macclesfield, England
Date of study initiation: June 1, 2006
GLP compliance: Yes (X) No ()
QA report: Yes (X) No ()
Drug, Lot number, purity: ZD6474, C357/7, 99.86%
Species: Male Han Wistar rats (AlpkHsdBrlHan;WIST)
Body weight and age : 239 to 262 g at 56 to 57 days old
Treatment:

ZD6474 Dose (mg/kg)	Number of Rats*
0	6
40	6
200	7
1000	5

*uneven number distribution among dose groups due to mis-dosing

Dose volume: 10 mL/kg
Vehicle: 0.5% w/v Hydroxypropyl methylcellulose (HPMC) containing 0.1% w/v Polysorbate 80

Results
Male rats received a single dose of vehicle, 40, 200, or 1000 mg/kg ZD6474 by oral gavage. A Functional Observation Battery (FOB) was performed 4 hours after dosing and findings were considered noteworthy when 3 rats displayed an activity or behavior that was not present in the vehicle- treated group or when numerical data from ZD6474-treated rats reached statistical significance ($p<0.05$).

			ZD6474 (mg/kg)			
			0	40	200	1000
	Parameter	Description	6 rats	6 rats	7 rats	5 rats
Sensorimotor	Approach Response	No reaction		3	1	1
	Grasping reflex	Grasp w/o manuevering hindlimbs onto bar			1	
		Not reaching for bar			1	
	Pupil response	Remains dilated or slow constriction			2	1
Landing food splay	Distance b/w feet (cm)	Pre-dose minus post-dose	-0.7	0	-0.6	-2.9*
Grip strength	Strength post-dose (g)		353.2	396	357.1	329
	Strength diff. pre- vs. post-dose (g)		32.2	79.5	21.1	-44
Open Field Activity	Time to exit circle (s)		61	136.8*	152.9*	133*
	Line crossings (counts)		9.2	3	0.7*	0.4*
	Supported rears (counts)		2.5	0.5	0.1*	0*
Body Weight	Weight gain pre- vs. post-dose (g)		0	-3.3	-8.4***	-12***
Misc.	Piloerection					5 rats

*P<0.05, ** P<0.01, ***P<0.001

Body temperature was unaffected by ZD6474 treatment at 4 hours post-dose.

Study Title: ZM382,561: Multi-observation test in the mouse

Key findings:
A single dose of 50 mg/kg ZM382,651, (also known as ZD6474) resulted in no changes in behavior and a mild increase in rectal temperature that was not statistically significant. This dose used in this study (≈150 mg/m^2) is approximately 0.81 times the 185 mg/m^2 dose that will be administered daily to humans.

Study number: TSM1124
Study report location: M4.2.1.3
Conducting laboratory and location: Zeneca Pharmaceuticals
Safety of Medicines Department
Cheshire, England
Date of study initiation: August 24, 1998
GLP compliance: Yes (X) No ()
QA report: Yes () No (X)
Drug: ZM382,561, Batch #13, purity not determined

Species: Alpk:APfCD-1 (AP mouse) mice
Dose volume: 10 mL/kg
Vehicle: 0.5% w/v hydroxypropyl methyl cellulose in 0.1% aqueous polysorbate 80

Results

Five male mice were treated with a single oral dose of ZM382,561 or vehicle. The mice were observed for changes in behavior and rectal temperature during a pre-dose period and for 1 hour post-dose. The study monitored for changes in muscle tone, stereotypy, straub tail, salivation, ptosis, tremors, righting reflex, mydriasis, convulsions, respiration, locomotor activity and lethality.

The sponsor noted that no abnormal behavioral signs occurred in the group that was administered ZM 382,561. The ZM 382,561-treated group had a mild increase in rectal temperature which did not reach statistical significance.

	Rectal temperature	
	Pre-dose	60 minutes post-dose
Vehicle	35.12 +/- 0.22	35.08 +/-0.41
50 mg/kg ZM382,561	34.62 +/- 0.45	35.9 +/- 0.59

Cardiovascular effects:

Study Title: The effect of ZD6474 on hERG potassium channel

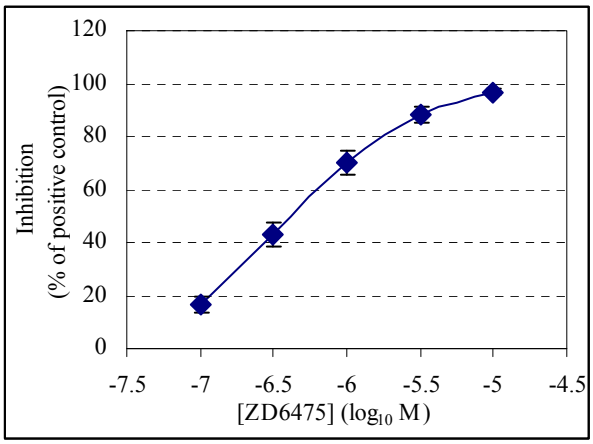
Key findings:
ZD6474 inhibited the hERG channel with -p[IC]$_{50}$=6.4 $\pm$ 0.1 which equates to 0.4 µM or 190 ng/mL under the conditions of this valid experiment.

Study number: TSZ36
Study report location: M4.2.1.3
Experiment initiation: July 5, 2001
Positive control: $3x10^{-6}$ M AR-C155039XX (dofetilide)
Vehicle: DMSO (0.1%)
Cell line: hERG-expressing human embryonic kidney cells (HEK)
Concentrations of ZD6474: $1x10^{-7}$ to $1x10^{-5}$ M in half-log increments

Results

HEK cells expressing the hERG potassium channel were tested by patch clamp technique to determine if ZD6474 inhibits the flow of potassium ions through the channel. Data are expressed as a percentage of the inhibition demonstrated by the positive control, $3x10^{-6}$ M AR-C155039XX.

hERG channel inhibition by ZD6474



Data expressed as mean of 5 recordings +/- SEM

ZD6474 inhibited the hERG channel with a -p[IC]$_{50}$ = 6.4 $\pm$ 0.1 which equates to 0.4 µM or 190 ng/mL.

Study Title: Study report for the effect of M382558 and M447882 on hERG potassium channel

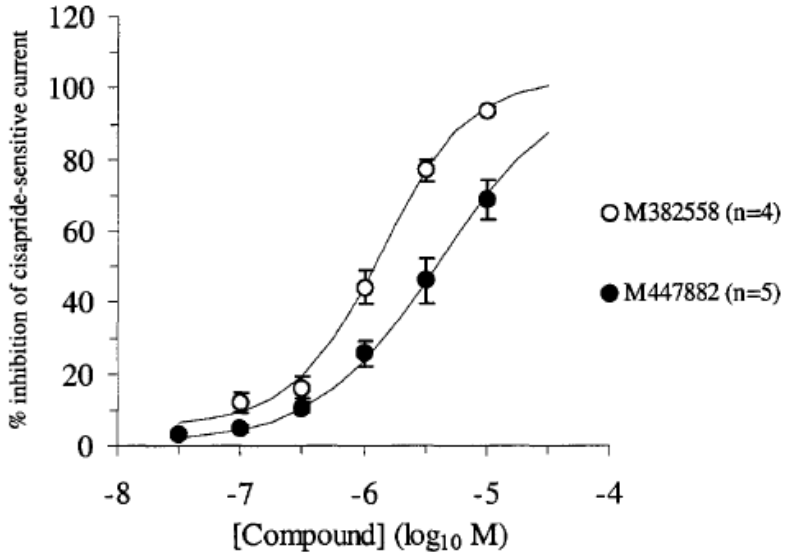
Key findings:
The N-desmethyl metabolite of ZD6474 (M382558) inhibited the hERG channel with a -p[IC]$_{50}$ = 5.9 $\pm$ 0.1 which equates to 1.3 µM or 600 ng/mL, and the N-oxide metabolite of ZD6474 inhibited the hERG channel with a -p[IC]$_{50}$ = 5.4 $\pm$ 0.1 which equates to 4.0 µM or 2038 ng/mL.

Study number: 0048SZ
Study report location: M4.2.1.3
Experiment initiation: unknown; Study dates: July, 16, 18, and 26, 2002
Positive control: 3x10^{-6} M cisapride
Vehicle: DMSO (0.1%)
Cell line: hERG-expressing human embryonic kidney cells (HEK)
Concentrations of M382558 and M447882: 3.16 x10^{-8} to 1x10^{-5} M in half-log increments

Results

HEK cells expressing the hERG potassium channel were tested by patch clamp technique to determine if the N-desmethyl (M382558) and N-oxide (M447882) metabolites of ZD6474 inhibit the flow of potassium ions through the channel. Data are expressed as a percentage of the inhibition produced by the positive control, 3x10^{-6} cisapride. The mean concentration-effect curves for the N-desmethyl (M382558) and N-oxide (M447882) metabolites of ZD6474 are shown in figure below. The N-desmethyl metabolite of ZD6474 (M382558) inhibited the hERG channel with a -p[IC]$_{50}$ = 5.9 $\pm$ 0.1 which equates to 1.3 µM or 600 ng/mL, and the N-oxide metabolite of ZD6474 inhibited the hERG channel with a -p[IC]$_{50}$ = 5.4 $\pm$ 0.1 which equates to 4.0 µM or 2038 ng/mL.

Mean concentration-effect curves for M382558 and M447882
(excerpted from sponsor's submission)



Study Title: ZD6474 and Ondansetron: The Effect on hERG Potassium Channel

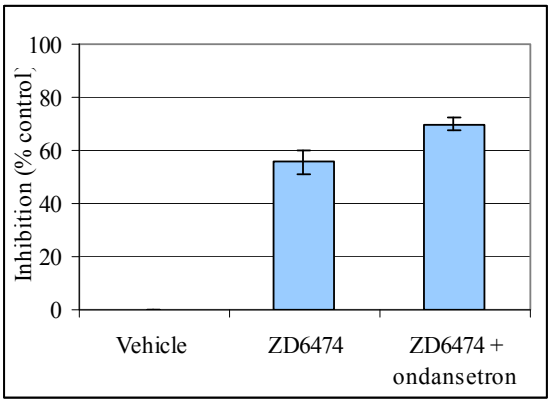
Key findings:
A combination of ZD6474 and ondansetron (at their respective IC_{50}) resulted in 70% inhibition of the hERG channel. Co-administration of ZD6474 and ondansetron inhibited the hERG channel more than either agent alone, but was sub-additive in extent of inhibition under the conditions of this assay.

Study number: 0102SZ
Study report location: M4.2.1.3
Experiment initiation: October 14, 2003
Positive control: $3x10^{-6}$ M cisapride
Negative control / Vehicle: DMSO (0.1%) / Saline (0.9% NaCl)
Cell line: hERG-expressing Chinese Hamster Ovary (CHO) cells
Concentrations of test articles for combination experiment:
ZD6474 : $4x10^{-7}$, the IC_{50} from previous study reports
Ondansetron: $1x10^{-6}$, the IC_{50} calculated from data in this study report

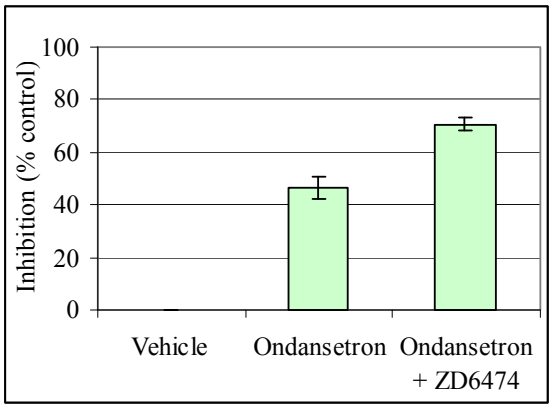
Rationale: ZD6474 may be co-administered with anti-emetics in the clinic. The sponsor tested the combination of ZD6474 and ondansetron, an anti-emetic that binds to the hERG channel. Both compounds were tested at previously determined IC_{50} values.

Results

hERG channel expressing CHO cells treated with ZD6474 prior to ondansetron*



hERG channel expressing CHO cells treated with ondansetron prior to ZD6474



*Data normalized to the inhibition of hERG current from $3x10^{-6}$ M cisapride

Study Title: Evaluation of effect on cardiac action potential in isolated canine purkinje fibres

Key findings:
Concentrations of 1 and 10 µM ZD6474 lengthened the action potential duration (APD) at 50, 70 and 90% of repolarization in canine isolated cardiac Purkinje cells at stimulation frequencies of 0.3 and 1 Hz under normal and low potassium Tyrode's buffers. The prologation was greater at 0.33 Hz stimulation and in low-potassium buffer. ZD6474 did not affect the resting potential, action potential amplitude or maximal rate of depolarization (Vmax) at any dose tested. ZD6474 appeared to have affected the cardiac potassium channels under the conditions of this assay.

Reference number/Study number: TSD1293/20010596
Study report location: M4.2.1.3
Conducting laboratory and location: (b) (4)

Date of study initiation: December 7, 2001
GLP compliance: Yes (x) No ()
QA report: Yes (x) No ()
Drug, lot #: ZD6474, Lot C268/1 Batch ADM65949A99,
% purity: 99% pure
Positive control: $3x10^{-7}$ Cisapride
Negative control: DMSO (0.1%)
Cell line: isolated canine Purkinje fibers obtained from 5 male Beagle dogs

The effect of ZD6474 on transmembrane action potential in isolated canine Purkinje cells was tested in concentrations ranging from 0.1×10^{-6} to 10×10^{-6} M in both normal and low-potassium Tyrode's buffer. The study used an intracellular microelectrode to evaluate action potential amplitude (APA), resting potential (RP), maximal rate of depolarization (Vmax) and Action Potential Duration (APD 50, APD70 and APD90) at 5 minute intervals.

The sponsor chose this concentration range because 28 days of 300 mg ZD6474 dosing in humans resulted in a C_{max} of 1457 ng/mL (3.07 µM) at steady state. Calculating for 90% protein binding, the sponsor estimates that the free-drug concentration is 0.31 µM in humans. The high concentration in this experiment is approximately 30 times higher than this calculated human exposure.

Results

(the following tables and graphs were excerpted from the sponsor's study report)

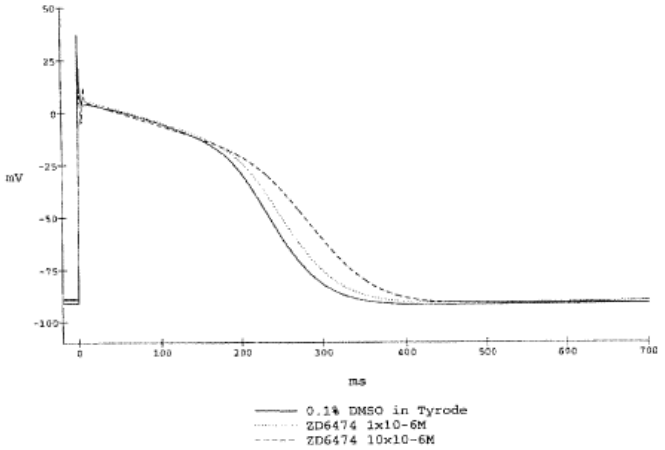
EFFECTS OF ZD6474 AND CISAPRIDE ON CARDIAC ACTION POTENTIAL IN ISOLATED CANINE PURKINJE FIBRES UNDER NORMAL STIMULATION RATE (1 Hz) DATA OBTAINED IN "NORMAL POTASSIUM" TYRODE'S SOLUTION

TABLE 1

TREATMENT		APA (mV)	RP (mV)	Vmax (V/s)	APD50 (ms)	APD70 (ms)	APD90 (ms)
Predose values (Tyrode)	Mean	125	-91	450	206	246	284
	SEM	3	0	43	12	10	10
	N	6	6	6	6	6	6
0.1% DMSO in Tyrode	Mean	2	0	44	-5	-5	-5
	SEM	3	0	29	4	2	2
	N	6	6	6	6	6	6
ZD6474 0.1×10^{-6} M	Mean	1	-1	32	-4	-4	-5
	SEM	4	0	32	6	3	3
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
ZD6474 0.3×10^{-6} M	Mean	-2	1	38	-2	-2	-1
	SEM	4	1	51	5	4	3
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
ZD6474 1×10^{-6} M	Mean	-1	0	30	7	9	12
	SEM	5	0	43	4	3	3
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	**
ZD6474 10×10^{-6} M	Mean	-1	0	-47	14	34	50
	SEM	6	0	43	7	5	5
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	**	**
Tyrode	Mean	-2	0	1	38	60	77
	SEM	5	0	43	8	7	6
	N	6	6	6	6	6	6
Cisapride 3×10^{-6} M	Mean	-5	0	-32	43	69	90
	SEM	5	0	46	12	10	9
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS

Predose values: control period with Tyrode.
ppm: pulses per minute.
APA: action potential amplitude.
RP: resting potential.
Vmax: maximal rate of depolarisation.
APD50: action potential duration at 50% of repolarisation.
APD70: action potential duration at 70% of repolarisation.
APD90: action potential duration at 90% of repolarisation.
Mean: mean value.
SEM: Standard Error of the Mean.
N: number of preparations.
Results are expressed as variation calculated in relation to values measured during the control period (Tyrode perfusion).
NS: $P > 0.05$, **: $P \leq 0.01$ when compared to the vehicle control period (0.1% DMSO in Tyrode): analysis of variance with NEWMAN KEULS test if $P \leq 0.05$.
Note: values of APA, RP, Vmax, APD50, APD70 and APD90 were analysed 25 minutes after starting each infusion period.

Effect of ZD6474 on cardiac action potential in isolated canine purkinje fibers under normal stimulation rat (1Hz) in normal potassium Tyrode's buffer -Representative tracing



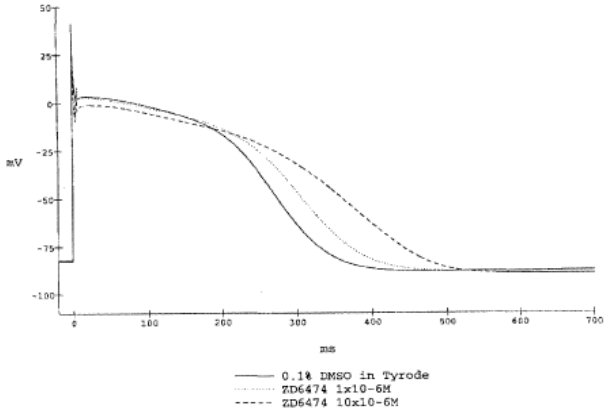
EFFECTS OF ZD6474 AND CISAPRIDE ON CARDIAC ACTION POTENTIAL IN ISOLATED CANINE PURKINJE FIBRES UNDER LOW STIMULATION RATE (0.33 Hz)
DATA OBTAINED IN "NORMAL POTASSIUM" TYRODE'S SOLUTION

TABLE 2

TREATMENT		APA (mV)	RP (mV)	Vmax (V/s)	APD50 (ms)	APD70 (ms)	APD90 (ms)
Predose values (Tyrode)	Mean	119	-85	450	223	276	317
	SEM	4	1	41	15	12	12
	N	6	6	6	6	6	6
0.1% DMSO in Tyrode	Mean	-1	0	18	-5	-8	-6
	SEM	4	2	41	4	4	4
	N	6	6	6	6	6	6
ZD6474 0.1x10⁻⁶M	Mean	-3	-1	-32	-1	-4	0
	SEM	4	1	51	8	5	4
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
ZD6474 0.3x10⁻⁶M	Mean	-4	0	23	2	-1	1
	SEM	5	2	36	5	4	3
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS
ZD6474 1x10⁻⁶M	Mean	-1	0	43	13	20	26
	SEM	6	1	26	3	3	4
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	*	**
ZD6474 10x10⁻⁶M	Mean	-1	-1	-20	23	58	93
	SEM	7	2	45	13	12	11
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	**	**
Tyrode	Mean	-7	-1	-30	68	118	154
	SEM	6	2	47	14	18	20
	N	6	6	6	6	6	6
Cisapride 3x10⁻⁶M	Mean	-7	0	9	79	123	160
	SEM	4	2	62	25	24	26
	N	6	6	6	6	6	6
	P	NS	NS	NS	NS	NS	NS

Predose values: control period with Tyrode.
ppm: pulses per minute.
APA: action potential amplitude.
RP: resting potential.
Vmax: maximal rate of depolarisation.
APD50: action potential duration at 50% of repolarisation.
APD70: action potential duration at 70% of repolarisation.
APD90: action potential duration at 90% of repolarisation.
Mean: mean value.
SEM: Standard Error of the Mean.
N: number of preparations.
Results are expressed as variation calculated in relation to values measured during the control period (Tyrode perfusion).
NS: $P > 0.05$, *: $P \leq 0.05$, **: $P \leq 0.01$ when compared to the vehicle control period (0.1% DMSO in Tyrode): analysis of variance with NEWMAN KEULS test if $P \leq 0.05$.
Note: values of APA, RP, Vmax, APD50, APD70 and APD90 were analysed 30 minutes after starting each infusion period.

Effect of ZD6474 on cardiac action potential in isolated canine purkinje fibers under normal stimulation rat (1Hz) in normal potassium Tyrode's buffer -Representative tracing



Purkinje cells treated with either 1 or 10 µM ZD6474 had statistically significant prolongation of action potential (AP) duration, a trend that was also apparent at 0.3 µM ZD6474 that did not reach statistical significance. AP prolongation was greater at 0.33 Hz than at 1 Hz stimulation. Prolongation was also greater in low-potassium Tyrode's solution than in buffer with normal potassium levels (data not shown).

Bioanalytical evaluation of samples confirmed the concentration of ZD6474 in each sample. Cisapride did not have the expected prolongation of action potential duration subsequent to ZD6474 treatment therefore some interaction between these compounds may have occurred.

Study Title: Telemetry report: The effect of ZD6474 on systolic and diastolic blood pressure in rats

Key findings:
Mean systolic and diastolic blood pressures were dose-dependently increased with single doses of 12.5 and 50 mg/kg ZD6474. Seven daily treatments with 12.5 mg/kg caused greater increases in systolic and diastolic blood pressure when compared to a single dose suggesting that daily dosing had a cumulative effect on BP in rats. Changes in blood pressure with ZD6474 treatment may be linked to effects of VEGF inhibition on vasculature.

Study number: Internal report #10
Study report location: M4.2.1.3
Conducting laboratory and location: [redacted] (b) (4)

Date of study initiation: August 5th, 2
GLP compliance: Yes () No (X)
QA report: Yes () No (X)
Drug: ZD6474, batch and purity not documented
Species: Male Wistar rats
Groups: 3/dose
Dose volume:

Vehicle: 1% polysorbate in water
Treatments:

Dose (mg/kg)	Schedule
12.5	Single
50	Single
12.5	Daily x 7

Results

Conscious male Wistar rats were dosed with ZD6474 by oral gavage. Blood pressure alterations were monitored using implanted radio telemetry equipment. The mean systolic and diastolic values were calculated from data spanning 40 minutes to 6 hours post-dosing. All animals were dosed with vehicle to gain a pre-dose baseline value. Changes in BP after ZD6474 treatment were then calculated as the difference between post-dose and baseline values. (the following table was adapted from the sponsor's study report).

ZD6474 increases systolic and diastolic blood pressure in male Wistar rats

ZD6474 dose (mg/kg)	Number of rats	# of doses	Change in Mean Systolic BP (mmHg)	Change in Mean Diastolic BP (mmHg)
50	3	1	13	11
12.5	3	1	5	5
12.5	3	7	12	13

The sponsor reports that heart rate was marginally decreased with ZD6474 treatment, though primary data was not submitted. Reduced heart rate may be functionally linked to the increase blood pressure after drug treatment.

Study Title: Zeneca ZD6474: Cardiovascular effects of ZD6474 in conscious, telemetered, beagle dogs
Study number: TKD1045
Study report location: M4.2.1.3
Previously reviewed in IND 60042 by Wendelyn Schmidt, Ph.D.

Study Title: ZD6474: Hemodynamic Effects in Anesthetized, Beagle Dogs

Key findings:
ZD6474 caused both QTcV prolongation and dose-dependent increases in T wave amplitude and polarity. Increases in QTcV (up to 15% compared to baseline values) were observed with doses of $\geq$ 2.0 mg/kg ZD6474. ZD6474 caused dose-dependent increases in T wave amplitude and polarity, with increases observed at doses $\geq$ 6.7 mg/kg and slight changes occasionally observed at lower doses (0.67 and 20 mg/kg). Additionally, PR intervals in the ZD6474-treated group were statistically longer compared to vehicle at many time points.

Study number: 0276SD
Study report location: M4.2.1.3

Conducting laboratory and location: Safety Assessment UK, AstraZeneca Macclesfield, England
Date of study initiation: November 5, 2003
GLP compliance: Yes (X) No ()
QA report: Yes (X) No ()
Drug, lot #, purity: ZD6474, ADM90862F02, 99.8 % pure
Species: Beagle Dogs
Weight and age: 9.25 to 13.21 kg at 10 to 14 months of age
Groups: 4 males in vehicle and 4 males in ZD6474-treated groups
Vehicle: Water for Injection containing 15% w/v hydroxypropyl-b-cyclodextrin and 1.6% w/v mannitol, adjusted to pH 7

Treatments: Beagle dogs were treated with either 5 doses of vehicle or 5 escalating doses of ZD6474 as noted below. (Table excerpted from sponsor's study report)

Table 3 Group 2 (ZD6474) dose levels and infusion rates

Dose	Dose levels							
	Bolus Dose			Continuous Infusion Dose (over 45 min)				Total Dose
	Dose level mg/kg	Formulation concentration mg/ml	Dose volume ml/kg	Infusion rate mg/kg/h	Infusion dose mg/kg	Formulation concentration mg/ml	Dose volume ml/kg	Dose level mg/kg
Dose 1	0.16	0.25	0.64	0.058	0.044	0.025	1.74	0.2
Dose 2	0.52	0.25	2.1	0.194	0.146	0.25	0.58	0.67
Dose 3	1.57	2.5	0.63	0.581	0.436	0.25	1.74	2.0
Dose 4	5.24	2.5	2.1	1.94	1.46	2.5	0.58	6.7
Dose 5	10.5	2.5	4.2	3.88	2.91	2.5	1.16	13.4

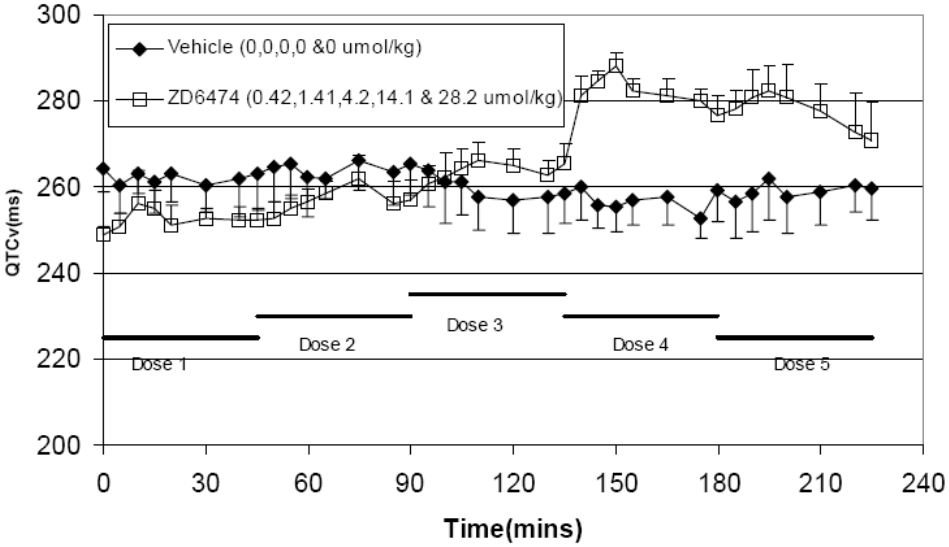
Time points: Hemodynamic parameters were measured at -20, -10, 0 (Start of infusion), 5, 10, 15, 20, 30, 40 and 45 minutes (end of infusion).

Results

Blood pressure, heart rate, left ventricular pressure, lead II ECG and blood flows were monitored in anesthetized beagle dogs after intravenous administration of ZD6474 as ascending doses (0.2, 0.67, 2, 6.7 and 13.4 mg/kg ZD6474). Parameters were evaluated for 45 minutes during infusion and the next dose was infused without a recovery period from the prior dose. Five doses were administered on a single day.

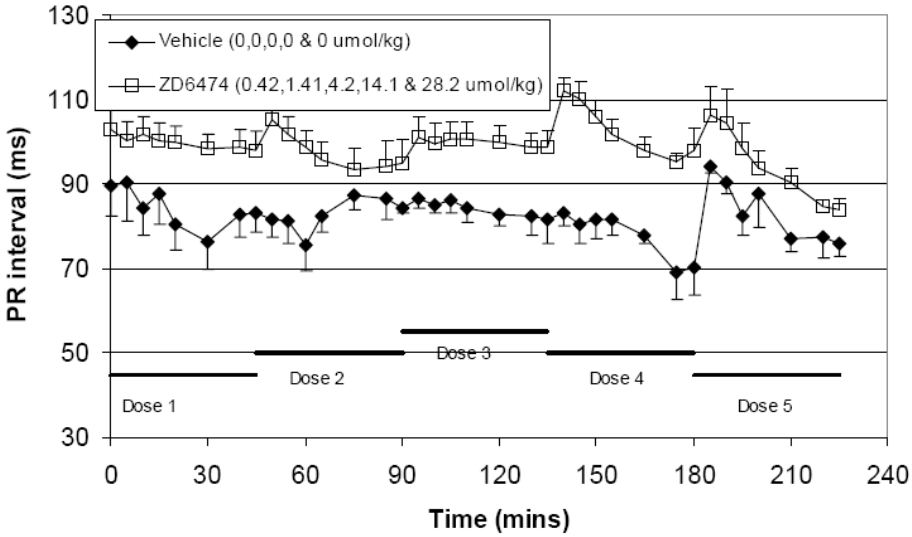
Drug exposure in animals showed significant inter-animal variation. ZD6474 had no clear effect on arterial blood pressure in this study. Results from the ECG and waveform analysis indicate that ZD6474 increased QTcV and PR intervals. Increases in QTcV (up to 15% compared to baseline values) were observed with doses of $\geq$ 2.0 mg/kg ZD6474. ZD6474 also caused dose-dependent increases in T wave amplitude and polarity, with increases observed at doses $\geq$ 6.7 mg/kg and slight changes occasionally observed at lower doses (0.67 and 2.0 mg/kg). PR intervals in the ZD6474-treated group were statistically longer compared to the vehicle group at many time points. The effects of vehicle and ZD6474 on QTcV interval and PR interval are shown in the figures below.

Effects of Vehicle and ZD6474 on QTcV interval
(excerpted from sponsor's submission)



Dose 1= 0.2 mg/kg, Dose 2= 0.67 mg/kg, Dose 3= 2.0 mg/kg, Dose 4= 6.7 mg/kg, Dose 5= 13.4 mg/kg

Effects of Vehicle and ZD6474 on PR interval
(excerpted from sponsor's submission)



Dose 1= 0.2 mg/kg, Dose 2= 0.67 mg/kg, Dose 3= 2.0 mg/kg, Dose 4= 6.7 mg/kg, Dose 5= 13.4 mg/kg

Study Title: ZD6474 and Ondansetron: QTc investigations in anaesthetised, beagle dogs (pilot study)

Key findings:
Treatment with ZD6474 caused a dose-related increase in QTcV interval and an increase in blood pressure. Additionally, ZD6474 caused a dose-dependent increase in T wave amplitude, which may be related to the QTcV prolongation. Ondansetron also caused a dose-related increase in QTcV interval.

Study number: 0257SD
Study report location: M4.2.1.3
Conducting laboratory and location: Safety Assessment UK, AstraZeneca Macclesfield, England
Date of study initiation: July 18, 2003
GLP compliance: Yes () No (X)
QA report: Yes () No (X)
Drug, lot #, purity: ZD6474, ADM90862F02, 99.5 % pure
Ondansetron, GA0021A
Species: Beagle Dogs
Weight: 11.58 to 14.68 kg
Groups: 2 males/group in ondansetron and ZD6474-treated groups
Vehicle: ZD6474: Water for Injection containing hydroxypropyl-β-cyclodextrin, mannitol, and 0.1 M HCl
Ondansetron: 0.9% w/v NaCl
Treatments: Beagle dogs were treated with either 3 escalating doses of ondansetron or 3 escalating doses of ZD6474 as noted below. (Tables excerpted from sponsor's study report)

Table 1 Ondansetron dose levels and infusion rates (Group 1)

Dose	Ondansetron Dose levels (as base compound)							
	Bolus Dose			Continuous Infusion Dose (over 45 min)				Total Dose
	Dose Level mg/kg	Formulation Concentration mg/ml	Dose Volume ml/kg	Infusion rate mg/kg/h	Infusion dose mg/kg	Formulation Concentration mg/ml	Dose Volume ml/kg	Dose Level mg/kg
1	0.525	0.25	2	0.64	0.48	0.25	2	1
2	0.525	0.25	2	1.28	0.96	0.5	2	1.5
3	1.05	0.5	2	2.96	2.2	1	2	3

Table 2 ZD6474 dose levels and infusion rates (Group 2)

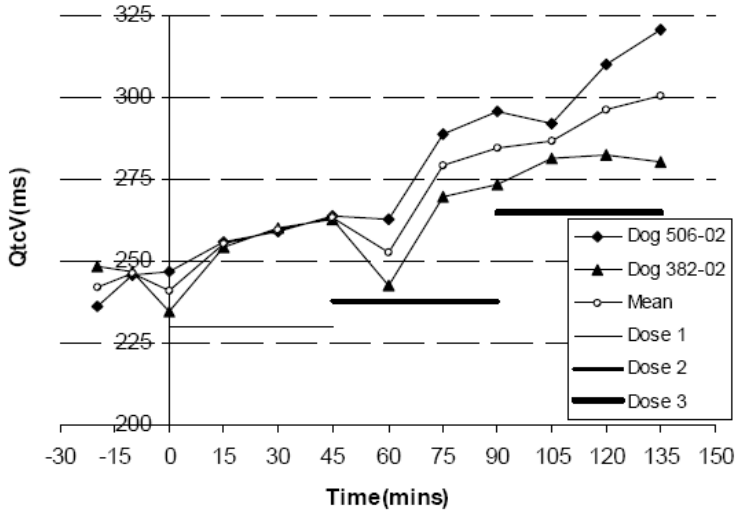
Dose	ZD6474 Dose levels (as base compound)							
	Bolus Dose			Continuous Infusion Dose (over 45 min)				Total Dose
	Dose Level mg/kg	Formulation Concentration mg/ml	Dose Volume ml/kg	Infusion rate mg/kg/h	Infusion dose mg/kg	Formulation Concentration mg/ml	Dose Volume ml/kg	Dose Level mg/kg
1	3.5	1.7	2	2.25	1.69	1.7	1	5.19
2	14	2.5	5.6	9	6.75	2.5	2.7	20.75
3	14	2.5	5.6	18	13.5	2.5	5.4	27.5

Rationale: ZD6474 may be co-administered with anti-emetics in the clinic. Both ZD6474 and ondansetron have been shown to prolong the QT interval in humans. This pilot was conducted to identify dose levels of ZD6474 and ondansetron alone which cause QT prolongation in the anesthetized dog. This information was used in a larger study (Study # 0258SD) to investigate the effects of these compounds in combination.

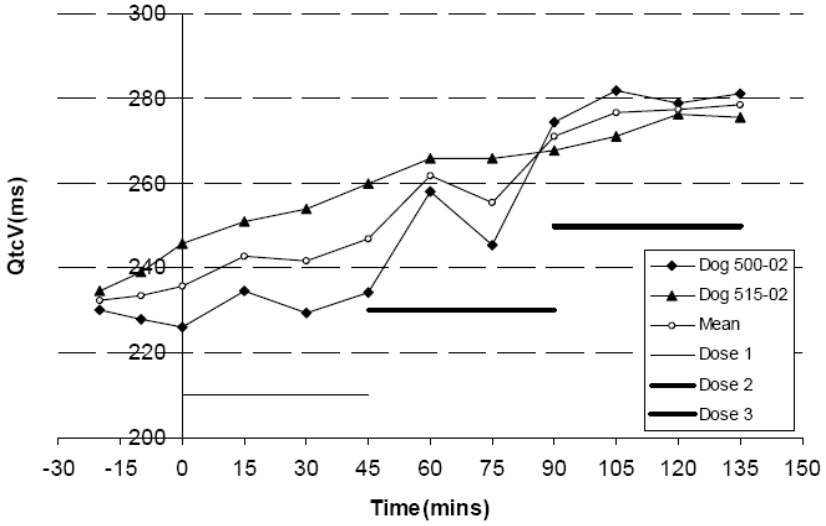
Results:

Blood pressure, heart rate, and lead II ECG were monitored in anesthetized beagle dogs after intravenous administration of ascending doses of ondansetron (1, 1.5, and 3.0 mg/kg) or ZD6474 (5.19, 20.75, and 27.5 mg/kg). Parameters were evaluated for 45 minutes during infusion and the next dose was infused without a recovery period from the prior dose. Three doses were administered on a single day. Treatment with ZD6474 caused a dose-related increase in QTcV interval and an increase in blood pressure. Additionally, ZD6474 caused a dose-dependent increase in T wave amplitude, which may be related to the QTcV prolongation. Ondansetron also caused a dose-related increase in QTcV interval. The effects of ZD6474 or ondansetron on QTcV interval and blood pressure are shown in the figures below.

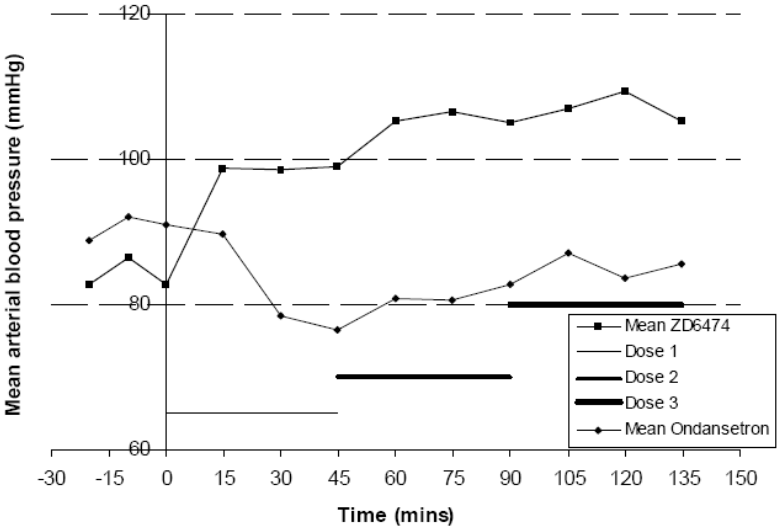
Effects of ZD6474 on QTcV
(excerpted from sponsor's submission)



Effects of ondansetron on QTcV
(excerpted from sponsor's submission)



Effects of ZD6474 or ondansetron on mean femoral arterial blood pressure
(excerpted from sponsor's submission)



Study Title: ZD6474 and Ondansetron: QTc investigations in anaesthetised, beagle dogs

Key findings:
Ondansetron caused a dose-related increase in QTcV, however, ZD6474 did not further increase the QTcV interval following ondansetron treatment. Treatment with ZD6474 did significantly increase diastolic blood pressure compared to treatment with vehicle. Additionally, ZD6474 caused an increase in T wave amplitude and polarity, despite having no effect on QTcV prolongation.

Study number: 0258SD
Study report location: M4.2.1.3
Conducting laboratory and location: Safety Assessment UK, AstraZeneca Macclesfield, England
Date of study initiation: September 25, 2003
GLP compliance: Yes (X) No ()
QA report: Yes (X) No ()
Drug, lot #, purity: ZD6474, P/4058/17
Ondansetron, GA0033A, GA0030A, GA0033B, GA0021A
Species: Beagle Dogs
Weight and age: 11 to 14.3 kg at 10 to 13 months
Groups: 4 males/group in vehicle and ZD6474-treated groups
Vehicle: ZD6474: Water for Injection containing 15% w/v hydroxypropyl-β-Cyclodextrin and 1.6% w/v mannitol, adjusted to pH7
Ondansetron: 0.9% NaCl
Treatments: Beagle dogs were treated with 3 escalating doses of ondansetron, in the absence and then presence of ZD6474 or vehicle as noted below. (Tables excerpted from sponsor's study report)

Table 1 Groups and dose levels

Group	Dose Number and Identity		
	Ondansetron Dose Response 1 (total doses)	Test Substance	Ondansetron Dose Response 2 (total doses)
1	1, 1.5 and 3 mg/kg	Vehicle	1, 1.5 and 3 mg/kg
2	1, 1.5 and 3 mg/kg	ZD6474 20.75 mg/kg	1, 1.5 and 3 mg/kg

Table 2 Individual dose details

Test substance	Dose levels							
	Bolus Dose			Continuous Infusion Dose (over 45 min)				Total Dose
	Dose level mg/kg	Formulation Concentration mg/ml	Dose Volume ml/kg	Infusion rate mg/kg/h	Infusion dose mg/kg	Formulation concentration mg/ml	Dose volume ml/kg	Dose level mg/kg
Ondansetron Dose 1	0.525*	0.25*	2*	0.64	0.48	0.25	2	1
Ondansetron Dose 2	0.525	0.25	2	1.28	0.96	0.5	2	1.5
Ondansetron Dose 3	1.05	0.5	2	2.96	2.2	1	2	3
Vehicle	0	0	5.6	9	0	0	2.7	0
ZD6474	14	2.5	5.6	9	6.75	2.5	2.7	20.75

* Bolus dose not required for the ondansetron second dose response curve (Dose 5), due to carry over of ondansetron levels from the first dose response curve. Only the 45 min infusion was required for Dose 5 in order to reach the total dose level of 1 mg/kg.

Time points: Hemodynamic parameters were measured at -20, -15, -10, -5, 0 (Start of infusion), 5, 10, 15, 20, 25, 30, 35, 40 and 45 minutes (end of infusion).

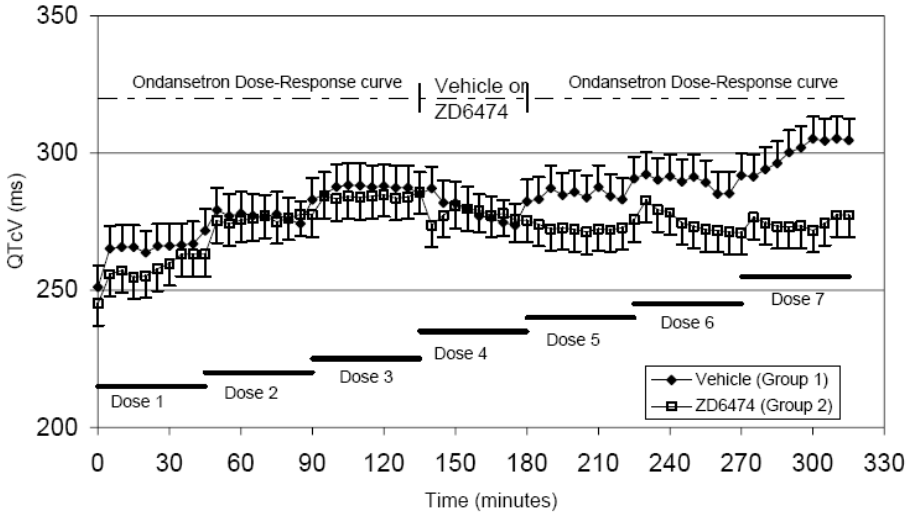
Rationale: ZD6474 may be co-administered with anti-emetics in the clinic. Both ZD6474 and Ondansetron have been shown to prolong the QT interval in humans. This study investigated the effects of these compounds in combination.

Results:

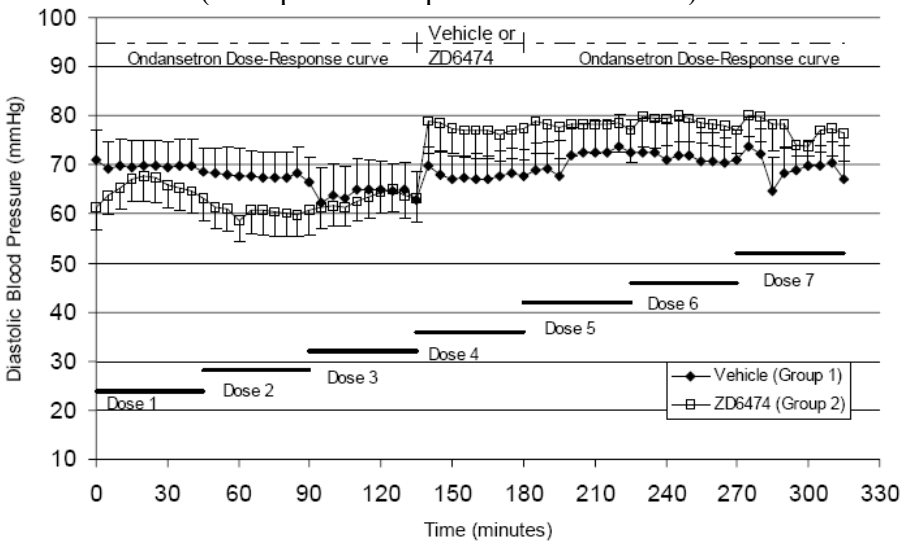
Blood pressure, heart rate, and lead II ECG were monitored in anesthetized beagle dogs after intravenous administration of ascending doses of ondansetron (1, 1.5, and 3.0 mg/kg), after intravenous administration of vehicle or ZD6474 (20.75 mg/kg), and after subsequent intravenous administration of ascending doses of ondansetron (1, 1.5, and 3.0 mg/kg). Parameters were evaluated for 45 minutes during infusion and the next dose was infused without a recovery period from the prior dose. Seven doses were administered on a single day. Ondansetron caused a dose-related increase in QTcV interval during the first dose escalation curve in both groups. The QTcV interval decreased slightly during administration of vehicle or ZD6474, indicating that ZD6474 did not further increase the QTcV interval following ondansetron treatment. Administration of the second dose escalation caused a further increase in QTcV in the vehicle group but not the ZD6474 group. Treatment with ZD6474 did significantly increase diastolic blood pressure compared to treatment with vehicle. Additionally, ZD6474 caused an increase in T wave amplitude and polarity, despite having no effect on QTcV prolongation. Subsequent administration of ondansetron did not further increase T wave

amplitude or polarity. The effects of ondansetron and vehicle or ZD6474 on QTcV interval and diastolic blood pressure are shown in the figures below.

Effects of ondansetron and vehicle or ZD6474 on QTcV interval
(excerpted from sponsor's submission)



Effects of ondansetron and vehicle or ZD6474 on femoral arterial diastolic blood pressure
(excerpted from sponsor's submission)



Pulmonary effects:

Study Title: ZD6474: Evaluation of Effect on Respiration in the Unrestrained Conscious Rat following Single Oral Administration

Key findings:
Peak expiratory flow, respiratory rate, tidal volume and minute volume were not affected by treatment with ZD6474. Males receiving 1000 mg/kg ZD6474 had increased peak inspiratory flow coupled with reduced inspiratory time that was statistically significant. Males in the 200 mg/kg group also had a decrease of inspiratory time that was not statistically significant.

Study number: 20060012PCR
Study report location: M4.2.1.3
Conducting laboratory and location: (b) (4)

Date of study initiation: Februaruy 2
GLP compliance: Yes (X) No ()
QA report: Yes (X) No ()
Drug, lot #: ZD6474, Batch ADM: 20091K04, purity not documented
Species: Male Wistar rats (RJ:WI (IOPS Han), 8 males per dose group
Weight and age: 302 to 342 grams at 9 to 12 weeks
Treatment:

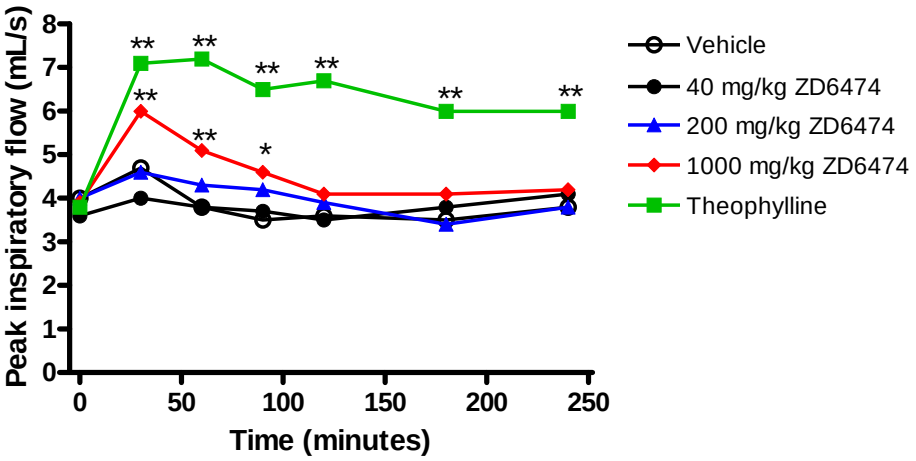
Compound	Dose
Theophillyne (pos. control)	100 mg/kg
ZD6474	0, 40, 200, 1000 mg/kg (Vehicle, 84.2, 420.7, 2103 µmol/kg)

Dose volume: 10 mL/kg
Schedule: Single dose
Vehicle: 0.5% hydroxypropylmethylcellulose with 0.1% Tween 80
Dose justification: Similar doses used in gastrointestinal safety pharmacology test.

Whole body plethysmography was used to evaluate the effect of ZD6474 on respiratory parameters including respiratory rate, peak inspiratory and expiratory flows, inspiration and expiration times, tidal volume and minute volume. Respiration was tested for 4 hours post-dose.

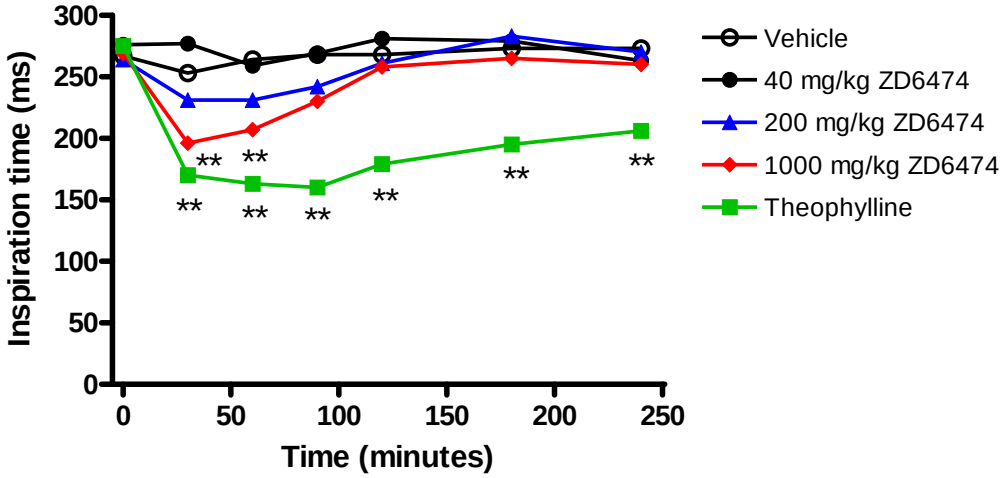
Results

The effect of ZD6474 treatment on peak inspiratory flow in male Wistar rat



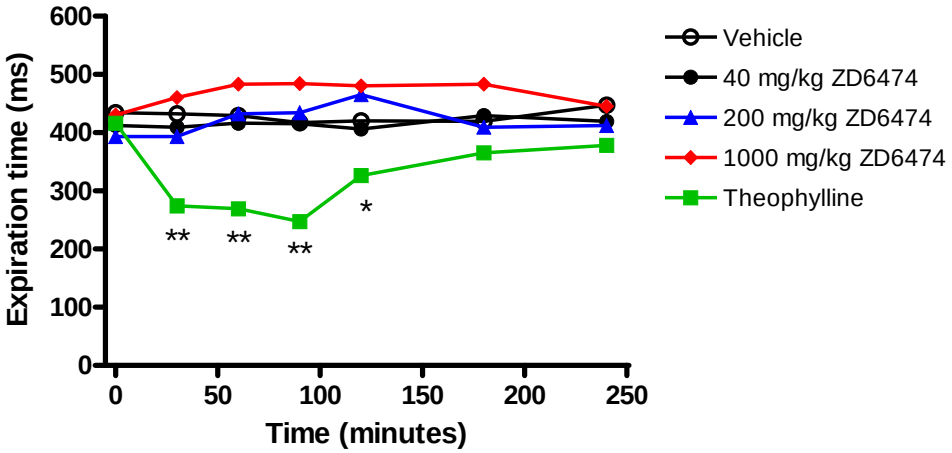
* Significantly different from control, p<0.05
** Significantly different from control, p<0.01

Effect of ZD6474 inspiration time in male Wistar rats



** Significantly different from control, p<0.01

Effect of ZD6474 on expiration time in male Wistar rats



* Significantly different from control, p<0.05
** Significantly different from control, p<0.01

Peak expiratory flow, respiratory rate, tidal volume and minute volume were all similar among control and ZD6474-treated groups.

Renal effects:

Study Title: ZM 382,561 – Diuretic Test

Key findings:
Female rats treated with 50 mg/kg ZM 382,561 (synonymous with ZD6474) had a reduction in sodium, potassium and chloride ions and an increase in total protein content in the urine by 24 hours post-dose. Urine volume, specific gravity, creatinine and glucose levels were unaffected in ZM-382,561-treated rats. Data suggest that ZM382,561 is mildly toxic to the kidneys, but the compound did not cause a diuretic or anti-diuretic effect in this study.

Study number: TSR2922
Study report location: M4.2.1.3
Conducting laboratory and location: Zeneca Pharmaceuticals
Safety of Medicines Dept.
Cheshire, England
Date of study initiation: August 18, 1998
GLP compliance: Yes (X) No ()
QA report: Yes (X) No ()
Drug, Batch: ZD6474, batch #13, purity not determined
Species: Wistar-derived rats (Alpk'APfSD (AP rat), females

Dose volume: 5 mL/kg
Dose schedule: Single dose
Vehicle: 0.5% w/v hydroxypropyl methulcellulose in 0.1% aquesous polysorbate 80

Results
Two groups of 5 female rats received either vehicle or 50 mg/kg ZM 382,561 and urine was collected over the first 6 hours and then between 6 and 24 hours. Urinalysis included measurement of urine volume, specific gravity, potassium, sodium, chloride, creatinine, glucose and total protein.

Effect of ZM 382,561 on urinalysis parameters in female rats

Parameter	0 to 6 hours post-dose		6 to 24 hours post-dose	
	Control	50 mg/kg ZM 382,561	Control	50 mg/kg ZM 382,561
Total Protein (mg/dL)	15.6 +/- 2.5	17.6+/-2.4	17.4+/-5.4	26.2+/-4.8*
Total Protein (mg/sample)	0.28+/- 0.07	0.34+/-0.07	1.2+/-0.3	2.3+/-0.9
Sodium (mmol/L)	92.8+/-62	89.6+/-14.2	197.2+/-16.3	139+/-75.3
Sodium (mmol/sample)	0.17+/- 0.13	0.17+/-0.02	1.4+/-0.3	1.1+/- 0.3
Potassium (mmol/L)	247.8+/- 85.5	214.4+/-35.5	401.9+/-33.3	247 +/- 112.6*
Potassium (mmol/sample)	0.42+/-0.1	0.4+/-0.06	2.8+/-0.2	2.0+/-0.6*

Parameter	0 to 6 hours post-dose		6 to 24 hours post-dose	
	Control	50 mg/kg ZM 382,561	Control	50 mg/kg ZM 382,561
Chloride (mmol/L)	196.6+/- 39.6	170.2 +/- 30.4	230.4 +/- 57.1	193.6 +/- 109.5
Chloride (mmol/sample)	0.35+/- 0.11	0.32+/-0.04	2.4+/-0.2	1.6+/-0.6*

*p < 0.05, 2-tail Student's t-test
Figures are mean +/- std. deviation

Females treated with ZM382-561 had similar urine volume, specific gravity, creatinine and glucose as rats treated with vehicle.

Gastrointestinal effects:

Study Title: ZD6474: Gastric emptying and intestinal motility in the rat following single, oral administration

Key findings:
Intestinal transit and gastric emptying were evaluated in male Wistar rats treated with a single oral dose of 0, 40, 200 or 1000 mg/kg ZD6474. ZD64764 dose dependently inhibited both intestinal transit and gastric emptying beginning at the lowest dose (40 mg/kg).

Study number: 1290SR
Study report location: M4.2.1.3
Conducting laboratory and location: Safety Assessment UK, AstraZeneca R&D Alderley, England
Date of study initiation: June 6, 2006
GLP compliance: Yes (X) No ()
QA report: Yes (X) No ()
Drug, lot #: ZD6474, ADM31130H05
% purity: 99.81% pure
Species: AzHsdBrlHan:Wistar rats, 10 males/dose group
Weight, age: 200 to 244 grams at 6 to 8 weeks
Treatment:

Compound	Dose
Atropine (pos. contrl)	20 mg/kg (34.6 µmol/kg)
ZD6474	0, 40, 200, 1000 mg/kg (Vehicle, 84.2, 420.7, 2103 µmol/kg)

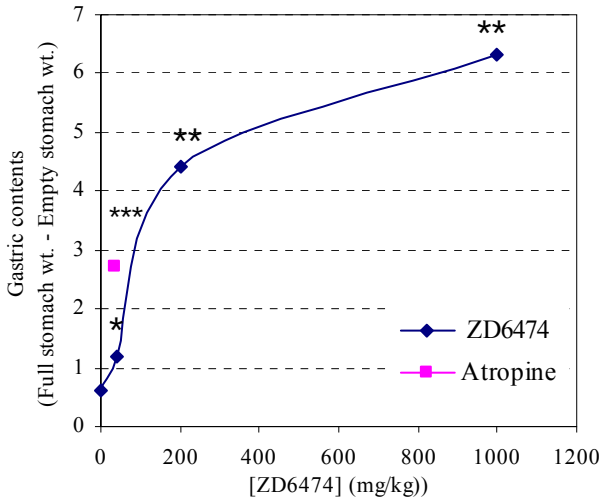
Dose volume: 10 mL/kg body weight
Vehicle: Water containing 0.5% w/v hydroxypropyl methyl cellulose and 0.1% w/v polysorbate 80.

Dose justification: 40 mg/kg expected to achieve similar free plasma concentrations as humans taking a 300 mg dose. The high dose, 1000 mg/kg, was chosen as the maximum tolerated single dose.

Results

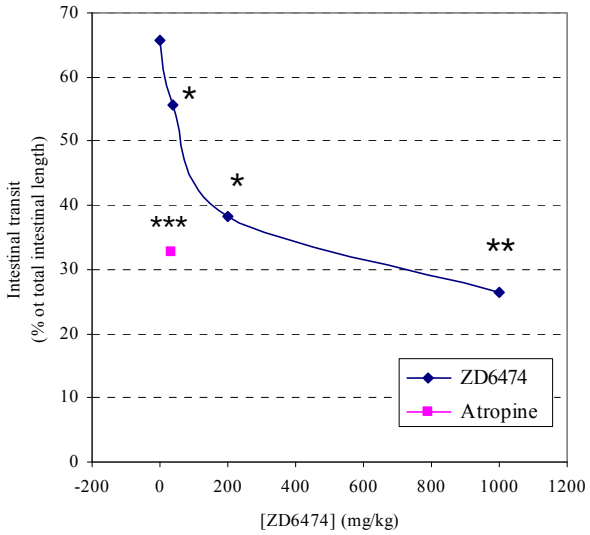
Animals were treated with vehicle, ZD6474, or atropine and fed charcoal meal 4 hours post-dose. Rats were anesthetized 15-20 minutes after the meal and the stomach and small intestines were removed for evaluation of intestinal transit and relative gastric emptying. For intestinal transit, the distance traveled by the charcoal front was measured and expressed as a percentage of the total intestinal length. Relative gastric emptying was expressed as the weight of the stomach contents.

Gastric emptying is dose dependently decreased
by ZD6474 treatment in male Wistar rats



* p<0.05, ** p<0.01, *** p<0.001

Intestinal transit is inhibited by ZD6474 treatment in male Wistar rats



* p<0.05, ** p<0.01, *** p<0.001

Abuse liability:
None provided

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Analytical methods and validation reports:

Studies were conducted to validate methods for determining concentrations of ZD6474, the N-desmethyl and N-oxide metabolites of ZD6474, and ondansetron. These studies and the methods and results are listed in the table below.

Validation Study #	Objective	Analyte	Species	Range (ng/mL)	Precision (% variation)	Accuracy (%)
KPV003	Validation of high performance liquid chromatography with mass spectrometry (HPLCMS-MS) method for determining ZD6474 concentrations in rat and dog plasma	ZD6474	Rat	5-500	ND	ND
			Dog	5-500	6.0-8.9	98.6-110
KPV033	Validation of an improved HPLCMS-MS method for determining ZD6474 concentrations in dog and rat plasma	ZD6474	Rat	5-1000	1.2-5.9	102-110
			Dog	5-1000	4.1-13.9	98.2-112
KPV039	Validation of a LC-MS/MS method for measurement of Ondansetron in dog plasma	Ondansetron	Dog	10-1000	5.5-13.3	92.2-104.8
KPV061	Validation of a LC-MS/MS method for the determination of N-oxide-ZD6474, N-desmethyl-ZD6474, and ZD6474 in rat plasma	ZD6474	Rat	5-1000	3.0-6.9	110-113
		N-desmethyl	Rat	1-200	8.3-13.7	99.3-116
		N-oxide	Rat	1-200	8.4-11.9	100-116

Validation Study #	Objective	Analyte	Species	Range (ng/mL)	Precision (% variation)	Accuracy (%)
KPV064	Validation of a LC-MS/MS method for the determination of N-oxide-ZD6474, N-desmethyl-ZD6474, and ZD6474 in dog plasma	ZD6474	Dog	5-1000	5.5-10.6	106-117
		N-desmethyl	Dog	1-200	7.5-13.9	94.8-107
		N-oxide	Dog	1-200	6.6-11.7	109-115
KPV065	Validation of a LC-MS/MS method for the determination of N-oxide-ZD6474, N-desmethyl-ZD6474, and ZD6474 in mouse plasma	ZD6474	Mouse	5-1000	5.6-10.5	95.6-101
		N-desmethyl	Mouse	1-200	5.4-11.4	87.9-118
		N-oxide	Mouse	1-200	7.0-12.5	109-116
KPV097	Partial validation of a LC-MS/MS method for the determination of N-oxide-ZD6474, N-desmethyl-ZD6474, and ZD6474 in mouse plasma	ZD6474	Mouse	5-1000	1.5-4.9	93.2-111
		N-desmethyl	Mouse	1-200	2.2-14.4	89.1-106
		N-oxide	Mouse	1-200	2.0-4.5	101-107
KPV076	Validation of a LC-MS/MS method for the determination of N-oxide-ZD6474, N-desmethyl-ZD6474, and ZD6474 in animal tissue	ZD6474	Rat	5-1000	3.9-35.4*	ND
		N-desmethyl	Rat	5-1000	2.7-38.1*	ND
		N-oxide	Rat	5-1000	2.8-46.8*	ND

ND= not determined
* data was for a variety of tissues (rat brain, liver, kidney, testes, heart, and lung)

Study title: Long term storage stability of ZD6474 and its two metabolites N-desmethyl-ZD6474 and N-oxide-ZD6474 in rat, dog, and mouse plasma for a period of 12 months at -20°C and -70°C
Study no.: KKI059
Study report location: M4.2.2.1

This study was conducted to determine the long term storage stability up to 12 months of ZD6474 and its N-desmethyl and N-oxide metabolites in rat, dog, and mouse plasma at storage temperatures of -20°C and -70°C. ZD6474 (15 and 800 ng/mL) and the N-desmethyl and N-oxide metabolites (3.0 and 160 ng/mL) were added to control rat, dog, and mouse plasma and samples were stored at -20°C and -70°C with stability assessed at intervals up to 12 months. The samples were analyzed based upon protein precipitation by using Isolute® PPT+ 96 well plates and high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) detection. Evaluation of stability sample results was done using the results of freshly prepared QC samples analyzed each run. Differences between fresh and stored samples are shown in the table below. Overall, samples stored up to 12 months at -20°C and -70°C differed < 15% from fresh samples, indicating that stability of ZD6474 and its N-desmethyl and N-oxide metabolites in rat, dog, and mouse plasma under these conditions appears to be within acceptable limits.

Summary of the stability up to 12 months in animal plasma
(excerpted from sponsor's submission)

Component	Concentration level (ng/mL)	Difference at -20°C (%)			Difference at -70°C (%)		
		Rat	Dog	Mouse	Rat	Dog	Mouse
ZD6474	15	6.4	-4.4	-16.0	2.8	-2.8	0.8
ZD6474	800	9.4	4.9	10.7	7.1	3.5	9.7
N-Desmethyl-ZD6474	3.00	6.5	-5.1	8.4	17.9	-14.5	15.1
N-Desmethyl-ZD6474	160	1.2	1.1	3.0	5.2	-8.2	1.1
N-Oxide-ZD6474	3.00	-7.9	-6.4	-12.3	-9.4	-14.9	-1.3
N-Oxide-ZD6474	160	6.0	-6.2	0.5	3.1	-5.0	2.2

Difference (%) = Difference between fresh and stored samples (%)

Lower limit (%) = 15%, Upper limit (%) = -15%

Absorption:

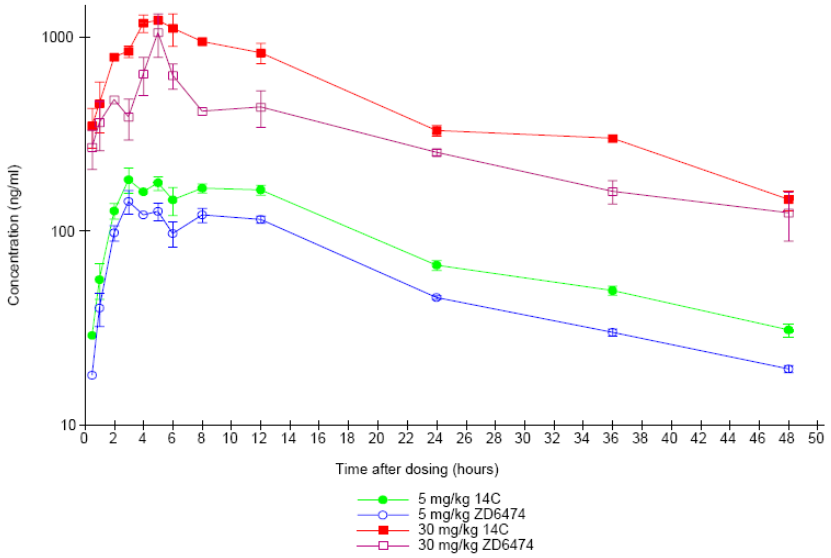
Study title: **Zeneca ZD6474: The distribution of radioactivity in the blood after oral and intravenous administration of [^{14}C]-Zeneca ZD6474 to rats**
Study no.: KKR007
Study report location: M4.2.2.2
Previously reviewed in IND 60042 by Wendelyn Schmidt, Ph.D. A separate review was conducted below.

The distribution of [^{14}C]-ZD6474 following administration of a single oral dose (5 and 30 mg/kg) and a single intravenous (i.v.) dose (5 mg/kg) was examined in rats and pharmacokinetics were determined. Exposure (AUC) of ZD6474 was higher in females than males.

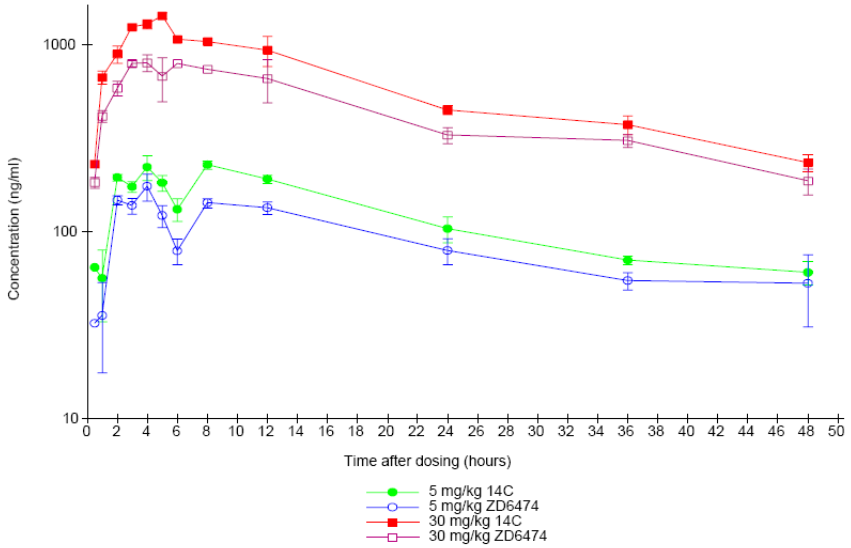
Pharmacokinetics of ZD6474 in rats following i.v. and oral administration						
Parameter	5 mg/kg i.v.		5 mg/kg oral		30 mg/kg oral	
	Males	Females	Males	Females	Males	Females
C_{max} (ng/mL)	-	-	142	175	1,050	800
t_{max} (hours)	-	-	3.0	4.0	5.0	4.0
AUC (ng.h/mL)	3,560	6,150	3,530	6,110	17,900	26,100
$AUC_{(0-36)}$ (ng.h/mL)	2,920	3,780	2,680	3,520	12,600	17,700
$T_{1/2}$ (hours)	15.6	31.0	19.6	25.3	19.6	20.3
Bioavailability (%)	-	-	91.8	93.1	71.9	78.0

Concentrations of radioactivity in the plasma exceeded the ZD6474 plasma concentrations, indicating the presence of circulating metabolites of ZD6474.

Mean plasma concentrations of total reactivity and ZD6474 following single oral doses of [^{14}C]-ZD6474 at 5 and 30 mg/kg to male rats (excerpted from sponsor's submission)



Mean plasma concentrations of total reactivity and ZD6474 following single oral doses of [^{14}C]-ZD6474 at 5 and 30 mg/kg to female rats (excerpted from sponsor's submission)



Study title: **The pharmacokinetics of ZD6474, N-desmethyl and N-oxide metabolites following single oral and intravenous doses of ZD6474 in male and female rats- Report amendment 1**
Study no.: KPR056
Study report location: M4.2.2.2
Study title: The pharmacokinetics of ZD6474, N-desmethyl and N-oxide metabolites following single oral and intravenous doses of ZD6474 to male dogs- Report amendment 1
Study no.: KPD057

Study report location: M4.2.2.2

Plasma pharmacokinetics of ZD6474 and the metabolites N-desmethyl and N-oxide were measured following a single intravenous or oral dose of ZD6474 in male and female rats in study KPRO56 and in male dogs in study KPD057. Exposure (AUC) of ZD6474 was higher in female rats than male rats for both intravenous and oral administration. Oral bioavailability was also higher in female rats (94.6%) than male rats (54.6%). Exposure (AUC) of ZD6474 following oral administration and oral bioavailability were similar in male rats and male dogs. For intravenous administration, plasma clearance was faster in male dogs (34.7 mL/min/kg) than rats and was slightly faster in male rats (17.1 mL/min/kg) than female rats (13.7 mL/min/kg). ZD6474 was extensively distributed in both rats and dogs, however, the volume of distribution was higher in dogs than rats. The plasma levels of the metabolites were lower than for ZD6474 in both rats and dogs. In study KPR056, samples collected from rats were alternately taken from the tail vein and by cardiac puncture. Higher concentrations of ZD6474 were observed in the cardiac samples, suggesting there were some differences in the concentration of ZD6474 in arterial and venous blood.

Intravenous administration of ZD6474 (7.5 mg/kg)

Parameter	ZD6474			N-desmethyl			N-oxide		
	Rat		Dog	Rat		Dog	Rat		Dog
	Male	Female	Male	Male	Female	Male	Male	Female	Male
C_0 or C_{inf} (ng/mL)	986	913	1,400	NA	NA	NA	NA	NA	NA
C_{max} (ng/mL)	NA	NA	NA	9.74	3.51	11.5	14.5	22.5	78.5
t_{max} (hours)	NA	NA	NA	8.0	12.0	34.0	0.11	0.49	0.39
AUC $_{(0-\infty)}$ (ng h./mL)	7,297	9,103	3,222	NC	NC	NC	NC	NC	557
AUC $_{(0-t)}$ (ng.h./mL)	7,029	8,841	2,900	311	126	503	126	152	485
$t_{½}$ (hours)	28.3	30.4	20.8	NC	NC	NC	NC	NC	21.7
Cl (mL/min/kg)	17.1	13.7	34.7	NC	NC	NC	NC	NC	NC
Vd_{ss} L/kg	27.2	27.9	43.6	NC	NC	NC	NC	NC	NC

NC=Not calculated
NA=Not applicable

Oral administration of ZD6474 (Rat: 10 mg/kg; Dog: 20 mg/kg)

Parameter	ZD6474			N-desmethyl			N-oxide		
	Rat		Dog	Rat		Dog	Rat		Dog
	Male	Female	Male	Male	Female	Male	Male	Female	Male
C_{max} (ng/mL)	326	368	267	6.03	4.41	46.0	5.66	8.56	76.8
t_{max} (hours)	2.0	4.0	3.25	8.0	4.0	38.5	3.0	3.0	2.25
AUC $_{(0-\infty)}$ (ng h./mL)	5,298	11,481	4,930	NC	NC	NC	NC	NC	987
AUC $_{(0-t)}$ (ng.h./mL)	5,083	11,091	4,355	168	136	2,286	53.8	185	1,036
$t_{½}$ (hours)	22.9	35.2	22.1	NC	NC	NC	NC	NC	20.5
Bioavailability (%)	54.5	94.6	56.4	NC	NC	NC	NC	NC	NC

NC=Not calculated

Distribution

Study title: **The tissue distribution of [^{14}C]-AstraZeneca ZD6474 in male rats- Report Amendment 1**
Study no.: KMR080
Study report location: M4.2.2.2
The concentrations of total radioactivity, ZD6474, and the metabolites N-desmethyl and N-oxide were measured in plasma and selected tissues (heart, liver, kidneys, brain, lungs, and testes) following a single oral administration of [^{14}C]-ZD6474 (10 mg/kg) in male rats. Plasma and tissue samples were collected at 1, 2, 6, 12, 24, 48, and 72 hours after dosing. Low concentrations of the metabolites N-desmethyl and N-oxide were detected in the plasma. The highest concentrations of ZD6474 were in the lungs, liver, and kidneys. The concentrations of ZD6474 increased slowly in the testes with t_{max} at 24 hours after dosing and then decreased slowly and was 75% of C_{max} at 72 hours. Low concentrations of ZD6474 were detected in the brain and heart. At 6 hours after dosing 1.36 µg/g of ZD6474 were observed in the heart. The metabolite N-desmethyl was detected in the liver only and concentrations of N-oxide were below the limit of detection in the tissues.

Pharmacokinetic parameters for ZD6474 and its metabolites in plasma following administration of single oral dose of [^{14}C]-ZD6474 in male rats (excerpted from sponsor's submission)

Parameter	ZD6474	N-desmethyl ZD6474	ZD6474 N-oxide
C_{max} (ng/mL)	318	11.4	6.56
t_{max} (h)	6.00	12.00	6.00
$AUC_{(0-\infty)}$ (ng.h/mL)	10520	465	174
$AUC_{(0-last)}$ (ng.h/mL)	9666	405	100
$t_{½}$ (h)	19.3	22.3	NC

NC = Not calculated

Pharmacokinetic parameters for ZD6474 in plasma and tissues derived following administration of single oral dose of [^{14}C]-ZD6474 in male rats (excerpted from sponsor's submission)

Parameter	Plasma	Heart	Liver	Kidney	Lungs	Brain	Testes
C_{max} (µg/mL or µg/g)	0.32	NC	76.6	27.1	113	3.23	6.56
t_{max} (h)	6.00	NC	6.00	6.00	12.0	6.00	24.0
$AUC_{(0-\infty)}$ (µg.h/mL or µg.h/g)	10.5	NC	2816	909	3196	NC	1192
$AUC_{(0-last)}$ (µg.h/mL or µg.h/g)	9.67	NC	2603	775	2776	97.7	372
$t_{½}$ (h)	19.3	NC	20.4	27.8	26.4	NC	NC

NC = Not calculated

Pharmacokinetic parameters for N-desmethyl ZD6474 in the liver derived following administration of single oral dose of [^{14}C]-ZD6474 in male rats

Parameter	Liver
C_{max} (µg/mL or µg/g)	4.99
t_{max} (hours)	12.0
AUC $_{(0-\infty)}$ (µg.h./mL or µg.h/g)	379
AUC $_{(0\text{-last})}$ (µg.h./mL or µg.h/g)	258
$t_{½}$ (hours)	46.0

Study title: **AZD6474: The tissue distribution of [^{14}C]-AstraZeneca ZD6474 in nude mice bearing LoVo Xenografts, Report amendment 1**
Study no.: KMM063
Study report location: M4.2.2.3

A single oral dose of [^{14}C]-ZD6474 (50 mg/kg) was administered to female nude mice bearing LoVo tumor xenografts. Mice were sacrificed at 2, 4, 8, 12, 24, 48, 96, and 144 hours after dosing, and plasma and tumor were taken from three mice at each time to determine concentrations of ZD6474, N-desmethyl ZD6474, and ZD6474 N-oxide using HPLC with tandem mass spectrometric detection (HPLC-MS/MS). Another two mice at each time point were analyzed by Quantitative Whole Body Autoradiography (QWBA). Additional tumor analysis was conducted from intact frozen tumor tissue from the animals not needed for QWBA with one mouse for each time point (except hour 12), and the radioactivity was determined by HPLC- MS/MS) using an ethyl acetate extraction method.

Radioactivity was widely distributed to the various tissues, with the highest concentrations of radioactivity observed at either 8 or 12 hours after dosing in most tissues. Concentrations were high in the intestinal tract, liver, and kidney, which may reflect the involvement of these tissues in excretion. The highest concentrations of radioactivity were in the spleen, and concentrations in the tumor and brain were higher than in blood.

Concentration of total radioactivity in selected tissues following a single oral dose of [^{14}C]-ZD6474 to female nude mice (excerpted from sponsor's submission)

	Concentrations of radioactivity (µg equiv/g)								
Animal No.	**005F**	**009F**	**014F**	**019F**	**020F**	**024F**	**029F**	**034F**	**040F**
Timepoint	**2 h**	**4 h**	**8 h**	**12 h**	**12 h**	**24 h**	**48 h**	**96 h**	**144 h**
Tumour	24.6	16.2	54.5	43.5	42.4	31.8	18.7	4.4	1.1
Adrenal cortex	74.0	101.9	120.8	88.3	79.6	62.2	55.0	44.7	11.9
Adrenal medulla	104.6	80.8	97.6	141.2	112.6	86.3	91.4	19.0	18.0
Adrenal (whole)	81.1	98.0	115.6	95.9	90.3	68.8	62.6	45.1	12.4
Bladder wall	41.2	44.4	62.8	24.6	115.0	24.2	NP	6.7	1.5
Blood (cardiac)	3.8	12.6	2.9	1.5	1.9	1.0	0.5	0.2	0.1
Bone marrow	51.1	47.8	85.6	53.8	74.5	43.4	19.7	8.5	2.6
Brain	10.0	11.2	12.1	13.0	12.3	6.8	3.2	1.2	0.5
Brown fat	29.2	28.6	35.7	35.9	24.5	31.6	20.9	7.3	3.1
Eye	2.9	4.5	7.5	7.3	6.6	5.1	2.7	3.1	0.3
Gall Bladder	54.3	160.6	88.0	NP	34.1	54.1	21.4	11.9	0.5
Harderian gland	88.9	84.2	142.4	145.8	128.5	62.9	32.4	12.0	4.3
Heart muscle	22.5	17.8	20.6	20.4	18.2	12.0	7.6	4.1	0.9
Kidney cortex	79.7	98.0	97.8	92.6	95.6	79.4	47.0	26.2	6.6
Kidney medulla	58.4	43.5	60.2	50.9	52.8	50.4	34.1	27.8	3.4
Kidney (whole)	75.3	81.7	84.0	73.9	89.4	69.2	41.9	26.8	6.0
Lachrymal gland	61.6	52.2	58.3	71.7	44.0	44.8	35.7	31.7	7.3
Large intestine wall	90.0	65.2	66.5	41.5	76.0	13.8	16.8	17.6	3.9
Liver	117.8	107.7	101.6	81.8	67.1	56.9	37.1	15.9	3.1
Lung	43.1	53.9	65.2	133.3	71.8	43.2	31.6	10.7	1.9
Lymph node	97.8	104.5	137.1	160.5	134.1	59.0	78.8	38.4	16.8
Ovary	68.8	102.1	155.1	122.3	123.4	29.6	109.7	86.6	23.9

	Concentrations of radioactivity (µg equiv/g)								
Animal No.	**005F**	**009F**	**014F**	**019F**	**020F**	**024F**	**029F**	**034F**	**040F**
Timepoint	**2 h**	**4 h**	**8 h**	**12 h**	**12 h**	**24 h**	**48 h**	**96 h**	**144 h**
Pancreas	58.2	48.5	61.2	54.0	56.6	40.6	42.6	26.0	7.9
Pituitary gland	38.4	35.0	27.1	32.3	45.0	44.3	24.6	5.8	1.0
Rectum wall	24.3	111.2	31.5	29.7	NP	8.4	7.6	3.8	0.8
Salivary gland	51.0	48.5	45.1	55.5	37.3	28.3	29.9	12.7	3.9
Skeletal muscle	8.4	6.5	8.5	6.9	8.5	4.7	2.4	1.0	0.3
Skin	16.0	19.3	31.2	48.4	44.5	27.4	19.1	9.5	1.9
Small intestine wall	180.9	48.4	68.6	114.1	72.6	94.4	38.4	13.6	3.2
Spinal cord	9.2	6.3	13.6	10.1	9.9	5.5	2.0	0.8	0.2
Spleen	137.9	160.7	217.0	252.9	133.3	145.2	87.4	43.9	9.0
Stomach wall	31.5	40.5	39.6	33.8	28.2	16.0	12.8	5.5	1.3
Thyroid gland	11.2	23.3	50.0	29.1	NP	36.3	15.5	4.9	1.1
Uterus	36.8	39.0	55.6	29.9	34.5	31.1	25.7	12.6	6.0
Uveal	10.8	15.5	23.4	21.2	16.6	11.6	8.6	5.0	1.0
White fat	3.7	8.0	11.8	6.6	1.3	2.6	2.6	1.5	0.3
Limit of reliable measurement:	0.1	0.2	0.2	0.1	0.0	0.1	0.1	0.1	0.0

NP = tissue not present in sections

Plasma concentrations of ZD6474 reached a maximum of 2.59 µg/mL at 12 hours after dosing. Metabolites were detected at very low concentrations compared to ZD6474.

Plasma concentrations of ZD6474 and metabolites following a single oral dose of dose of [^{14}C]-ZD6474 to female nude mice

Time after dose (h)	Concentration in plasma (µg/mL)		
	ZD6474	N-desmethyl	N-oxide
2	2.03	0.03	0.03
4	2.23	0.05	0.03
8	2.16	0.05	0.03
12	2.59	0.06	0.03
24	0.96	0.02	0.01
48	0.60	0.02	0.01
96	0.25	0.01	0.00
144	0.15	0.01	0.00

In the original analysis of tumor concentrations of ZD6474 and metabolites, only ZD6474 was detected with a maximum concentration of 3.95 µg/mL at 12 hours after dosing. An additional analysis was conducted using a different method and frozen tumor tissue from animals not used for QWBA. The concentrations of ZD6474 were higher than in the original analysis with a maximum observed dose of 15.6 µg/mL at 8 hours after dosing. Concentrations of N-oxide were detected with a maximum value of 3.43 µg/mL at 8 hours after dosing.

Tumor concentrations of total radioactivity, ZD6474, and metabolites for original analysis and additional analysis following a single oral dose of [^{14}C]-ZD6474 to female nude mice
(excerpted from sponsor's submission)

	Concentration (µg equiv/g or µg/g)							
	QWBA*	Original analysis			Additional analysis*			
Time after dose (h)	Radioactivity	ZD6474	N-desmethyl ZD6474	ZD6474 N-oxide	Radioactivity	ZD6474	N-desmethyl ZD6474	ZD6474 N-oxide
2	24.6	1.77 ± 0.17	NQ	NQ	27.9	8.91	NQ	1.45
4	16.2	3.30 ± 0.47	NQ	NQ	29.6	6.90	NQ	1.89
8	54.5	3.44 ± 0.12	NQ	NQ	32.7	15.6	NQ	3.43
12	43.5, 42.4	3.95 ± 0.43	NQ	NQ	NS	NS	NS	NS
24	31.8	2.46 ± 0.33	NQ	NQ	27.5	12.3	NQ	1.31
48	18.7	1.51 ± 0.13	NQ	NQ	21.0	6.20	NQ	1.43
96	4.4	0.58 ± 0.15	NQ	NQ	4.96	2.15	NQ	NQ
144	1.1	0.21 ± 0.03	NQ	NQ	2.56	1.19	NQ	NQ

* n=1
NS = no sample
NQ = Non-quantifiable (ie, below the LLOQ of the assay)

Study title: **AstraZeneca ZD6474: The tissue distribution of [^{14}C]-AstraZeneca ZD6474 in the male pigmented rat and albino rat following single oral administration at 5 mg/kg (quantitative whole body phosphor imaging)**
Study no.: KMR014
Study report location: M4.2.2.3

Male pigmented (Hooded Lister) and albino (Sprague Dawley) rats were administered a single oral dose of 5 mg/kg [^{14}C]-ZD6474 (5 mL/kg dose volume). Tissue concentrations of radioactivity were quantified by quantitative whole body phosphor imaging (QWBPI) from single rats at 2, 6, 24, 72, 168, and 336 hours after dosing for pigmented rats and at 2, 24, 72, and 168 hours after dosing for albino rats.

Radioactivity was extensively distributed in both pigmented and albino rats following a single oral dose of [^{14}C]-ZD6474, with high levels of radioactivity measured in the contents of the gastrointestinal tract. Maximum tissue concentrations for most tissues were observed at 2, 6, or 24 hours after dosing in the pigmented rat and at 2 or 24 hours after dosing in albino rats. In the pigmented rat the highest concentrations of radioactivity were in the pigmented eye, the Harderian gland, pituitary gland, adrenal medulla, and spleen. In the albino rat the highest concentrations of radioactivity were found in the liver, adrenal cortex, adrenal medulla, and spleen. At 168 hours after dosing, levels of radioactivity associated with [^{14}C]-ZD6474 or its metabolites were considerably reduced in the gastrointestinal tract and other tissues, indicating the radioactivity was in the process of being eliminated. At 336 hours after dosing, radioactivity was mostly eliminated from the tissues in the pigmented rat with tissue levels measurable in only the pigmented eye, pigmented skin/fur, Harderian gland, kidney cortex, spleen, testes, and whole eye.

Tissue concentrations of radioactivity in pigmented rats at various times following oral administration of [^{14}C]-ZD6474 (2-336 hours). Results expressed as ng equiv/g. (excerpted from sponsor's submission)

Tissue/organ	Rat 1 (2 h)	Rat 2 (6 h)	Rat 3 (24 h)	Rat 4 (72 h)	Rat 5 (168 h)	Rat 6 (336 h)
LOQ	21.0	14.7	15.4	53.9	3.59	13.2
Adrenal cortex	16451	15625	18451	2264	320	bld
Adrenal medulla	11111	16188	26014	1660	nd	bld
Bone	403	278	448	47.6	48.8	bld
Bone marrow	4444	11808	6603	986	176	bld
Brain	849	1210	785	127	17.4	bld
Brown fat	1994	1882	2249	277	52.1	bld
Caecum contents	1808	40779	13516	2472	134	bld
Caecum wall	1953	nd	2288	380	nd	bld
Cardiac blood	333	321	254	bld	4.94	bld
Cardiac muscle	2310	2076	2250	262	32.3	bld
Epididymis	720	199	nd	444	90.5	bld
Harderian gland	5350	15819	17142	31979	11013	526
Kidney cortex	9771	11793	9594	967	176	54.4

LOQ denotes limit of quantification; **nd** denotes sample is not distinguishable from surrounding tissue; **bld** denotes below limit of detection

Tissue/organ	Rat 1 (2 h)	Rat 2 (6 h)	Rat 3 (24 h)	Rat 4 (72 h)	Rat 5 (168 h)	Rat 6 (336 h)
LOQ	21.0	14.7	15.4	53.9	3.59	13.2
Kidney medulla	8025	11601	8661	1723	253	nd
Large intestine contents	173	36744	19613	4856	258	49.6
Large intestine wall	1973	nd	nd	nd	nd	nd
Liver	9481	8941	6169	799	105	bld
Lung	10507	15996	9935	919	49.6	bld
Nasal mucosa	2061	3748	2738	378	132	bld
Pancreas	4639	5818	3129	504	113	bld
Pigmented eye	7185	34865	130105	121189	208325	157894
Pigmented fur/skin	720	2583	3893	2573	15402	6669
Pineal body	1115	13917	16177	1634	bld	bld
Pituitary gland	nd	20166	30802	10381	1473	bld
Preputial gland	3974	11049	11184	634	11.1	bld
Seminal vesicles	414	791	861	116	nd	bld
Skeletal muscle	816	1032	841	85.5	18.1	bld

LOQ denotes limit of quantification; **nd** denotes sample is not distinguishable from surrounding tissue; **bld** denotes below limit of detection

Tissue/organ	Rat 1 (2 h)	Rat 2 (6 h)	Rat 3 (24 h)	Rat 4 (72 h)	Rat 5 (168 h)	Rat 6 (336 h)
LOQ	21.0	14.7	15.4	53.9	3.59	13.2
Small intestine contents	67192	32642	18037	2002	190	bld
Small intestine wall	124870	66274	45340	5227	439	110
Spinal cord	791	882	639	63.3	8.20	bld
Spleen	16140	31918	23709	2418	585	199
Stomach contents	42786	18860	14137	464	12.4	bld
Stomach wall	2327	2842	2446	505	nd	bld
Submaxillary salivary gland	6711	11090	8239	694	104	bld
Testes	494	1426	5213	3726	1070	280
Thymus	3303	7266	7069	833	146	bld
Thyroid gland	10542	7177	8077	787	266	bld
Urine	482	ns	ns	51.8	bld	bld
White fat	314	421	282	58.7	19.5	bld
Whole eye	68.7	111	nd	454	252	952
Blood*	155	259	132	21.2	3.80	bld

LOQ denotes limit of quantification; **nd** denotes sample is not distinguishable from surrounding tissue; **bld** denotes below limit of detection; **ns** denotes no sample on tissue section; * value obtained by sample combustion

Tissue concentrations of radioactivity in albino rats at various times following oral administration of [^{14}C]-ZD6474 (2-168 hours). Results expressed as ng equiv/g. (excerpted from sponsor's submission)

Tissue/organ	Rat 7 (2 h)	Rat 8 (24 h)	Rat 9 (72 h)	Rat 10 (168 h)
LOQ	33.5	15.8	9.20	5.03
Adrenal cortex	14759	12956	5116	1455
Adrenal medulla	9455	14637	2835	nd
Bone	384	176	106	nd
Bone marrow	4229	8069	1542	798
Brain	577	434	76.8	108
Brown fat	3368	1145	405	299
Caecum contents	528	9804	2729	798
Caecum wall	1447	1038	nd	nd
Cardiac blood	384	115	16.1	22.7
Cardiac muscle	1843	1141	242	255
Epididymis	897	2347	319	235
Fur/skin	nd	1085	nd	nd
Harderian gland	3505	54317	46395	93272
Kidney cortex	6341	5755	1270	911

LOQ denotes limit of quantification; **nd** denotes sample is not distinguishable from surrounding tissue;

Tissue/organ	Rat 7 (2 h)	Rat 8 (24 h)	Rat 9 (72 h)	Rat 10 (168 h)
LOQ	33.5	15.8	9.20	5.03
Kidney medulla	8375	5632	1716	1685
Large intestine contents	225	19442	3296	1659
Large intestine wall	1668	2079	1067	nd
Liver	110889	2865	990	769
Lung	5800	4158	974	241
Nasal mucosa	340	1355	337	nd
Pancreas	5205	2650	772	542
Pineal body	6249	1529	nd	nd
Pituitary gland	5025	9716	4025	1582
Preputial gland	3598	8185	2218	2798
Seminal vesicles	396	393	246	956
Skeletal muscle	693	308	49.5	99.2

LOQ denotes limit of quantification; **nd** denotes sample is not distinguishable from surrounding tissue

Tissue/organ	Rat 7 (2 h)	Rat 8 (24 h)	Rat 9 (72 h)	Rat 10 (168 h)
LOQ	33.5	15.8	9.20	5.03
Small intestine contents	134317	8722	2980	1758
Small intestine wall	150907	13416	2262	6129
Spinal cord	488	342	42.6	77.4
Spleen	11146	14773	3024	3302
Stomach contents	176097	8769	3600	1471
Stomach wall	2174	924	680	392
Submaxillary salivary gland	5324	4164	845	573
Testes	445	3061	3998	5728
Thymus	2542	4160	1157	677
Thyroid gland	10170	8626	3006	1389
Urine	1281	ns	ns	ns
White fat	246	190	65.9	118
Whole eye	36.0	80.5	64.8	158
Blood *	101	95.5	14.8	9.86

LOQ denotes limit of quantification; **bdl** denotes below limit of detection; **ns** denotes no samples on tissue section;
* value obtained by sample combustion

Study title: **Bi-directional CACO-2 permeability of ZD6474**
Study no.: 02-ASTR-UK.P01R1
Study report location: M4.2.2.3

The bi-directional permeability of ZD6474 in the Caco-2 cell culture system was determined at three concentrations (1, 10, and 50 µM). Results are shown in the tables below (excerpted from the sponsor's submission). ZD6474 appears to be highly permeable in the Caco-2 cell line.

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Table 3.1 Recovery and Permeability (10^{-6} cm/s) of ZD6474 at 1 uM Concentration

Percent Recovery[C]			Papp[D] Blank	Papp, A-to-B				Papp, B-to-A				$Papp^{B-A}/Papp^{A-B}$ Ratio	Absorption Potential[A]	Significant Efflux[B]
Blank	A-to-B	B-to-A		Rep. 1	Rep. 2	Rep. 3	Avg	Rep. 1	Rep. 2	Rep. 3	Avg			
85	52	52	4.0	18.5	16.9	18.3	17.9	17.6	11.2	13.2	14.0	0.78	High	No

Table 3.2 Recovery and Permeability (10^{-6} cm/s) of ZD6474 at 10 uM Concentration

Percent Recovery[C]			Papp[D] Blank	Papp, A-to-B				Papp, B-to-A				$Papp^{B-A}/Papp^{A-B}$ Ratio	Absorption Potential[A]	Significant Efflux[B]
Blank	A-to-B	B-to-A		Rep. 1	Rep 2	Rep. 3	Avg	Rep. 1	Rep. 2	Rep. 3	Avg			
84	55	64	42.7	23.9	23.8	23.7	23.8	19.4	16.1	18.0	17.8	0.75	High	No

Table 3.3 Recovery and Permeability (10^{-6} cm/s) of ZD6474 at 50 uM Concentration

Percent Recovery[C]			Papp[D] Blank	Papp, A-to-B				Papp, B-to-A				$Papp^{B-A}/Papp^{A-B}$ Ratio	Absorption Potential[A]	Significant Efflux[B]
Blank	A-to-B	B-to-A		Rep. 1	Rep. 2	Rep. 3	Avg	Rep. 1	Rep. 2	Rep. 3	Avg			
76	60	84	46.5	31.2	32.2	30.2	31.2	15.5	15.8	19.1	16.8	0.54	High	No

(A) Absorption Potential Classification:
Papp(A-to-B) ≥ 1.0 x 10^{-6} cm/s — High
Papp(A-to-B) > 0.5 x 10^{-6} cm/s, Papp <1.0 x10^{-6} cm/s — Medium
Papp(A-to-B) < 0.5 x 10^{-6} cm/s — Low

(B) Efflux considered significant if:
Papp (B-to-A) ≥ 1.0 x 10^{-6} cm/s and Ratio Papp(B-to-A)/Papp(A-to-B) ≥ 3.0

(C) Low recoveries caused by non-specific binding, etc. can affect the measured permeability

(D) A low rate of diffusion (< 20 x10^{-6} cm/s) through the cell-free membrane indicates a lack of free diffusion, which may affect the measured permeability.

Study title: **Zeneca ZD6474: The binding of [^{14}C]-Zeneca ZD6474 to plasma proteins**
Study no.: KPJ010
Study report location: M5.3.2.1
Previously reviewed in IND 60042 by Wendelyn Schmidt, Ph.D.

Study title: **Determination of the involvement of human transport proteins MDR1, BCRP and MRP1 in the transport of ZD6474**
Study no.: KMN070
Study report location: M5.3.2.2

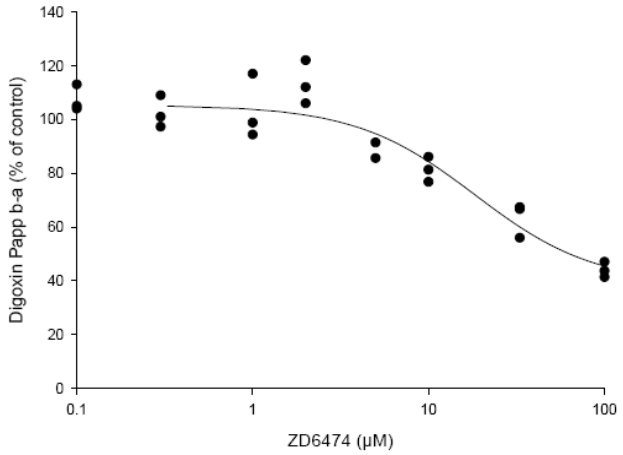
The possible interaction of ZD6474 with the human transport proteins MDR1, BCRP, and MRP1 was investigated in MDCKII cells. Monolayers of MDCKII cells were transfected with human MDR1, human BCRP, or human MRP1 and cultured in Transwells to study the directional transport of ZD6474 (1, 10, 25, and 50 µM). The direct interaction of ZD6474 with the transporter associated ATPase activity was studied in isolated membranes containing high levels

of either human MDR1, human BCRP, or human MRP1. Reference compounds and inhibitors were used to validate the system. Based on the results of transport experiments in MDCKII cells, ZD6474 was not a substrate of MDR1. However, in the stimulation of MDR1-associated ATPase experiments there was some evidence with high concentrations of ZD6474 that ZD6474 was a low affinity substrate of MDR1. ZD6474 was not a substrate for BCRP or MRP1. In the transporter associated ATPase activity experiments, ZD6474 inhibited BCRP at high concentrations and showed inhibition of MRP1 with the amount of inhibition differing for individual MRP1 substrates. In transfected MDCKII cells, ZD6474 may or may not be a low affinity substrate for MDR1, and appears to be an inhibitor of BCRP and MRP1.

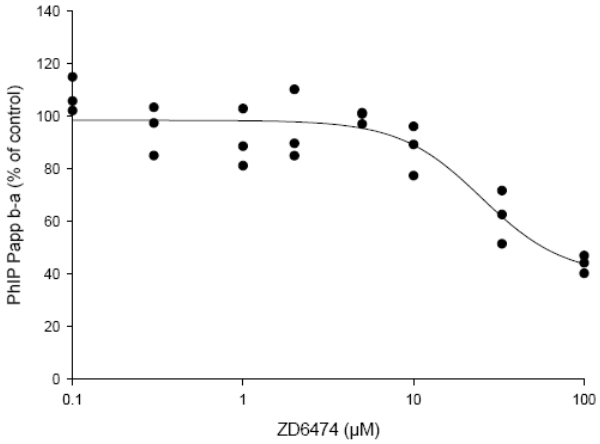
Study title: **Determination of the potential of ZD6474 to inhibit transport via the human multidrug resistance 1 protein (MDR1), human breast cancer resistance protein (BCRP) and human multidrug resistance protein 1 (MRP1)**
Study no.: KMN096
Study report location: M5.3.2.2

The potential of ZD6474 to inhibit the human transport proteins MDR1, BCRP, and MRP1 was investigated *in vitro* in MDCKII cells. The bidirectional transport of marker substrates [^{3}H]-digoxin (MDR1) and [^{14}C]-PhIP (BCRP) across control-, MDR1- and BCRP-transfected MDCKII cell monolayers was measured in the presence and absence of 8 concentrations of ZD6474 (0.1-100 µM), and known inhibitors ketoconazole (MDR1) and Ko143 (BCRP). For MRP1, the efflux of the MRP1 substrate calcein from control- and MDR1-Transfected MDCKII cells was determined in the absence and presence of 8 concentrations of ZD6474 (0.1-100 µM) and in the presence of the known MRP1 transport inhibitor MK571. ZD6474 inhibits human MDR1 in the MDR1-transfected MDCKII cells with an estimated IC_{50} value of 18.3 µM, and inhibits human BCRP in the BCRP-transfected MDCKII cells with an estimated IC_{50} value of 25.1 µM. These findings are shown in the figures below. Although ZD6474 produced a reduction of calcein efflux from the basolateral side in both contol- and MRP1-transfected cells, indicating that ZD6474 may inhibit MRP1, the variability in the data was high. Therefore, it was not possible to determine if ZD6474 inhibits human MRP1 in MRP1-transfected MDCKII cells.

Inhibition of the basolateral to apical transport of [^{3}H]-digoxin by ZD6474 in MDR1-transfected MDCKII cells (excerpted from sponsor's submission)



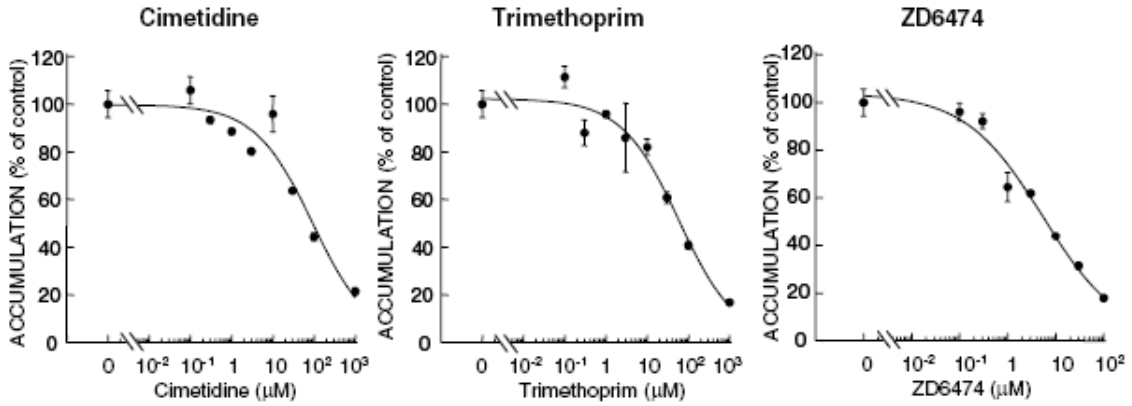
Inhibition of the basolateral to apical transport of [^{14}C]-PhIP by ZD6474 in BCRP-transfected MDCKII cells (excerpted from sponsor's submission)



Study title: **ZD6474- *in vitro* assessment of the involvement of the human transport protein OCT2 (SLC22A2) in the transport of ZD6474**
Study no.: KMX083
Study report location: M5.3.2.2

The possible interaction of ZD6474 with the human transport protein OCT2 was assessed *in vitro* by measuring uptake and inhibition in HEK293 cells. Uptake of [^{14}C]-ZD6474 (0.01-500 µM) and the selective marker substrate [^{14}C]-creatinine by control- and OCT2-transfected HEK293 cell monolayers was measured at various concentrations and time points (1-15 min) in both the absence and presence of the prototypical OCT2 inhibitor, 1-methyl-4-phenylpyridinium (MPP^+). To assess inhibition, the uptake of [^{14}C]-creatinine by HEK-transfected cell monolayers was determined in the presence of a range of concentrations of ZD6474 (0.1-100 µM) and the positive control inhibitors cimetidine and trimethoprim (0.1-1000 µM for both). Accumulation of [^{14}C]-ZD6474 was greater in OCT2-transfected HEK 293 cells compared to control HEK 293 cells, however, addition of the OCT2 inhibitor MPP^+ did not inhibit [^{14}C]-ZD6474 uptake into OCT2-transfected HEK293 cells. This suggests that the [^{14}C]-ZD6474 accumulation observed was not due to OCT2, therefore, ZD6474 appears to not be a substrate of OCT2. In the inhibition experiment, ZD6474 inhibited the transport of [^{14}C]-creatinine in a concentration-dependent manner, with an IC_{50} value of 5.5 µM. The inhibition of [^{14}C]-creatinine uptake by ZD6474 and the positive controls cimetidine and trimethoprim is shown below. ZD6474 is an inhibitor, but not a substrate of human OCT2 expressed in HEK293 cells under the conditions of this experiment.

Inhibition of human OCT2-mediated uptake of [^{14}C]-creatinine uptake by cimetidine, trimethoprim, and ZD6474 into HEK293 cells (excerpted from sponsor's submission)



Metabolism

Study title: **AstraZeneca ZD6474. The disposition of [^{14}C]-AstraZeneca ZD6474 in the mouse**
Study no.: KMM068
Study report location: M4.2.2.4
A single oral dose of 50 mg/kg [^{14}C]-ZD6474 was administered to male and female mice. Urine and feces were collected for 7 days after dosing and all samples were analyzed for total radioactivity. Selected excreta samples were examined chromatographically. The carcasses were retained for analysis. The urine and feces were analyzed by both HPLC and TLC. The mean total recovery of radioactivity was 83% and approximately 17% of the dosed radioactivity was not accounted for. The majority of radioactivity was eliminated in feces (55-63%), while an additional 9-10% was recovered in urine. Approximately 52% of the total recovered radioactive material was accounted for by 48 hours after dosing. The metabolite profile in urine and feces was similar by HPLC and TLC detection. Percentages are the proportion of chromatogram radioactivity. The two major components detected in mouse urine were ZD6474 (26-44%) and the N-oxide metabolite (34-46%), and two minor components detected were the N-desmethyl metabolite (5-10%) and a component tentatively identified as O-desalkyl ZD6474 glucuronide (2-10%). Other minor urinary components remain unidentified. Three components were detected in feces: ZD6474 (75.7-83.7%), the N-desmethyl metabolite (7-11%), and an unidentified chemical (1-2%).

Data for the recovery of radioactivity in excreta and the proportion of HPLC-RAD chromatogram radioactivity in urine and feces for both male and female mice following oral administration is presented along with the data from the rat and dog disposition studies in the tables entitled "Recovery of radioactivity in excreta following single oral administration of [^{14}C]-ZD6474", "Proportion of HPLC-RAD chromatogram radioactivity in urine following single oral administration of [^{14}C]-ZD6474", and "Proportion of HPLC-RAD chromatogram radioactivity in feces following single oral administration of [^{14}C]-ZD6474" located following study KMD037.

Study title: **Zeneca ZD6474: The disposition of [^{14}C]-Zeneca ZD6474 in the rat**
Study no.: KMR006
Study report location: M4.2.2.4
Previously reviewed in IND 60042 by Wendelyn Schmidt, Ph.D. Separate review below.

Single oral and intravenous doses of 5 mg/kg [^{14}C]-ZD6474 were administered to male and female rats. Urine and feces were collected for 5 days after dosing and all samples were analyzed for total radioactivity. Expired CO_2 was collected for 24 hours after oral dosing. The urine and feces were analyzed by both HPLC and TLC. Results were similar for oral and intravenous administration, with total recovery of radioactivity of between 80-90%. As in the mouse, the majority of radioactivity was eliminated in feces (58-70%), while an additional 7-12% was recovered in urine. No radioactivity was detected in the expired air. The metabolite profile in feces was similar by HPLC and TLC detection and between oral and intravenous administration. In feces, the majority of the radioactivity was associated with ZD6474 (65-79%). There were three other components detected in the feces, a polar metabolite accounting for 11-23% of the radioactivity and two less polar chemicals accounting for 3-12% and 3-6% of radioactivity. In urine, the majority of the radioactivity was associated with a polar metabolite (49-87%). ZD6474 accounted for approximately 33% of the radioactivity with intravenous administration and approximately 10% with oral administration. There were two other components each accounting for less than 5% of the radioactivity.

Data for the recovery of radioactivity in excreta for both male and female rats following oral administration is presented along with the data from the mouse and dog disposition studies in the table entitled "Recovery of radioactivity in excreta following single oral administration of [^{14}C]-ZD6474" located following study KMD037.

Study title: **AstraZeneca ZD6474. The disposition of [^{14}C]-AstraZeneca ZD6474 in the rat**
Study no.: KMR038
Study report location: M4.2.2.4

Single oral and intravenous doses of 5 mg/kg [^{14}C]-ZD6474 were administered to male and female rats. In one set of animals, urine and feces were collected for 7 days after dosing and all samples were analyzed for total radioactivity. The carcasses were retained for analysis. In a second set of animals, plasma samples were collected at 3, 8, and 24 hours after dosing for metabolite profile analysis. All samples were analyzed for total radioactivity and selected samples were examined chromatographically with HPLC-RAD. Total recovery of radioactivity was 93% following oral administration, with the majority of radioactivity eliminated in the feces (83%) and an additional 6% recovered in the urine. Following intravenous administration, total recovery of radioactivity was lower (74%), with (65%) eliminated in the feces and 6% recovered in the urine. The rate of elimination after oral administration was moderate, with approximately 70% of the total recovered radioactivity accounted for by 48 hours after dosing. Three components were detected in the urine following oral and intravenous administration. The major component was the N-oxide metabolite, which accounted for 65-78% of the chromatogram radioactivity. The two minor components were ZD6474 (9-16%) and the N-desmethyl metabolite (1-4%). Two components were detected in feces following oral and intravenous administration. The major component was ZD6474 (71-76%) and the minor component was the N-desmethyl metabolite (5-8%).

Data for the recovery of radioactivity in excreta and the proportion of HPLC-RAD chromatogram radioactivity in urine and feces for both male and female rats following oral administration is presented along with the data from the mouse and dog disposition studies in the tables entitled "Recovery of radioactivity in excreta following single oral administration of [^{14}C]-ZD6474", "Proportion of HPLC-RAD chromatogram radioactivity in urine following single oral administration of [^{14}C]-ZD6474", and "Proportion of HPLC-RAD chromatogram radioactivity in feces following single oral administration of [^{14}C]-ZD6474" located following study KMD037.

Study title: **Zeneca ZD6474: The disposition of [^{14}C]-ZD6474 in the dog following oral and intravenous administration**
Study no.: KKD005
Study report location: M4.2.2.2

Previously reviewed in IND 60042 by Wendelyn Schmidt, Ph.D. Separate review below.

Single oral and intravenous doses of 5 mg/kg [^{14}C]-ZD6474 were administered to male dogs. Urine and feces were collected for 5 days after dosing and blood samples were taken at various times up to 2 days. For both oral and intravenous administration, the total recovery of radioactivity was 90%. As in both the mouse and rat, the majority of radioactivity was eliminated in feces (81-85%), while an additional 4-8% was recovered in urine. The urine and feces were analyzed by both HPLC and TLC. There were two components in the feces: ZD6474 co-chromatographed with another chemical and accounted for 76-82% of the radioactivity and another chemical accounting for 15-19% of the radioactivity. In urine, there were also two components. TLC showed ZD6474 co-chromatographed with another chemical accounted for 24% of the radioactivity and the other chemical accounted for 66% of the radioactivity. The pharmacokinetic data from blood samples is presented below.

Individual animal pharmacokinetic parameters derived following single oral and intravenous doses of [^{14}C]-ZD6474 to male dogs (excerpted from sponsor's submission)

	Individual animal pharmacokinetic parameters					
	Intravenous dose			Oral dose		
Parameter	M1	M2	M3	M1	M2	M3
C_{max} (µg/ml)	0.349	0.277	0.266	0.023	0.024	0.037
t_{max} (hours)	0.083	0.083	0.083	6	6	4
AUC (µg.h/ml)	1.075	1.133	0.790	NC	NC	NC
AUC_{0-12} (µg.h/ml)	0.756	0.738	0.659	0.193	0.230	0.281
$t_{½}$ (hours)	10.9	9.09	4.78	NC	NC	NC
Absolute bioavailability (%)	-	-	-	25.5	31.2	42.6
Clearance (ml/min/kg)	77.6	73.5	105	-	-	-
Volume of distribution (l/kg)	48.7	46.5	39.9	-	-	-

Absolute bioavailability calculated using AUC_{0-12}
NC Not calculated

Data for the recovery of radioactivity in excreta for male dogs following oral administration is presented along with the data from the mouse and rat disposition studies in the table entitled "Recovery of radioactivity in excreta following single oral administration of [^{14}C]-ZD6474" following study KMD037.

Study title: **AstraZeneca ZD6474. The disposition of [^{14}C]-AstraZeneca ZD6474 in female dogs**
Study no.: KMD037
Study report location: M4.2.2.4

Single oral and intravenous doses of 5 mg/kg [^{14}C]-ZD6474 were administered to female dogs. Urine and feces were collected for 7 days after dosing. Plasma was collected at 2, 4, 8, and 12 hours after oral dosing and at 30 minutes, 2, 8, and 12 hours after intravenous dosing. All samples were analyzed for total radioactivity and metabolite profiles were determined in selected plasma, urine, and feces samples using HPLC-RAD. Total recovery of radioactivity was 74% following oral administration and 77% following intravenous administration, with the majority of radioactivity eliminated in the feces (66-70%) and an additional 7% recovered in the urine. For oral administration approximately 57% of the total recovered radioactivity accounted for by 48 hours after dosing. The elimination rate was slightly slower for intravenous administration with approximately 47% of the total recovered radioactivity accounted for by 48 hours after dosing. The plasma concentrations of radioactivity following oral and intravenous administration are presented below.

Plasma concentrations of radioactivity (excerpted from sponsor's submission)

Time (hours)	Concentration of radioactivity (ng equiv/g)	
	Oral	Intravenous
0.5	NS	601 ± 15
2	130 ± 10	300 ± 10
4	119 ± 5	NS
8	76 ± 3	114 ± 9
12	58 ± 5	72 ± 5

NS = No sample

Five components were detected in the urine following oral and intravenous administration. The major component was the N-oxide metabolite, which accounted for 47-63% of the chromatogram radioactivity. ZD6474 (22-35%) and the N-desmethyl metetabolite (3-4%) were also present. Two other unidentified components were present, accounting for 3-4% and 2-3% of the radioactivity. Two components were detected in the feces following oral and intravenous administration, ZD6474 (81%) and the N-desmethyl metabolite (8%).

Data for the recovery of radioactivity in excreta and the proportion of HPLC-RAD chromatogram radioactivity in urine and feces for female dogs following oral administration is presented in the tables below along with the data from the mouse and rat disposition studies.

Recovery of radioactivity in excreta following single oral administration of [^{14}C]-ZD6474

Study #	Proportion of dose recovered (%)							
	KMM068		KMR006		KMR038		KKD005	KMD037
Dose of [^{14}C]-ZD6474	50 mg/kg		5 mg/kg		5 mg/kg		5 mg/kg	5 mg/kg
Days of urine and feces collection	7		5		7		5	7
Species	Mouse		Rat		Rat		Dog	Dog
Sex	Male	Female	Male	Female	Male	Female	Male	Female
Urine	10.06	9.44	8.18	7.57	5.3	6.0	4.37	6.64
Feces	55.12	62.90	70.03	66.29	82.4	84.4	84.62	65.56
Cage wash	7.48	8.24	5.67	6.31	1.4	2.2	1.22	1.46
Carcass	10.21	2.81	4.69	7.86	2.0	1.7	NA	NA
Total recovery	82.87	83.38	88.56	88.03	91.1	94.2	90.21	73.65

NA= not available

Proportion of HPLC-RAD chromatogram radioactivity in urine following single oral administration of [^{14}C]-ZD6474

Study #	Proportion of HPLC-RAD chromatogram radioactivity (%)				
	KMM068		KMR038		KMD037
Dose of [^{14}C]-ZD6474	50 mg/kg		5 mg/kg		5 mg/kg
Days of urine collection	7		2		2
Species	Mouse		Rat		Dog
Sex	Male	Female	Male	Female	Female
ZD6474	42.01	26.07	13.9	16.1	35.4
N-oxide metabolite	33.50	45.68	65.7	64.7	47.3
N-desmethyl metabolite	6.53	5.01	3.5	1.8	4.4
O-desalkyl ZD6474 glucuronide	2.31	9.51	-	-	-

Proportion of HPLC-RAD chromatogram radioactivity in feces following single oral administration of [^{14}C]-ZD6474

Study #	Proportion of HPLC-RAD chromatogram radioactivity (%)				
	KMM068		KMR038		KMD037
Dose of [^{14}C]-ZD6474	50 mg/kg		5 mg/kg		5 mg/kg
Days of feces collection	7		5		5
Species	Mouse		Rat		Dog
Sex	Male	Female	Male	Female	Female
ZD6474	75.74	83.72	73.2	73.1	81.1
N-oxide metabolite	-	-	-	-	-
N-desmethyl metabolite	11.05	6.62	8.0	3.0	8.4
O-desalkyl ZD6474 glucuronide	-	-	-	-	-

Study title: **AstraZeneca ZD6474: Investigation of biliary secretion and enterohepatic recirculation of [^{14}C]-AstraZeneca ZD6474 in the bile of cannulated male rats**
Study no.: KMR013
Study report location: M4.2.2.4

To determine the disposition and metabolism of [^{14}C]-ZD6474, a single oral dose of 5 mg/kg [^{14}C]-ZD6474 was administered to bile duct cannulated male Sprague Dawley rats. Bile, urine, and feces were collected for 3 days after dosing. To investigate the possibility of enterohepatic recirculation, a pooled bile sample (prepared from selected bile samples from rats dosed orally with [^{14}C]-ZD6474) was infused into the duodenum of bile duct cannualated male rats at 1 mL/hour for 6 hours. Bile, urine, and feces were collected for 3 days. Selected samples from both groups of rats were examined chromatographically by LC-MS. The total radioactivity recovered and the mean recovery of radioactivity in urine, feces, cage wash, and bile for male bile duct cannulated rats administered a single oral or intraduodenal dose are in the tables below. Following single oral administration, the amount of radioactivity recovered in urine (20.9%), feces (20.8%), and bile (26.9%) was similar. This contrasts with findings in other disposition studies where the majority radioactivity was eliminated in the feces. Following the single intraduodenal dose, the majority of the radioactivity was eliminated in the feces (72.2%), with 5% in the urine and 7% in the bile. The chromatography results for the amount of ZD6474 and the N-oxide and N-desmethyl metabolites in urine, bile, and feces presented in a table below. The data is presented as a % of the dose and not the chromatogram. Following single oral administration of [^{14}C]-ZD6474, ZD6474 was the major component in the feces (12.4%) and urine (17.3%), while the N-oxide metabolite was the major component in the bile (23.4%) and present in the urine (5.41%). The rats that received the single intraduodenal administration were dosed with the bile obtained from the single oral dose rats and therefore, received mostly the N-

oxide metabolite. Following the intraduodenal administration, ZD6474 was the major component in the feces (42.2%). This indicates that the N-oxide metabolite present in the bile administered to the rats was reduced back to ZD6474 in the gut. The N-oxide and N-desmethyl metabolites were present along with other unidentified components. These results suggest that following administration of ZD6474, biliary elimination is the major route of excretion and that up to 14% of the material excreted in the bile may be reabsorbed and recirculated.

Mean recovery of radioactivity in excreta samples following a single oral administration of [^{14}C]-ZD6474 to male bile duct cannulated rats (excerpted from sponsor's submission)

	Percentage Dose Radioactivity Recovered									
Time Point	Urine		Faeces		Cage Wash		Bile		Total	
(hours)	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE
0-24	12.7	0.73	4.58	1.76	2.76	0.50	18.4	2.54	38.5	3.40
24-48	5.21	0.57	9.64	0.44	1.51	0.60	6.08	0.69	22.5	1.06
48-72	2.94	0.39	6.58	1.63	0.99	0.24	2.37	0.16	12.9	2.05
Total (0-72)	20.9	0.84	20.8	2.29	5.26	1.06	26.9	3.32	73.8	3.27

All values show the mean ± SE obtained from five animals (animal 003M removed from all mean calculations as absence of 0-12 hour post-dose bile samples may have affected the route of excretion)
The limit of detection (LOD), taken as 50 dpm once background has been subtracted
Any individual values below the limit of detection (LOD) are taken as 0.00 in mean calculations

Mean recovery of radioactivity in excreta samples following a single intraduodenal administration of bile collected following oral administration of [^{14}C]-ZD6474 to male bile duct cannulated rats (excerpted from sponsor's submission)

	Percentage Dose Radioactivity Recovered									
Time Point	Urine		Faeces		Cage Wash		Bile		Total	
(hours)	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE
0-24	2.66	0.29	52.4	7.19	0.75	0.11	4.18	0.62	60.0	7.15
24-48	1.46*	0.30*	16.3*	7.08*	0.62*	0.07*	1.61*	0.33*	20.0*	7.43*
48-72	0.75*	0.14*	2.81*	0.82*	0.53*	0.12*	0.84*	0.21*	4.94*	0.96*
Total (0-72)	4.78*	0.72*	72.2*	3.62*	1.91*	0.20*	6.96*	1.11*	85.8*	3.58*

Each value shows the mean ± SE obtained from six animals
* = Each value shows the mean ± SE obtained from five animals
The limit of detection (LOD), taken as 50 dpm once background has been subtracted
Any individual values below the limit of detection (LOD) are taken as 0.00 in mean calculations

Radioactvity profiles in urine, bile, and feces following a single oral administration of [^{14}C]-ZD6474 to male bile duct cannulated rats

Component	Single oral administration			Single intraduodenal administration		
	% of [^{14}C]-ZD6474 dose			% of [^{14}C]-bile pool dose		
	Urine*	Bile	Feces	Urine*	Bile	Feces
ZD6474	17.3	0.58	12.4	1.70	-	42.2
N-oxide metabolite	5.41	23.4	0.15	3.16	-	-
N-desmethyl metabolite	2.25	-	1.50	0.27	-	4.44

*= includes cage wash

Study title: **Zeneca ZD6474: Metabolism of Zeneca ZD6474 in rat, dog, and human hepatocytes**
Study no.: KMN008
Study report location: M4.2.2.4
Previously reviewed in IND 60042 by Wendelyn Schmidt, Ph.D.

According to the review, results showed that "the metabolism of ZD6474 was minimal in rat and dog and negligible in human." The data are shown in the table below excerpted from the review.

% of total radioactivity/peak			
Species	Peak 1 (RT 0.88)*	Peak 2 (RT 1.37)	Parent
Rat	6.9%	1.1%	92.0%
Dog	1.0%	---	99.0%
Human	---	---	100.0%

*RT = retention time

Study title: **Comparison of the metabolism of [^{14}C]-AstraZeneca ZD6474 in the rat and dog**
Study no.: KMN012
Study report location: M4.2.2.4

This study was conducted to further investigate the metabolism of ZD6474 using samples from studies KMN008, KKD005, KMR006, and KKR007. Selected samples were re-analyzed using an improved HPLC method, designed to separate ZD6474 and the major urinary metabolite. The other aims of the study were to examine the metabolite profile in plasma, and characterize the major metabolite in dog urine. Analysis of the plasma samples from rats and dogs did not yield any notable metabolite information. Chromatography of the 0-6 hour urine samples from male dogs administered single oral and intravenous doses of 5 mg/kg [^{14}C]-ZD6474 (study # KKD005) showed that there were two components. The major component previously unidentified was determined to be the N-oxide metabolite. This finding is consistent with the results of study KMR037 conducted with the same dose in female dogs.

Study title: **The analysis of ZD6474, N-desmethyl-ZD6474 and N-oxide-ZD6474 in human urine samples by HPLC-MS/MS**
Study no.: KMN094
Study report location: M5.3.2.2

This was an exploratory analysis to access relative concentrations of ZD6474 and the two metabolites (N-desmethyl and N-oxide) in human urine samples. Samples were collected in January 2004 from 4 subjects in AstraZeneca study D4200C00025 and were analyzed after the established stability period using HPLC-MS/MS detection. ZD6474, the N-oxide metabolite, and the N-desmethyl metabolite were all detected in the urine of all four subjects. The N-oxide metabolite was present at concentrations similar to or lower than the concentrations of ZD6474, and the concentrations of the N-desmethyl metabolite were very low compared to concentrations of ZD6474.

Study title: ***In vitro* assessment of human liver cytochrome P450 inhibition potential of ZD6474 and the N-desmethyl metabolite of ZD6474**
Study no.: KMX095
Study report location: M5.3.2.2
The potential of ZD6474 and the N-desmethyl metabolite of ZD6474 to inhibit cytochrome P450 enzymes (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2D6, CYP2E1, and CYP3A4/5) in human liver microsomes was assessed *in vitro*. Reversible inhibition of cytochrome P450 enzymes was investigated by measuring the rate of formation of cytochrome P450 isoform-specific metabolites following co-incubation of the N-desmethyl metabolite of ZD6474 (0.03, 0.1, 1, 3, and 10 μM) with human liver microsomes, model substrate for each cytochrome P450 enzyme, and NADPH. Time-dependent inhibition of cytochrome P450 enzymes was initially investigated by pre-incubating ZD6474 (30 μM) or the N-desmethyl metabolite of ZD6474 (3 μM) for 30 minutes with human liver microsomes in the presence of NADPH. For the full time-dependent inhibition experiment, k_{inact} and K_I values were determined with ZD6474 (0.3, 1, 3, 10, and 30 μM) preincubated (0, 1, 5, 10, 20, or 30 minutes) with NADPH. Metabolism of model substrates was determined using HPLC-MS/MS. Relevant control incubations were performed, and cytochrome P450 isoform-selective inhibitors were used as positive controls.

The N-desmethyl metabolite of ZD6474 did not reversibly inhibit the cytochrome P450 enzymes tested and did not produce time-dependent inhibition of these enzymes at a concentration of 3 μM. In the preliminary time-dependent experiment, ZD6474 inhibited CYP2C9-mediated diclofenac 4'-hydroxylation (65% reduction) and CYP3A4/5-mediated testosterone 6β-hydroxylation (25% reduction) when incubated with NADPH. However, the full time-dependent inhibition experiment did not show any NADPH- and time-dependent inhibition, therefore, ZD6474 does not appear to produce time dependent inhibition on the cytochrome P450 enzymes tested (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2D6, CYP2E1, and CYP3A4/5).

Study title: **ZD6474: Investigation of the potential inhibitory effect of AstraZeneca ZD6474 on the metabolism of cytochrome P450 (CYP) model substrates**
Study no.: KMX020
Study report location: M5.3.2.2

To evaluate the potential of ZD6474 to inhibit of cytochrome P450 isozymes (CYP1A2, CYP2D6, CYP2C9, CYP2C19, and CYP3A4) and cause drug interactions, human liver microsomal protein was incubated with selective P450 substrates in the presence of ZD6474 (0.025-100 μg/mL). The inhibitory potential of ZD6474 was studied for each of these human P450 enzymes, and K_i values were derived from values of IC_{50} where appropriate. The effect of ZD6474 on the activity of the human liver P450 isozymes is shown in the table below. ZD6474 clearly inhibited CYP2D6 activity, with only 14% of vehicle control activity remaining in the presence of 100 μg/mL ZD6474. CYP1A2, CYP2C9, CYP2C19, and CYP3A4 (testosterone 6β-hydroxylase) activities were inhibited to a lesser extent with 55-89% of vehicle control activity remaining in the presence of 100 μg/mL ZD6474. For CYP1A2, CYP2C9, CYP2C19, and CYP3A4, the IC_{50} values were greater than the highest concentration tested (100 μg/mL), therefore K_i values could not be estimated. The IC_{50} determined against CYP2D6 was 25.3 μg/mL, and the K_i was 12.6 μg/mL. Therefore, at the maximum expected plasma dose (5

µg/mL), ZD6474 may inhibit the clearance of compounds which are metabolized by CYP2D6 and possibly increase the steady state plasma concentration of the co-administered compound.

Effect of ZD6474 on the activity of human P450 isozymes (excerpted from sponsor's submission)

ZD6474 Conc. (µg/mL)	Ethoxyresorufin O-deethylase (CYP1A2)*	Tolbutamide 4'-hydroxylase (CYP2C9)	S-Mephenytoin 4'-hydroxylase (CYP2C19)	Bufuralol 1'-hydroxylase (CYP2D6)	Testosterone 6β-hydroxylase (CYP3A4)	Midazolam 1'-hydroxylase (CYP3A4)
0.025	96.8	92.0	87.7	110.1	105.0	109.7
0.1	99.5	88.0	99.9	108.0	116.3	112.7
0.25	89.6	89.7	86.5	107.6	117.6	95.0
1	93.7	93.5	82.0	103.9	107.4	93.2
2.5	95.3	89.7	79.6	94.9	116.1	92.5
10	90.0	91.4	77.6	80.8	104.2	110.6
25	81.2	87.0	75.6	57.0	94.2	120.7
100	55.1	89.1	67.0	14.2	78.0	100.6

Results are presented as the mean (n=2* or 3) percentage of vehicle control enzyme activity for each specific marker substrate assay.

Excretion

Study title: **ZD6474. The excretion of drug-related material in the milk following oral administration of [^{14}C]-ZD6474 to lactating rats**
Study no.: KMR071
Study report location: M4.2.2.5

This study was conducted to investigate the transfer of drug related material into milk following oral administration of [^{14}C]-ZD6474 to lactating rats. A single oral dose of [^{14}C]-ZD6474 (10 mg/kg; 21 µmol/kg) was administered to 6 female lactating rats (Hans Wistar) 14 days post-partum. The dams were separated from their litters and received a dose of oxytocin (4IU/kg, i.p.) 5 to 10 minutes prior to milking to stimulate milk to let down. Dams were milked and blood samples were taken at 1, 2, 4, 6, 8, 12, and 24 hours after dosing. Control samples of milk were collected from each female the day before dosing. Total radioactivity was measured in milk and blood samples. Chromatographic profiles were generated using HPLC with ultraviolet and radiochemical detection (HPLC-UV-RAD) and metabolites identified by HPLC coupled with mass spectrometry (HPLC-MS) for 1, 4, 8, and 24 hour milk samples.

After oral administration of [^{14}C]-ZD6474 to lactating rats, ZD6474 and trace amounts of the N-desmethyl and N-oxide metabolites of ZD6474 were excreted in milk with peak concentrations observed at 8 hours after dosing. Concentrations of ZD6474 were higher in milk than blood at all time points except 1 hour after dosing. Mean concentrations of radioactivity in milk and blood following administration of [^{14}C]-ZD6474 are presented in the table below.

Mean concentrations of radioactivity in milk and blood following oral administration of [^{14}C]-ZD6474 to lactating rats

(excerpted from sponsor's submission)

Time (hours)	Concentration of radioactivity (ng equiv/g)		
	Milk	Blood	Ratio milk : blood
1	463 ± 108	211 ± 37.8	2.19:1
2	1610 ± 184	394 ± 32.1	4.09:1
4	1929 ± 406	448 ± 12.4	4.31:1
6	1822 ± 391	411 ± 6.67	4.43:1
8	3447 ± 608	346 ± 12.1	9.97:1
12	1330 ± 259	304 ± 13.2	4.38:1
24*	1443 ± 445	180 ± 5.19	8.03:1

Each value represents the mean ± SE obtained from three animals except * where six animals were used to calculate the result

6 General Toxicology

6.1 Single-Dose Toxicity

Study title: **Zeneca ZD6474: Acute toxicity (limit) study in mice: Oral administration**
Study no.: TLM1135
Study report location: M4.2.3.1
Previously reviewed in IND 60042 by Wendelyn Schmidt, Ph.D.

Study title: **Zeneca ZD6474: Acute toxicity (limit) study in rats: Oral administration**
Study no.: TLR2935
Study report location: M4.2.3.1
Previously reviewed in IND 60042 by Wendelyn Schmidt, Ph.D.

Study title: **Zeneca ZD6474: Acute toxicity (limit) study in mice: Intravenous administration**
Study no.: TLM1136
Study report location: M4.2.3.1
Previously reviewed in IND 60042 by Wendelyn Schmidt, Ph.D.

6.2 Repeat-Dose Toxicity

Study title: **Zeneca ZD6474: One month oral toxicity study in rats**
Study no.: TAR2937
Study report location: M4.2.3.2
Previously reviewed in IND 60042 by Wendelyn Schmidt, Ph.D. See summary.

Study title: **Zeneca ZD6474: Dog oral pilot toxicity study**
Study no.: TAD1041
Study report location: M4.2.3.2
Previously reviewed in IND 60042 by Wendelyn Schmidt, Ph.D.

Study title: **Zeneca ZD6474: One month oral toxicity study in dogs**
Study no.: TAD1042
Study report location: M4.2.3.2
Previously reviewed in IND 60042 by Wendelyn Schmidt, Ph.D. See summary.

The one month repeat dose toxicology studies in rats (Study TAR2937) and dogs (Study TAD1042) were previously reviewed under IND 60042 (Wendelyn Schmidt, Ph.D.; 2000). In the one month studies vandetanib was administered daily for 29 days in rats (0, 5, 25, or 75 mg/kg/day; 0, 30, 150 or 450 mg/m^2/day) and dogs (0, 5, 15, or 40 mg/kg/day; 0, 100, 300, or 800 mg/m^2/day). In both studies dosing was stopped early in the high dose group, on Day 25 in rats and Day 14 in dogs. Mortality was also observed in both studies, with 9 deaths (4 males and 5 females) in the high dose group in the rat and 6 dogs (3 males and 3 females) in the high dose group euthanized for poor condition. Decreased body weight and food consumption compared to controls and clinical signs of loose feces, thinness, loss of skin tone, and subdued behavior were observed in both species. Target organs of toxicity were the liver, kidney, ovaries/uterus, bone, and teeth in the rats, and the liver, gastrointestinal tract, and bone in the dogs.

In the one month studies, liver toxicities included increases in ALT in both the rat and dog, decreases in liver weight in the rat, and histopathology findings in the liver including reduced hepatocyte glycogen vacuolation, centrilobular hepatocyte vacuolation, foamy macrophages, and necrosis. Since liver histopathology findings were observed at the high doses only in the one month studies (450 mg/m^2/day in rat; 800 mg/m^2/day in dog), results suggest that vandetanib may only produce liver toxicity at these higher doses, even with a longer treatment time. Toxicity in the ovaries and uterus were also observed in the one month rat study.

Study title: **Zeneca ZD6474: Six month oral toxicity study in rats**

Key study findings:

- Mortality:
  - Mortality was observed at all doses of ZD6474 and in the control group; rats were either found dead or euthanized due to poor health.
  - Probable causes of mortality include toxicities of the lung (pleuritis, abscess, foreign body pneumonia, and alveolar congestion and/or edema), esophagitis in the esophagus, pancreatitis in the pancreas, and cholangitis in the bile duct.
- The highest dose was initially 20 mg/kg/day, however, due to significant toxicity (mortalities and significant reductions in food intake and body weight gain), this dose was reduced to 10 mg/kg/day starting at Week 13 of the study.
- Target organs of toxicity include the adrenal gland, bile duct, heart, kidney, lungs, pancreas, mesenteric lymph node, skin, spleen, teeth, and thymus.
- Two toxicities of interest are teeth abnormalities and masses observed in the clinical signs and the gross pathology and/or the histopathology.

- Increases in WBC and neutrophils and histopathology findings of inflammatory cell infiltration in multiple organs indicate inflammation.
- Histopathology changes in the kidney and increases in urea and creatinine levels in the blood indicate renal toxicity.

Study no.: TPR2939
Study report location: M4.2.3.2
Conducting laboratory and location: AstraZeneca UK Limited
Safety Assessment UK Alderley
Alderley Park
Macclesfield
Cheshire SK10 4TG
U.K.

Histopathology:
(b) (4)

Date of study initiation: March 10, 2000 (Study plan review),
March 20, 2000 (Protocol approved)

GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: ZD6474, batch # C268/1, purity: 99.0%

Methods

Species/strain:	Alpk:AP$_f$SD (Wistar derived) rat
Number/sex/group or time point:	Main study: 20/sex/group Recovery: 10/sex/group, control and 20/10 mg/kg/day groups only
Age:	39-41 days old at start of dosing
Weight:	Males: 134-227 grams at start of dosing Females: 121-177 grams at start of dosing
Schedule:	Once daily for 26 weeks, 13 week recovery
Doses:	0, 1, 5, or 20/10 mg/kg/day ZD6474 (at Week 13 the 20 mg/kg/day dose was reduced to 10 mg/kg/day*)
Vehicle/control:	0.5% w/v hydroxypropyl methylcellulose solution containing 0.1% w/v polysorbate 80
Route:	oral gavage
Volume:	1 mL/kg; during week 13 following the dose reduction from 20 mg/kg/day to 10 mg/kg/day, the high dose group was administered a dose volume of 0.5 mL/kg

***** The highest dose was initially 20 mg/kg/day, however, due to significant toxicity (mortalities and significant reductions in food intake and body weight gain), this dose was reduced to 10 mg/kg/day starting at Week 13 of the study.

Observations and times:

Mortality:	Twice daily
Clinical signs:	Twice daily for gross abnormality Weekly for detailed observations
Body weights:	Prior to study (day -7), Day 1 , and weekly for the remainder of the study
Food consumption:	Continuously for 7 days pre-study and throughout the remainder of the study (exception: not recorded for males during week 9)
ECG	Not conducted
Ophthalmoscopy:	Pretest and week 26 (all animals), weeks 5 and 14 (control and 20/10 mg/kg/day groups), and during last week of recovery (recovery animals)
Hematology:	Weeks 13 and 26 (10 animals/sex/group), and week 38 (recovery animals)
Clinical chemistry:	Weeks 13 and 26 (10 animals/sex/group), and week 38 (recovery animals)
Coagulation:	Not conducted
Urinalysis:	Weeks 12 and 25 (up to10 animals/sex/group) and week 37 (recovery animals)
Gross pathology:	At necropsy (Main study: Week 27; Recovery: Week 40)
Organ weights:	At necropsy (Main study: Week 27; Recovery: Week 40)
Histopathology:	At necropsy (Main study: Week 27; Recovery: Week 40)
Toxicokinetics:	Collected from 3 animals/sex/group at each timepoint • At 0.5, 1, 2, 4, 8, and 24 hours after first dose for the 5 mg/kg/day group • At 0.5, 1, 2, 4, 8, and 24 hours after dosing during week 26 for the 1, 5, and 20/10 mg/kg/day groups • At 28, 32, and 48 hours after the final dose in the 20/10 mg/kg/day group

Other:

- To assess male fertility, all male animals from the main study groups were paired with virgin females from study number TGR/3138 for 7 days during week 9. All data related to mating performance and fertility indices are reported separately under study number TGR/3138.

Data and statistics:

- Data for body weight, food consumption, hematology, clinical chemistry, urinalysis, and organ weights were presented with the medians for each group.
- Statistics were conducted using a step-down version of the Jonckheere-Terpstra non-parametric trend test with critical values approximated by the student t-distribution. Statistical significance attached to the groups relate to the trend up to and including that group

Results

Mortality:

Dose of ZD6474	Animal #	Sex	Found dead or Euthanized	Week	Findings/Clinical signs
Control	26	Male	Found dead	6	• No clinical signs observed
	112	Female	Found dead	23	• No clinical signs observed • Histopathology: Moderate acute pleuritis in lungs, probable cause of death
1 mg/kg/day	31	Male	Found dead	13	• No clinical signs observed • Histopathology: Severe multifocal alveolar congestion and moderate multifocal alveolar edema, probable cause of death
	32	Male	Found dead	13	• No clinical signs observed • Histopathology: Moderate multifocal alveolar congestion and moderate multifocal alveolar edema in lungs, probable cause of death
	35	Male	Found dead	13	• No clinical signs observed • Histopathology: Severe multifocal alveolar congestion and moderate multifocal alveolar edema in lungs, probable cause of death
	42	Male	Found dead	27	• Open wounds on the head and swelling of right ear observed from Week 26 • Histopathology: Moderate multifocal alveolar congestion and moderate multifocal alveolar edema in lungs • Died on the morning of scheduled necropsy and was processed as a termainal necropsy
	50	Male	Euthanized	5	• Loss of skin tone, hunched, thin, and decreased activity observed during Week 5 • Weight loss from Days 22-31
5 mg/kg/day	162	Female	Found dead	18	• Decreased activity and hunched posture observed in Week 18 • Histopathology: Severe acute

Dose of ZD6474	Animal #	Sex	Found dead or Euthanized	Week	Findings/Clinical signs
					pleuritis in lungs, severe multifocal alveolar congestion, and moderate multifocal alveolar edema in lungs, probable cause of death
20/10 mg/kg/day	88	Male	Euthanized	38	• Piloerection, pale, respiration dyspnea, and loss of skin tone • Histopathology: Large adjacent tissue abscess in lungs, probable cause of condition • Weight loss
	97	Male	Found dead	17	• No clinical signs observed • Histopathology: Severe acute foreign body pneumonia, severe multifocal alveolar congestion, and moderate multifocal alveolar edema in lungs, probable cause of death
	180	Female	Euthanized	17	• Thin, hunched posture, and loss of skin tone observed during Week 17
	186	Female	Euthanized	14	• Decreased activity, cold, thin, salivation reflex, hunched posture, and staining on muzzle observed during Week 14 • Histopathology: Moderate multifocal acute esophagitis in esophagus, probable cause of condition
	189	Female	Euthanized	33	• Piloerection, pale, respiration depth increased respiration rate decreased, bleeding from vagina and/or ano-genital region • Histopathology: Moderate multifocal decidual reaction associated with luminal hemorrhage, necrosis, and mixed inflammatory cell infiltration in uterus, probable cause of condition
	194	Female	Euthanized	12	• Left eye half shut/shut, piloerection, cold, distended abdomen, hunched posture, staining in uro/ano-genital region,

Dose of ZD6474	Animal #	Sex	Found dead or Euthanized	Week	Findings/Clinical signs
					clear discharge from both eyes observed on Week 12 • Histopathology: Severe acute cholangitis (ulcerative) in bile duct, probable cause of condition
	195	Female	Found dead	12	• No clinical signs observed • Histopathology: Severe acute cholangitis in bile duct and severe diffuse acute pancreatitis in pancreas, probable causes of death
	196	Female	Euthanized	13	• Abdominal swelling • Histopathology: Severe acute cholangitis (ulcerative) in bile duct, probable cause of condition
	199	Female	Euthanized	2	• Left ear swollen and discolored (Week 2) and open/wet wound on head starting on Week 1 which failed to heal
	200	Female	Found dead	10	• No clinical signs observed • Histopathology: Moderate acute cholangitis in bile duct, probable cause of death

Clinical signs:
Clinical signs observed in particular rats prior to death or euthanasia are listed in the mortality table above. Below is a table with the total number of rats in each group with various clinical signs and the week or weeks of the study in which they were observed. Two clinical signs of interest are teeth abnormalities and masses.

- Teeth abnormalities (see table for specifics) were observed in the 20/10 mg/kg/day group (10 males and 13 females) from Weeks 8-22, and as a result, these animals were fed a moistened or ground diet.

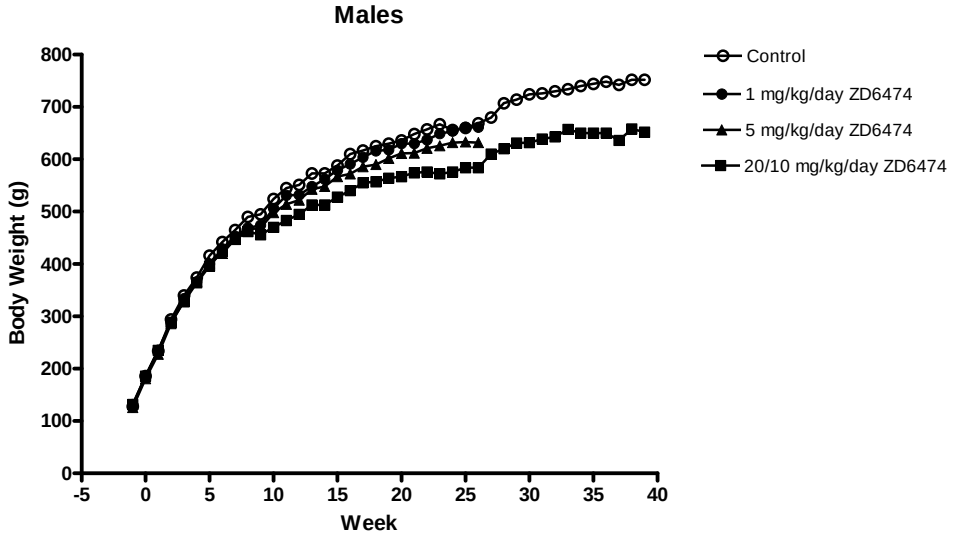
- Masses were observed on the lower abdomen of 1 male in the 5 mg/kg/day group during Week 24 and 7 males and 1 female in the 20/10 mg/kg/day group from weeks 13-40. Masses were also seen on the head of 1 male from the 20/10 mg/kg/day group during Weeks 21 and 22.

Clinical Sign	No. of animals affected (Week (s) observed relative to start date)							
	Males				**Females**			
Dose of ZD6474 (mg/kg/day)	Control	1	5	20/10	Control	1	5	20/10
Number of animals examined	30	20	20	30	30	20	20	30
Activity decreased	-	1 (5)	-	-	-	-	1 (18)	1 (14)
Teeth broken	-	-	-	7 (12-22)	-	-	-	12 (8-19)
Teeth missing	-	-	-	6 (8-13)	-	-	-	4 (8-17)
Teeth loose	-	-	-	1 (8)	-	-	-	-
Teeth discoloration	-	-	-	-	-	-	-	4 (14-18)
Piloerection	-	-	-	1 (38)	-	-	-	3 (11-33)
Pale	-	-	-	1 (38)	-	-	-	1 (33)
Thin	-	1 (5)	-	-	-	-	-	6 (8-40)
Cold	-	-	-	-	-	-	-	2 (12-14)
Respiration dyspnea	-	-	-	1 (38)	-	-	-	-
Respiration rate decreased	-	-	-	-	-	-	-	1 (33)
Respiration depth increased	-	-	-	-	-	-	-	1 (33)
Skin lesion open wound	1 (24-27)	1 (26)	2 (17-18)	4 (13-26)	-	-	-	1 (1-2)
Hunched posture	-	1 (5)	-	-	-	-	1 (18)	4 (11-17)
Loss of skin tone	-	1 (5)	-	1 (38)	-	-	-	2 (9-17)
Mass	-	-	1 (24)	7 (13-40)	-	-	-	1 (21-27)
Distended abdomen	-	-	-	-	-	-	-	2 (12-13)
Red discharge	-	-	-	-	-	-	-	4 (4-11)

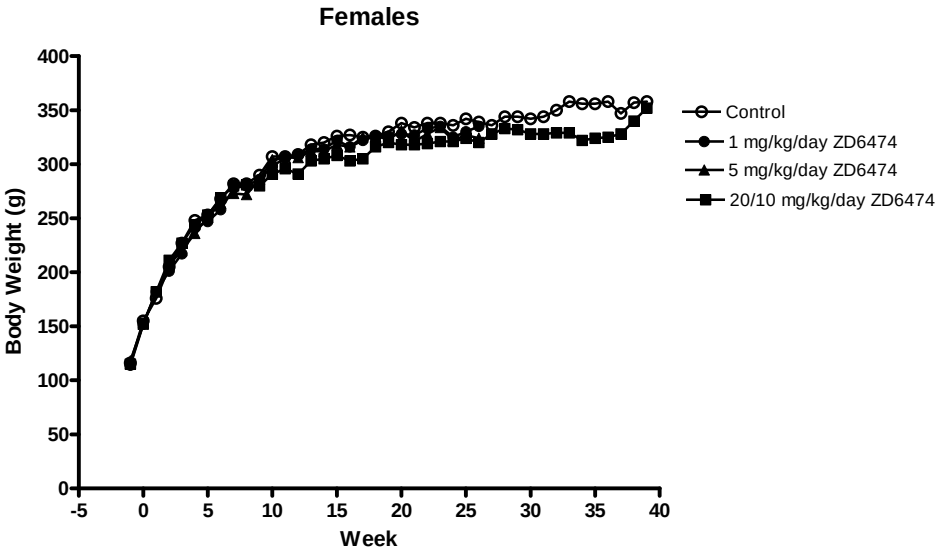
- = no test-article related changes

Body weights:

Below are the median body weights for males and females. In males, body weight was significantly lower in the 5 mg/kg/day and 20/10 mg/kg/day treatment groups than controls. In females, body weight was significantly lower in the 20/10 mg/kg/day treatment group.



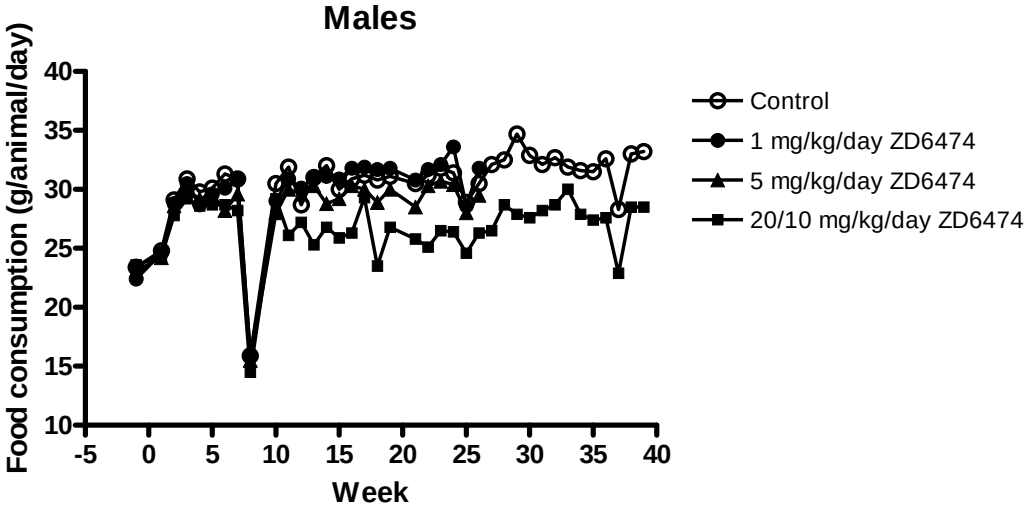
5 mg/kg/day: Statistically significant Weeks12-26, p<0.05 or p<0.01
20/10 mg/kg/day: Statistically significant Weeks 8-39, p<0.05, p<0.01, or p<0.001



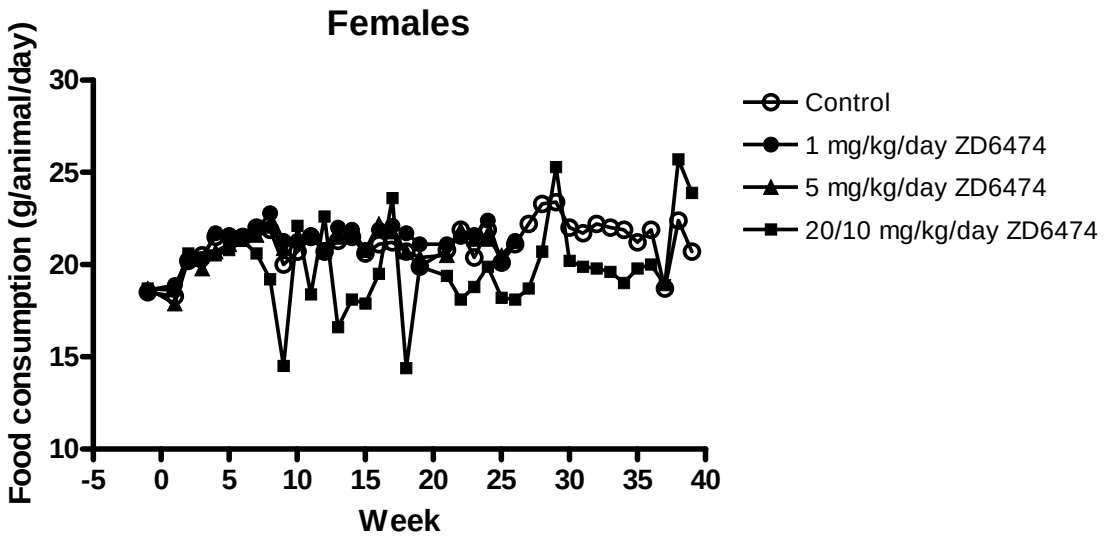
20/10 mg/kg/day: Statistically significant Weeks 10-17, 20-26, and 36, p<0.05 or p<0.01

Food consumption:

Food consumption appears to have been measured for each cage with 5 rats per cage, and calculated as g/animal/day for each cage. The median values are presented. Food consumption was not measured for males on Week 9. There is also no data for Week 20 for either males or females with no explanation.



5 mg/kg/day: Statistically significant Weeks 6 and 11, p<0.05
20/10 mg/kg/day: Statistically significant Weeks 2-3, 6-8, 11, and 13-26, p<0.05, p<0.01, or p<0.001
No statistcal analyses conducted for Weeks 27-39



20/10 mg/kg/day: Statistically significant Weeks 4, 7-8, 13-15, 17-18, 24 and 26, $p<0.05$, or $p<0.01$
No statistcal analyses conducted for Weeks 27-39

Ophthalmoscopy: Appears to be unremarkable, however, the ophthalmology report and individual data are not included in the study report. The report says "Individual animal findings are archived with the other raw data generated during the study."

EKG: Not conducted

Hematology:

- RBC, hemoglobin, and hematocrit were slightly increased (4-10%) compared to controls in both males and females treated with 20/10 mg/kg/day at Weeks 13 and 26
- Platelets were increased compared to controls in males (11%) and females (21%) treated with 20/10 mg/kg/day at Week 13.
- WBC were increased compared to controls in females treated with 5 mg/kg/day (39-59%) at Weeks 13 and 26, males treated with 20/10 mg/kg/day (27%) at Week 13, and females treated with 20/10 mg/kg/day (37-88%) Weeks 13, 26, and 38.
- Neutrophils were increased compared to controls in females treated with 5 mg/kg/day (50-58%) at Weeks 13 and 26, males treated with 20/10 mg/kg/day (75%) at Week 13, and females treated with 20/10 mg/kg/day (104-170%) Weeks 13, 26, and 38.

Data analyzed was expressed as the median value for each group. The following outliers were observed for the data for the indexes listed above:

Index	Group (dose, sex)	Week	Median	Outliers
WBC	1 mg/kg/day, females	26	4.4	12.0
	20/10 mg/kg/day, females	26	6.2	19.3
Neutrophils	1 mg/kg/day, females	26	1.02	5.96
	5 mg/kg/day, males	26	2.86	9.00, 9.07
	20/10 mg/kg/day, females	26	2.35	11.40

Clinical chemistry:

- Urea was slightly increased compared to controls in males treated with 1 mg/kg/day (16%) at Week 13 and females treated with 20/10 mg/kg/day (8-17%) at Weeks 13, 26, and 38.
- Creatinine was slightly increased compared to controls in males treated with 1 mg/kg/day and females treated with 20/10 mg/kg/day at Week 13 (10-11%), and in males treated at all doses of ZD6474 (4-9%) and females treated with 5 and 20/10 mg/kg/day (10-12%) at Week 26.
- ALT and/or AST were increased in both males and females treated at all doses of ZD6474 at Weeks 13 and 26 (29-160%), and in males and females treated with 20/10 mg/kg/day at Week 38 (42-93%). These increases did not correspond to histopathology findings in the liver.
- Albumin was decreased by 9% in females treated with 20/10 mg/kg/day at Week 13.
- Cholesterol was decreased in males treated with 5 and 20/10 mg/kg/day (14-25%) at Weeks 13 and 26.

Data analyzed was expressed as the median value for each group. The following outliers were observed for the data for the indexes listed above:

Index	Group (dose, sex)	Week	Median	Outliers
ALT	Control, males	26	70	198
	20/10 mg/kg/day, females	38	106	452
AST	Control, males	26	109	305
	Control, females	26	98	338
	20/10 mg/kg/day, females	38	257	700

Urinalysis:

- Urine volume was decreased by 29% in both males and females treated with 20/10 mg/kg/day compared to controls Week 12 (data analyzed expressed as the median value for each group).
- Protein levels in urine were increased in rats treated with 20/10 mg/kg/day ZD6474 compared to controls Weeks 12 and 25. This finding is demonstrated in the separate tables for males and females below.

Males

Treatment-Related Urinalysis Findings		No of animals affected											
Dose		0 mg/kg/day			1 mg/kg/day			5 mg/kg/day			20/10 mg/kg /day		
Number of animals examined		10	10	10	10	7	0	10	10	0	10	10	10
Week		Week 12	Week 25	Week 37	Week 12	Week 25	Week 37	Week 12	Week 25	Week 37	Week 12	Week 25	Week 37
Protein	Negative	1	1	-	4	-	NA	1	-	NA	-	-	-
	Trace	1	-	-	-	2	NA	2	-	NA	-	-	-
	+	7	5	2	5	4	NA	5	3	NA	1	1	-
	+ +	1	3	3	1	-	NA	2	4	NA	3	2	3
	+ + +	-	1	5	-	1	NA	-	3	NA	6	7	7

- = no observations of this finding
NA= not available

Females

Treatment-Related Urinalysis Findings		No of animals affected											
Dose		0 mg/kg/day			1 mg/kg/day			5 mg/kg/day			20/10 mg/kg /day		
Number of animals examined		10	10	10	10	10	0	10	10	0	10	8	7
Week		Week 12	Week 25	Week 37	Week 12	Week 25	Week 37	Week 12	Week 25	Week 37	Week 12	Week 25	Week 37
Protein	Negative	5	9	2	6	9	NA	2	7	NA	-	4	1
	Trace	4	1	1	2	1	NA	4	1	NA	-	-	2
	+	1	-	6	1	-	NA	3	2	NA	4	4	2
	+ +	-	-	1	1	-	NA	1	-	NA	6	-	2
	+ + +	-	-	-	-	-	NA	-	-	NA	-	-	-

- = no observations of this finding
NA= not available

Gross pathology:

Treatment-Related Macroscopic Findings		No. of animals affected							
		Males				**Females**			
Dose (mg/kg/day)		0	1	5	20/10	0	1	5	20/10
Number of animals examined		1*/19/**10**	5*/15/**0**	0/20/**0**	2*/19/**9**	1*/19/**10**	0/20/**0**	1*/19/**0**	8*/15/7
Adrenal glands	Discoloration	-	-	0/1/**0**	0/1/**0**	-	-	0/1/**0**	1*/1/**0**
	Adhesion to liver	-	-	-	-	-	0/1/**0**	-	-
Bile duct	Mass/nodule	-	-	-	-	-	-	-	2*/0/**0**
Esophagus	Material present (yellow gelantinous), attached to lung	-	-	-	1*/0/**0**	-	-	-	-
	Dilatation/distension	-	-	-	-	-	-	-	1*/0/**0**
	Mass/nodule, adjacent to bronchus	-	-	-	-	-	-	-	1*/0/**0**
Intestine	Mass/nodule	-	-	-	-	-	-	-	1*/0/**0**
Heart	Enlarged atria (2-3 x), right atrium or bilateral	1*/0/**0**	2*/1/**0**	0/2/**0**	-	-	-	-	1*/0/**0**
	Dilatation/distension of atria	-	1*/0/**0**	-	-	-	-	-	-
	Discoloration, diffuse pallor throughout	-	-	-	-	-	-	-	1*/0/**0**
Kidney	Dilatation/distension, pelvic region (slight-moderate)	0/2/**0**	1*/3/**0**	0/4/**0**	1*/1/**1**	-	-	0/1/**0**	1*/0/**0**
Liver	Mass/nodule	-	-	0/2/**0**	-	-	-	-	-
Lungs	Irregular cream surface	-	-	-	0/1/**0**	-	-	-	0/1/**0**
	Mass/nodule	-	-	0/1/**0**	1*/0/**0**	-	-	0/1/**0**	1*/0/**0**
	Adhesion of lobe(s), adhered to rib cage	-	-	0/1/**0**	1*/0/**1**	-	-	0/1/**0**	-
Lymph node,	Mass/nodule	-	-	0/1/**0**	0/13/**1**	-	-	-	0/5/**0**

Treatment-Related Macroscopic Findings		No. of animals affected							
		Males				**Females**			
Dose (mg/kg/day)		0	1	5	20/10	0	1	5	20/10
Number of animals examined		1*/19/**10**	5*/15/**0**	0/20/**0**	2*/19/**9**	1*/19/**10**	0/20/**0**	1*/19/**0**	8*/15/7
mesenteric	Discoloration	-	-	0/9/**0**	2*/6/**5**	-	-	0/1/**0**	5*/8/**3**
	Enlarged	-	-	0/1/**0**	0/4/**3**	-	-	-	3*/4/**2**
Oral cavity	Discoloration	-	-	-	-	-	-	-	1*/0/**0**
	Mis-shapen	-	-	-	-	-	-	-	1*/0/**0**
Pancreas	Mass/nodule	-	-	-	0/0/**1**	-	-	-	1*/0/**0**
	Discoloration, pale throughout	-	-	-	-	-	-	0/1/**0**	1*/0/**0**
Skin	Wound, head-dorsal and left ear	-	-	-	-	-	-	-	1*/0/**0**
Thymus	Mass/nodule	-	-	-	1*/0/**0**	-	-	-	-
Urinary bladder	Dilatation/distension with urine (marked)	-	0/1/**0**	-	-	-	-	-	-

Number of animals examined and affected: Early deaths*/ Terminal necropsy / **Recovery necropsy**
- = no test-article related changes

Organ weights: Unremarkable

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

Histopathology was conducted on all tissues for the control and 20/10 mg/kg/day groups in the main study and from animals found dead or euthanized early. An exception was male #42 in the 1 mg/kg/day group, which died on the morning of the scheduled necropsy and was processed as a terminal necropsy. In the table below, this rat (#42) was included as an early death for the 1 mg/kg/day group, since he died prior to the terminal necropsy. In the 1 and 5 mg/kg/day groups and the recovery groups histopathology was conducted on the adrenals glands, kidneys, lungs, mesenteric lymph node, pancreas, skin, and teeth. Histopathology was not conducted on the masses observed in gross pathology.

Treatment-Related Microscopic Findings			No. of animals affected							
			Males				**Females**			
Dose (mg/kg/day)			0	1	5	20/10	0	1	5	20/10
Number of animals examined			1*/19/**10**	5*/15/**0**	0/20/**0**	2*/19/**9**	1*/19/**10**	0/20/**0**	1*/19/**0**	8*/15/7
Adrenal glands	Hemorrhage, cortical		-	-	-	-	0/0/**2**	-	-	4*/0/**0**
	Hemocyst, cortical		-	-	-	0/0/**1**	0/1/**3**	0/2/**0**	0/6/**0**	1*/1/**2**
	Vacuolation, cortical		0/0/**2**	2*/0/**0**	-	-	-	-	-	2*/0/**0**
Aorta	Inflammatory cell infiltration	Mild	-	NA	NA	1*/0/**NA**	-	NA	NA	1*/2/**NA**
Bile duct	Cholangitis	Total	NA	NA	NA	NA	NA	NA	NA	4*/NA
		Moderate	NA	NA	NA	NA	NA	NA	NA	1*/NA
		Severe	NA	NA	NA	NA	NA	NA	NA	3*/NA
Bone and marrow (Sternum)	Inflammatory cell infiltration, adjacent tissue	Mild-moderate	-	NA	NA	-	-	NA	NA	0/2/**NA**
	Bone marrow hemorrhage	Mild	-	NA	NA	-	-	NA	NA	1*/0/**NA**
Bronchus	Acute inflammatory cell infiltration, adjacent tissue	Mild	-	NA	NA	-	-	NA	NA	0/1/**NA**
	Acute bronchitis	Moderate	-	NA	NA	-	-	NA	NA	1*/0/**NA**
Harderian glands	Dacryoadenitis		0/1/**0**	NA	NA	0/5/**NA**	0/5/**NA**	NA	NA	1*/9/**NA**
Heart	Pericarditis	Mild-moderate	-	NA	NA	-	-	NA	NA	0/2/**NA**

Treatment-Related Microscopic Findings			No. of animals affected							
			Males				**Females**			
Dose (mg/kg/day)			0	1	5	20/10	0	1	5	20/10
Number of animals examined			1*/19/**10**	5*/15/**0**	0/20/**0**	2*/19/**9**	1*/19/**10**	0/20/**0**	1*/19/**0**	8*/15/7
	Ventricular myocardial fibrosis	Minimal-mild	0/1/**0**	NA	NA	0/3/**NA**	-	NA	NA	-
	Inflammatory cell infiltration	Minimal-mild	-	NA	NA	1*/0/**NA**	-	NA	NA	1*/0/**NA**
Kidney	Cortical epithelial brown pigmentation	Total	-	-	-	-	0/18/**10**	0/20/**0**	0/19/**0**	4*/15/**7**
		Minimal	-	-	-	-	0/10/**2**	0/7/**0**	0/4/**0**	-
		Mild	-	-	-	-	0/8/**7**	0/13/**0**	0/15/**0**	4*/10/**3**
		Moderate	-	-	-	-	0/0/**1**	-	-	0/5/**4**
	Tubular dilatation, cortical	Minimal-mild	0/1/**0**	-	-	0/3/**0**	0/1/**0**	-	-	1*/1/**0**
	Tubular basophilia, cortical	Minimal-mild	0/2/**3**	2*/2/**0**	0/4/**0**	0/14/**1**	1*/1/**1**	0/1/**0**	0/3/**0**	1*/0/**1**
	Inflammatory cell infiltration	Minimal	-	0/2/**0**	0/1/**0**	-	-	0/2/**0**	0/4/**0**	1*/0/**1**
Lungs	Foamy alveolar macrophages	Total	0/1/**2**	1*/2/**0**	0/7/**0**	0/13/**3**	0/3/**2**	0/1/**0**	0/10/**0**	7*/14/**1**
		Minimal	0/1/**2**	0/2/**0**	0/6/**0**	0/3/**3**	0/3/**2**	0/1/**0**	0/8/**0**	2*/12/**0**
		Mild	-	1*/0/**0**	0/1/**0**	0/10/**0**	-	-	0/2/**0**	3*/2/**1**
		Moderate	-	-	-	-	-	-	-	2*/0/**0**
	Pleuritis	Mild-severe	-	-	0/1/**0**	-	1*/0/**0**	-	1*/0/**0**	1*/3/**0**
	Alveolar edema	Moderate	-	4*/0/**0**	-	1*/0/**0**	-	-	1*/0/**0**	-
	Foreign body pneumonia	Moderate-severe	-	-	-	1*/0/**0**	-	-	-	1*/0/**0**
	Foreign body granuloma	Minimal-moderate	-	-	0/1/**0**	0/0/**1**	-	-	0/1/**0**	1*/2/**0**
	Inflammatory cell infiltration	Minimal-mild	-	-	-	0/0/**1**	-	-	-	1*/0/**0**
Lymph node, mesenteric	Sinusoidal hemorrhagic cystic degeneration	Total	-	-	0/5/**0**	0/19/**7**	-	-	-	1*/11/**2**
		Minimal	-	-	0/1/**0**	-	-	-	-	0/2/**0**
		Mild	-	-	0/4/**0**	0/1/**1**	-	-	-	1*/5/**1**
		Moderate	-	-	-	0/16/**5**	-	-	-	0/4/**1**
		Severe	-	-	-	0/2/**0**	-	-	-	-
		Not specified	-	-	-	0/0/**1**	-	-	-	-
	Agonal hemorrhage	Total	-	0/1/**0**	0/7/**0**	2*/0/**0**	0/2/**0**	-	0/1/**0**	4*/3/**0**
		Minimal	-	-	0/1/**0**	-	0/2/**0**	-	-	1*/0/**0**
		Mild	-	0/1/**0**	0/3/**0**	2*/0/**0**	-	-	0/1/**0**	0/1/**0**
		Moderate	-	-	0/3/**0**	-	-	-	-	2*/2/**0**
		Not specified	-	-	-	-	-	-	-	1*/0/**0**
Pancreas	Pancreatitis	Total	0/1/**1**	0/1/**0**	0/1/**0**	0/4/**1**	0/2/**0**	0/1/**0**	0/1/**0**	3*/3/**2**
		Minimal	0/1/**1**	0/1/**0**	0/1/**0**	0/2/**1**	0/1/**0**	0/1/**0**	0/1/**0**	0/2/**2**
		Mild	-	-	-	0/2/**0**	0/1/**0**	-	-	0/1/**0**
		Moderate	-	-	-	-	-	-	-	2*/0/**0**
		Severe	-	-	-	-	-	-	-	1*/0/**0**
Skin	Folliculitis	Total	0/0/**1**	0/1/**0**	0/5/**0**	0/3/**1**	0/2/**1**	0/1/**0**	0/2/**0**	1*/9/**0**
		Minimal	-	-	-	-	0/0/**1**	-	-	0/1/**0**
		Mild	0/0/**1**	-	0/5/**0**	0/1/**1**	0/2/**0**	-	0/2/**0**	1*/8/**0**
		Moderate	-	0/1/**0**	-	0/2/**0**	-	0/1/**0**	-	-
	Inflammatory cell infiltration	Minimal-mild	0/0/**2**	0/3/**0**	0/9/**0**	0/0/**4**	0/0/**1**	-	0/9/**0**	0/1/**3**
Spleen	Extramedullary hematopoiesis	Moderate	-	NA	NA	-	-	NA	NA	4*/0/**NA**
Teeth	Dental dysplasia		-	-	-	-	-	-	-	4*/0/**0**
	Adjacent tissue abscess		-	-	-	0/3/**0**	-	-	-	-
Thymus	Atrophy	Mild-moderate	0/1/**0**	NA	NA	1*/1/**NA**	-	NA	NA	4*/0/**NA**
	Inflammatory cell infiltration	Mild-moderate	-	NA	NA	-	-	NA	NA	1*/2/**NA**
Urinary bladder	Luminal coagula		1*/2/**NA**	1*/NA	NA	1*/5/**NA**	-	NA	NA	-

Number of animals examined and affected: Early deaths*/ Terminal necropsy / **Recovery necropsy**
- = no test-article related changes
NA= not available, tissue/organ not examined in this group except for early deaths

Toxicokinetics:

Toxicokinetics was conducted on ZD6474 following a single administration on Day 1 (5 mg/kg/day) and following chronic repeated administration during Week 26 (1, 5, and 20/10 mg/kg/day). Mean values are presented for each group with males and females combined in the table excerpted from the sponsor's submission, and the mean AUC_{0-24} values for males and females in each group are presented in a separate table below .

- C_{max} and AUC_{0-24} increased with dose for both males and females, and increases were roughly proportional to the increase in dose
- AUC_{0-24} is slightly higher in females than males treated with the low dose (1 mg/mg/day) and the high dose (20/10 mg/kg/day), however, this difference dose not occur at the mid dose (5 mg/kg/day) at either time point
- AUC_{0-24} for the 5 mg/kg/day group increased approximately 3-fold form Day 1 to Week 26, indicating drug accumulation following repeated administration

Toxicokinetics for males and females combined (table excerpted from sponsor's submission)

Parameter	Day 1	Week 26		
	Group III (5 mg/kg/day)	Group II (1 mg/kg/day)	Group III (5 mg/kg/day)	Group IV (10 mg/kg/day)
AUC_{0-24} (ng.h/ml)	1338	799	4257	8114
Half-life (h)	NC	NC	NC	55.2
C_{max} (ng/ml)	71.2	39.6	225	427
t_{max} (h)	24	8	2	2
C_{min} (ng/ml)	NA	25.9	133	292

NC - Not Calculated
NA - Not Applicable

AUC_{0-24} (ng.h/mL) for males and females separately

Sex	Day 1	Week 26		
	5 mg/kg/day	1 mg/kg/day	5 mg/kg/day	20/10 mg/kg/day
Male	1345	650	4327	7609
Female	1331	907	4169	9456

Other: Sperm analysis was conducted including sperm count/g cauda, vas deferens total sperm, vas deferens non-motile sperm, and motility (%). Results were unremarkable.

Study title: **Zeneca ZD6474: Nine month oral toxicity study in dogs**

Key study findings:

- The most common clinical sign observed in this study was abnormal feces in dogs treated with 20/15 mg/kg/day ZD6474. Due to the high incidence of abnormal feces, the 20 mg/kg/day dose was reduced to 15 mg/kg/day starting at Week 17 for main study animals and Week 18 for recovery animals. This was a dose-limiting toxicity, and as a result of the dose reduction other possible toxicities were not observed.
- Target organs of toxicity include the intestine (jejunum, rectum, and ileo-caecal junction), kidney, spleen, stomach, and thymus.
- Gastrointestinal toxicities include abnormal feces and gross pathology and histopathology findings in the intestine and stomach.

Study no.: TPD1043
Study report location: M4.2.3.2
Conducting laboratory and location: AstraZeneca UK Limited
Safety Assessment UK Alderley
Alderley Park
Macclesfield
Cheshire SK10 4TG
U.K.

Date of study initiation: March 14, 2000 (Protocol approved)
Experiment start date: April 10, 2000
GLP compliance: Yes (FDA compliance claimed for work performed from July 1, 2000 onwards; deviations do not appear to affect interpretation of study)
QA report: yes (X) no ( )
Drug, lot #, and % purity: ZD6474, batch # C268/1, purity: 99.0%

Methods

Species/strain:	Alderley Park Beagle dogs
Number/sex/group or time point:	Main study: 4/sex/group Recovery: 3/sex/group, control and 20/15 mg/kg/day groups only
Age:	10-15 months old at start of dosing
Weight:	Males: 8.1-12.8 kg at start of dosing Females: 7.3-12.4 kg at start of dosing
Schedule:	Once daily for 40 weeks, 13 week recovery
Doses:	0, 1, 5, or 20/15 mg/kg/day ZD6474 (at Week 17 for main study animals and Week 18 for recovery animals the 20 mg/kg/day dose was reduced to 15 mg/kg/day*)
Vehicle/control:	0.5% w/v hydroxypropyl methylcellulose solution containing 0.1% w/v polysorbate 80
Route:	oral gavage
Volume:	1 mL/kg

***** The highest dose was initially 20 mg/kg/day, however, due to a high incidence of abnormal feces and adverse effects on body weight, this dose was reduced to 15 mg/kg/day starting at Week 17 for main study animals and Week 18 for recovery animals.

Observations and times:

Mortality:	Twice daily
Clinical signs:	Twice daily for gross abnormality Weekly for detailed observations
Body weights:	Prior to study (weekly for 3 weeks), Day -1 , and weekly for the remainder of the study
Food consumption:	Prior to study (daily for 3 weeks) and daily for the remainder of the study
ECG	Prior to study and Weeks 13, 25, and 40 (all animals), and Week 53 (recovery animals)
Ophthalmoscopy:	Pretest and Weeks 5, 13, 26, and 40 (all animals), and during last week of recovery (recovery animals)
Hematology:	Prior to study and Weeks 5, 13, 26, and 40 (all animals) and Week 53 (recovery animals)
Clinical chemistry:	Prior to study and Weeks 5, 13, 26, and 40 (all animals) and Week 53 (recovery animals)
Coagulation:	Prior to study and Weeks 5, 13, 26, and 40 (all animals) and Week 53 (recovery animals)
Urinalysis:	Prior to study and Weeks 13, 26, and 40 (all animals) and Week 53 (recovery animals)
Gross pathology:	At necropsy (Main study: Week 41; Recovery: Week 54)
Organ weights:	At necropsy (Main study: Week 41; Recovery: Week 54)
Histopathology:	At necropsy (Main study: Week 41; Recovery: Week 54)
Toxicokinetics:	Collected from 4 animals/sex/group at each time point • At 0.5, 1, 2, 4, 8, and 24 hours after first dose and after 6 months for the 5 mg/kg/day group • At 0.5, 1, 2, 4, 8, and 24 hours at end of dosing at 9 months for the 1, 5, and 20/15 mg/kg/day groups Collected from 3 animals/sex/group at each time point • At 28, 33, and 48 hours after the final dose in the 20/15 mg/kg/day recovery animals

Data and statistics:

- Data for body weight, food consumption, ECG, hematology, clinical chemistry, urinalysis, and organ weights were presented with the medians for each group.
- Statistics were conducted using a step-down version of the Jonckheere-Terpstra non-parametric trend test with critical values approximated by the student t-distribution. Statistical significance attached to the groups relate to the trend up to and including that group.

Results

Mortality:

Dose of ZD6474	Animal #	Sex	Found dead or Euthanized	Week	Findings/Clinical signs
1 mg/kg/day	276	Female	Euthanized	40	• Sustained bite injuries from fighting with or being attacked by other dogs • Subsequent swelling of the right hind leg and bruising/swelling of the rear abdomen • Death not related to drug treatment

Clinical signs:
Abnormal feces

- The most common clinical sign observed in this study was abnormal feces in dogs treated with 20/15 mg/kg/day ZD6474. Incidences of abnormal feces (loose/liquid feces and red liquid feces) were recorded starting Week 2 in males and from Week 10 in females, and was observed throughout the remainder of the dosing period. There were some isolated occurrences in females in Weeks 2 and 7. Abnormal feces were also observed in dogs treated with 5 mg/kg/day and in control males. The incidences of abnormal feces observed when animals were single housed and group housed are listed in the table below.
- Due to the high incidence of abnormal feces, the 20 mg/kg/day dose was reduced to 15 mg/kg/day starting at Week 17 for main study animals and Week 18 for recovery animals. Following the dose reduction, there was a slight decrease in incidence, however, observations continued throughout the dosing period.
- Recovery for this clinical sign occurred rapidly during the recovery period with no observations recorded after Week 41 for males and Week 42 for females.
- Two dogs in the 20/15 mg/kg/day group received enteral anti-microbial therapy due to clinical signs related to abnormal feces. Clinical signs observed and the response to the enteral anti-microbial therapy suggest opportunistic intestinal infection, secondary to the effects of ZD6474 on gastrointestinal function in this dose group. The following signs were listed for these animals on day of veterinary treatment
    - Male (#311): Liquid feces, reduced food consumption, and abdomen tense on palpation on Day 11
    - Female (#301): Red stained feces on floor, red staining around anus, and soft red-feces in rectum at rectal examination on Day 122

Incidences of Abnormal Feces	No. of observations (Single housed/Group housed)							
	Males				Females			
Dose mg/kg/day	0	1	5	20/15	0	1	5	20/15
Week								
1	-	-	-	-	-	-	-	-
2	-	-	1/0	7/1	-	-	-	0/2
3	-	-	-	7/3	-	-	-	-
4	-	-	-	4/1	-	-	-	-
5	-	-	-	1/1	-	-	-	-
6	-	-	1/0	4/0	-	-	-	-
7	-	-	-	4/0	-	-	-	1/0
8	-	-	-	8/3	-	-	-	-
9	-	-	-	17/2	-	-	-	-
10	-	-	-	7/4	-	-	-	1/6
11	1/0	-	1/1	8/7	-	-	-	3/6
12	1/0	-	1/0	16/8	-	-	-	7/12
13	-	-	-	16/7	-	-	-	8/9
14	-	-	-	15/10	-	-	1/0	15/11
15	3/0	-	1/0	16/8	-	-	2/0	13/12
16	-	-	-	16/14	-	-	0/1	6/16
17	2/0	-	2/1	21/25	-	-	-	29/16
18	1/0	-	1/1	14/22	-	-	-	19/17
19	1/0	-	1/1	20/11	-	-	1/0	11/23
20	-	-	-	11/14	-	-	3/0	7/16
21	1/0	-	2/0	8/17	-	-	-	2/16
22	0/2	-	2/0	10/18	-	-	-	5/12
23	2/1	-	4/0	7/19	0/1	-	0/2	2/14
24	3/1	-	1/0	11/24	-	-	-	3/20
25	2/0	-	2/1	7/20	-	-	-	5/18
26	2/0	-	3/0	6/19	-	-	-	3/18
27	2/0	-	1/0	4/16	-	0/2	-	4/13
28	1/0	-	3/0	4/21	-	-	1/0	4/13
29	2/1	-	3/0	3/15	-	-	-	4/11
30	1/0	-	3/0	6/19	-	-	-	3/13
31	-	-	-	5/18	-	-	-	5/7
32	1/0	-	-	3/25	-	-	0/1	2/13
33	1/0	-	-	2/21	-	-	-	3/9
34	1/0	-	-	6/22	-	-	-	5/19
35	3/0	-	4/1	4/14	-	-	-	5/22
36	1/0	-	2/1	3/17	-	-	-	12/18
37	2/0	-	1/0	4/15	-	-	-	2/14
38	2/0	-	3/0	1/9	-	-	-	2/12
39	-	-	2/0	4/11	-	-	1/0	2/18

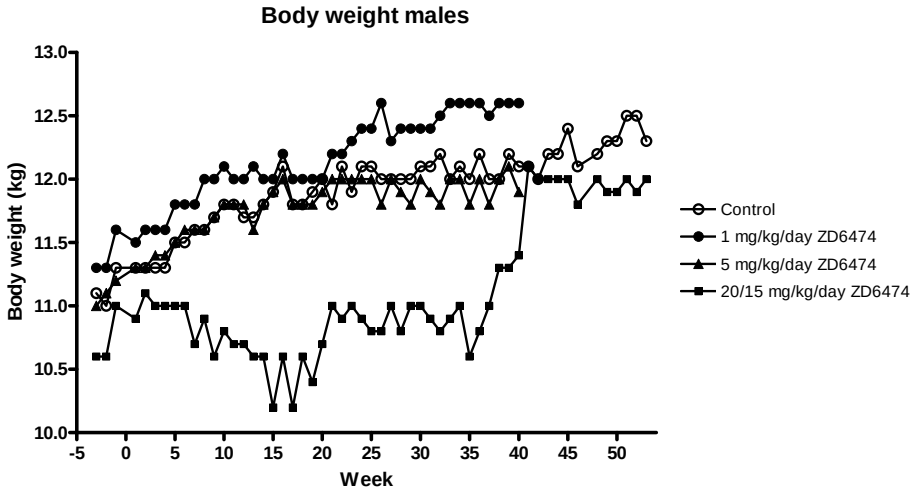
Incidences of Abnormal Feces	No. of observations (Single housed/Group housed)							
	Males				Females			
Dose mg/kg/day	0	1	5	20/15	0	1	5	20/15
Week								
40	-	-	3/1	1/13	-	-	-	5/16
41	-	-	-	3/11	-	-	-	8/5
42	3/0	-	-	-	-	-	-	0/1
43	-	-	-	-	-	-	-	-
44	-	-	-	-	-	-	-	-
45	-	-	-	-	-	-	-	-
46	-	-	-	-	-	-	-	-
47	-	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-	-
49	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-
51	-	-	-	-	-	-	-	-
52	3/0	-	-	-	-	-	-	-
53	-	-	-	-	-	-	-	-

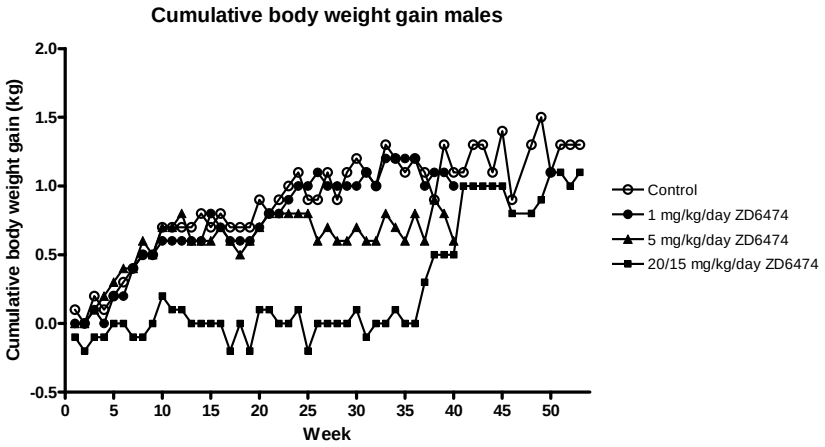
- = no test-article related changes

Note: A table of the incidences of abnormal feces and a table of the veterinary examination and treatment are included in the study report, however, there is no other listings of clinical signs.

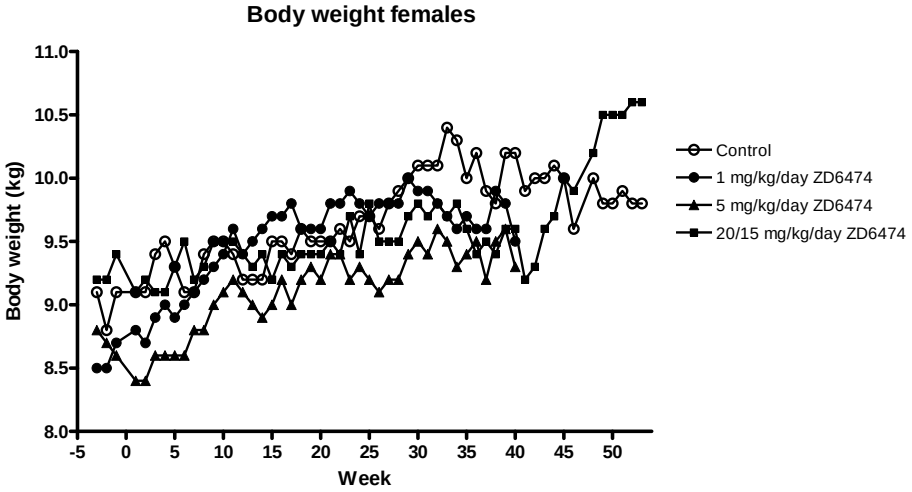
Body weights:

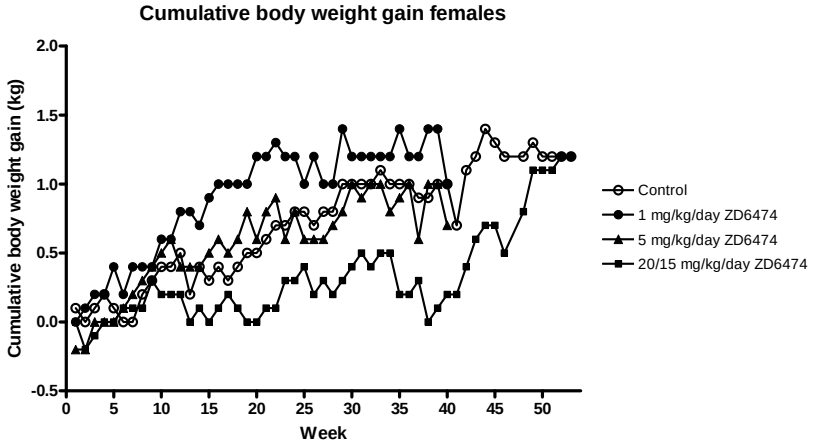
Below are the median body weights and the median cumulative body weight gains for males and females. There is no data for Week 47 for either males or females with no explanation. Body weight gain was significantly lower than controls for both males and females treated with 20/15 mg/kg/day ZD6474.





20/15 mg/kg/day: Statistically significant Weeks 1 and 3-40, p<0 05, p<0 01, or p<0.001
Statistics not conducted for Weeks 41-53

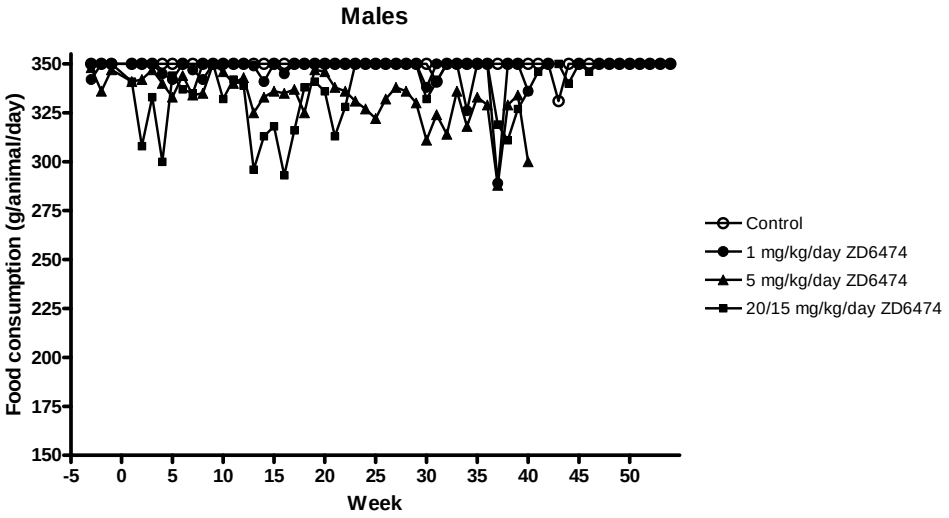


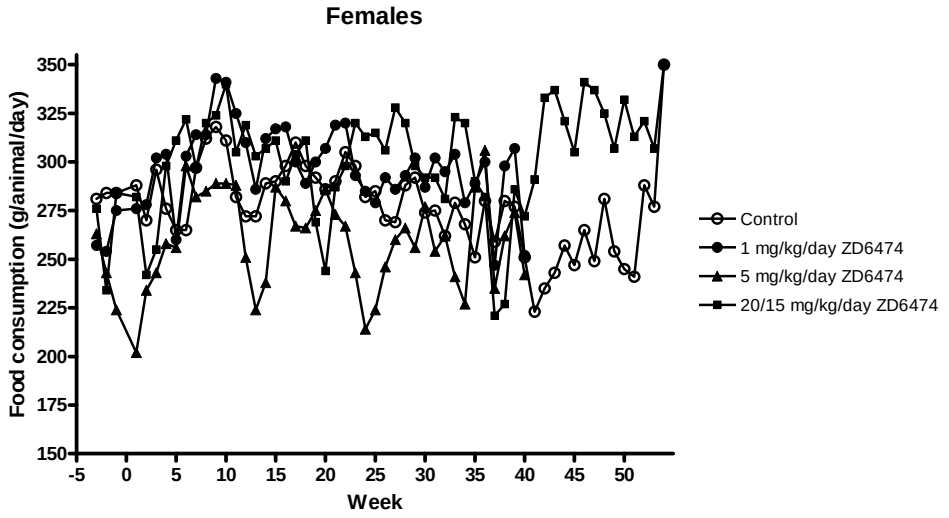


20/15 mg/kg/day: Statistically significant Weeks 2-4,15, 19-20, and 22-40, p<0 05, p<0 01, or p<0.001
Statistics not conducted for Weeks 41-53

Food consumption:

The median food consumption for males and females is shown in the graphs below. Group median food consumption was not affected by treatment with ZD6474, however, individual dogs treated with 20/15 mg/kg/day and one female dog treated with 5 mg/kg/day showed periods of reduced food consumption compared to pre-study. Food supplements were given to 8 of the 14 dogs treated with 20/15 mg/kg/day (4 males and 4 females) during the dosing period due to reduced food consumption and/or body weight loss. Generally the dogs consumed at least 300 g of supplements offered with the following ranges of grams consumed: Dog meat and diet: 334-608 grams; wet diet: 76-450 grams; liquivite and diet: 380-1000 grams. The table below lists the dogs and the weeks they showed reduced food consumption and/or received food supplementation.





Dogs with reduced food consumption and/or food supplementation

Dose of ZD6474	Animal #	Sex	Weeks of reduced food consumption	Food supplementation
5 mg/kg/day	26	Female	24-26	None
20/15 mg/kg/day	311	Male	2 (Days 10 and 11)	Week 2 (Days 10-11)
	323	Male	Not reduced	Week 20 (Days 137-138 and 140) Week 21 (Day 141) Week 37 (Days 254-259) Week 38 (Days 260-266) Week 39 (Days 267-268)
	328	Male	13-21 and 36-38	Week 16 (Days 107-108) Week 17 (Days 117-119) Week 18 (Days 120-121 and 123-126) Week 19 (Days 127-131 and 133) Week 20 (Days 134-136) Week 36 (Days 247-252) Week 37 (Days 253-259) Week 38 (Days 260-261)
	368	Male	16-21	Week 17 (Days 114-115) Week 18 (Days 124-126) Week 19 (Days 127-128, 130-131, and 133) Week 20 (Days 134-138 and 140) Week 21 (Days 141-143)
	40	Female	19-22 and 37-39	Week 20 (Days 134-138 and 140) Week 21 (Days 141-143) Week 37 (Days 254-259) Week 38 (Days 260-266) Week 39 (Days 267-268)
	47	Female	13, 19-21, and 37	Week 18 (Days 123-126) Week 19 (Days 127-133) Week 20 (Days 134-136) Week 36 (Days 247-252) Week 37 (Days 253-259) Week 38 (Days 260-261)
	198	Female	37	Week 36 (Days 247-252) Week 37 (Days 253-259) Week 38 (Days 260-261)
	301	Female	18-20	Week 17 (Days 117-119) Week 18 (Days 120-121 and 123-126) Week 19 (Days 127-131 and 133) Week 20 (Days 134-136)

Ophthalmoscopy: The ophthalmology report and individual data are not included in the study report. The report says "Individual animal findings are archived with the other raw data generated during the study."

ECG:

- Increases in QT interval were observed in both males and females treated with 20/15 mg/kg/day and were particularly increased at Week 13 compared to baseline. A few dogs in the control and 1 and 5 mg/kg/day treatment groups also showed increased QT at Week 13. However, heart rate was decreased at Week 13, so corrected QT was not increased compared to baseline for most of these dogs. Individual data for heart rate (HR), QT, and corrected QT (QTc) for dogs showing increases in QT is presented in the QT table below.
- QT prolongation was observed in one male treated with 20/15 mg/kg/day (#353) during Week 25. This data is presented in the table below.
- The median PR interval for the females treated with 20/15 mg/kg/day was significantly decreased by 10% compared to controls at Week 25, however, PR interval was also lower in this group than controls at baseline.
- According to the results section in the study report, PR variability was observed in one female control (# 207), one female treated with 5 mg/kg/day (# 26), and one female treated with 20/15 mg/kg/day (#40), "resulting in these animals having 1º atrio-ventricular heart block in Weeks 25 and/or 40 (PR greater than 130ms)". While there is some variability, this statement and finding is not clear from the individual data since none of the dogs had a PR greater than 130 ms and there are 0 values for #207 and #40 at Week 40. The individual PR interval data for these dogs is presented in the PR table below.
- PR interval was increased throughout the dosing period in one male treated with 1 mg/kg/day (#365) particularly at Week 40, and according to the results section in the study report, "this animal was recorded as having one ventricular premature complex in Week 40". The individual PR interval data for this dog is presented in the PR table below.

Increases in QT: Individual values

Dose of ZD6474	Sex	Animal #	Baseline			Individual values for week (s) of QT increase			
			HR	QT	QTc	Week	HR	QT	QTc
Control	Male	326	112	186	229	13	92	204	235
						25	96	200	234
						53	96	210	246
	Female	186	120	176	222	13	92	200	231
1 mg/kg/day	Female	276	160	164	227	13	100	188	223
5 mg/kg/day	Male	304	124	169	215	13	140	186	247
	Female	41	112	182	224	13	92	204	235
20/15 mg/kg/day	Male	303	208	164	248	13	88	192	218
		311	120	182	229	13	104	196	235
						25	112	200	246
		328	160	166	230	13	96	196	229

Dose of ZD6474	Sex	Animal #	Baseline			Individual values for week (s) of QT increase			
			HR	QT	QTc	Week	HR	QT	QTc
		353	148	174	235	25	128	210	**270**
	Female	40	120	182	229	13	80	208	229
						25	80	202	222
						40	92	204	235
						53	104	198	238
		47	168	156	220	13	184	164	238
		167	140	152	202	13	124	191	243
						25	124	186	237
		198	176	158	224	13	128	172	221
						25	140	180	239
						40	120	203	256

Individual PR values

Dose of ZD6474	Sex	Animal #	Individual PR values			
			Baseline	Week 13	Week 25	Week 40
Control	Female	207	106	110	114	0
1 mg/kg/day	Male	365	88	99	98	106
5 mg/kg/day	Female	26	104	120	92	115
20/15 mg/kg/day	Female	40	96	108	110	0

Hematology:

- Platelets were increased by 40% compared to controls in both males and females treated with 20/15 mg/kg/day at Week 13. Platelet levels remained increased (31-43%) in males treated with 20/15 mg/kg/day at Weeks 26 and 40.
- WBC were increased by 22-26% and neutrophils were increased by 42-44% compared to controls in both in males and females treated with 5 mg/kg/day at Week 40. Individual dogs in this group and the other ZD6474 treatment groups showed increases in WBC and neurtophils at Week 13 and/or 40 during drug treatment and their values are listed in the table below.

Data analyzed was expressed as the median value for each group. The following individual values were observed for WBC and neutrophils:

Dose of ZD6474	Sex	Week	Group Median		Animal #	Individual values	
			WBC	Neutrophils		WBC	Neutrophils
1 mg/kg/day	Male	40	13.3	8.78	370	23.0	17.02
	Female	40	12.4	9.35	206	24.9	19.38
5 mg/kg/day	Male	40	14.2	10.48	391	19.6	16.54
	Female	13	11.8	8.52	41	20.1	14.57
	Female	40	12.7	9.24	41	18.5	13.41

Dose of ZD6474	Sex	Week	Group Median		Animal #	Individual values	
			WBC	Neutrophils		WBC	Neutrophils
20/15 mg/kg/day	Male	13	11.3	7.34	328	18.6	13.31

Clinical chemistry:

- Albumin was decreased compared to controls in females treated with 20/15 mg/kg/day at Week 5 (10%), and both males (7-11%) and females (10-19%) treated with 20/15 mg/kg/day at Weeks 13, 26, and 40.
- Total protein was decreased (15-16%) compared to controls in females treated with 20/15 mg/kg/day at Weeks 26 and 40
- Triglycerides were increased compared to controls in females treated with 20/15 mg/kg/day at Weeks 5, 13, 40, and 53 (34-102%).
- Total calcium was decreased compared to controls in females treated with 20/15 mg/kg/day at Weeks 5, 13, 26, and 40 (5-10%).
- Multiple liver enzymes were increased compared to controls in females treated with 1 mg/kg/day at Week 40 including ALP (38%), ALT (317%), AST (117%), and GLDH (540%). These increases were observed in 3 of 4 females treated at this dose. These increases did not correspond to histopathology findings in the liver and were not observed in males of the same dose groups or in dogs treated with 5 or 20/15 mg/kg/day.

Data analyzed was expressed as the median value for each group. The following outliers were observed for the data for the indexes listed above:

Index	Group (dose, sex)	Week	Median	Outliers
ALT	Control, females	53	48	228
	1 mg/kg/day, females	40	150	47
	20/15 mg/kg/day, males	5	64	207
		26	48	174
AST	1 mg/kg/day, females	40	76	584
ALP	1 mg/kg/day, females	40	263	727
GLDH	Control, females	53	5	67
	1 mg/kg/day, females	40	32	8
	20/15 mg/kg/day, males	5	8	25
		26	6	17
Triglycerides	Control, females	5	0.43	1.59
		26	0.49	1.21, 1.76
	5 mg/kg/day, females	40	0.46	1.20
	20/15 mg/kg/day, females	26	0.54	1.49

Urinalysis: Unremarkable

Gross pathology:

Treatment-Related Macroscopic Findings		No. of animals affected							
		Males				**Females**			
Dose (mg/kg/day)		0	1	5	20/15	0	1	5	20/15
Number of animals examined		0/4/**3**	0/4/**0**	0/4/**0**	0/4/**3**	0/4/**3**	1*/3/**0**	0/4/**0**	0/4/**3**
Intestine, Ileo-caecal junction	Discoloration, patchy red	-	-	-	-	-	-	-	0/2/**0**
Intestine, jejunum	Discoloration, red area (s) (mucosal surface)	-	-	-	-	-	-	0/1/**0**	0/1/**0**
Intestine, rectum	Discoloration, patchy red (mucosal surface)	-	-	-	0/0/**2**	-	0/1/**0**	-	0/1/**0**
Kidney	Mass/nodule	-	-	-	-	-	-	0/1/**0**	-
Lymph node, mesenteric	Discoloration, patchy red or dark red	0/0/**1**	-	-	0/1/**0**	-	1*/0/**0**	0/2/**0**	0/1/**1**
Muscle, skeletal	Material present, dark red (thoracic region adherent to rib cage)	-	0/1/**0**	-	-	-	-	-	-
	Discoloration, red (left hindlimb)	-	-	-	-	-	-	-	0/1/**0**
Spleen	Discoloration, cream (parietal surface anterior pole) and dark red area	-	-	-	-	-	-	0/1/**0**	-
Stomach	Irregular surface, multiple raised areas throughout pyloric region	-	-	-	-	-	-	-	0/1/**0**
Skin	Discoloration, dark red area (left hindlimb)	-	0/1/**0**	-	-	-	-	-	-
Thymus	Discoloration, dark red	-	-	-	-	-	-	-	0/1/**0**

Number of animals examined and affected: Early deaths*/ Terminal necropsy / **Recovery necropsy**
- = no test-article related changes

Organ weights: Unremarkable

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

Histopathology was conducted on all tissues of main study animals. Tissues from recovery animals were not examined, therefore, the table below only includes information for the early death and main study (terminal necropsy) animals.

Treatment-Related Microscopic Findings			No. of animals affected							
			Males				**Females**			
Dose (mg/kg/day)			0	1	5	20/15	0	1	5	20/15
Number of animals examined			0/4	0/4	0/4	0/4	0/4	1*/3	0/4	0/4
Brain	Meningeal macrophage infiltration	Minimal	-	-	-	0/1	-	-	-	-
Heart	Focal chronic right ventricular arteritis	Mild	-	-	-	-	-	0/1	-	-
Intestine, Ileo-caecal-colic junction	Focal mucosal congestion	Mild	-	-	-	-	-	-	-	0/1
Intestine, jejunum	Multifocal mucosal congestion	Mild	-	-	-	-	-	-	-	0/1
Kidney	Multifocal bilateral cortical epithelial brown pigment	Minimal	-	-	0/2	0/1	-	-	-	-
	Solitary medullary protein cast		-	-	-	0/1	-	-	-	-
	Bilateral papillary congestion	Minimal	-	-	-	-	-	-	-	0/1

Treatment-Related Microscopic Findings			No. of animals affected							
			Males				**Females**			
Dose (mg/kg/day)			0	1	5	20/15	0	1	5	20/15
Number of animals examined			0/4	0/4	0/4	0/4	0/4	1*/3	0/4	0/4
Liver	Multifocal brown pigment macrophages	Mild	-	-	-	-	-	-	-	0/1
Lungs	Inflammatory cell infiltration	Minimal	-	0/1	-	-	-	0/2	0/1	-
Lymph node, bronchial	Hemorrhage	Mild	-	-	0/1	0/2	-	-	-	0/1
Pancreas	Acinar single cell necrosis	Mild	-	-	-	-	-	-	0/1	-
Spinal cord	Multifocal nerve root sheath mineralization	Minimal	-	0/1	0/1	0/1	-	-	0/1	-
Spleen	Focal siderofibrosis	Moderate	-	-	-	-	-	-	0/1	-
	Focal perivascular hemosiderosis	Mild	-	-	-	0/1	-	-	-	-
	Multifocal brown pigment	Mild	-	-	-	0/2	-	-	-	-
Stomach	Lamina propria mononuclear cell infiltration	Minimal-mild	0/1	0/1	0/2	0/4	-	0/1	0/2	0/3
Thymus	Interstitial hemorrhage	Moderate-severe	-	-	-	-	-	-	0/1	0/1

Number of animals examined and affected: Early deaths*/ Terminal necropsy
- = no test-article related changes

Toxicokinetics:

Toxicokinetics was conducted on ZD6474 following a single administration on Day 1 (5 mg/kg/day), following 6 months of daily administration (5 mg/kg), and following 9 months of daily administration (1, 5, and 20/15 mg/kg/day). Mean values for C_{max} and AUC_{0-24} for each group are presented in the table below.

- C_{max} and AUC_{0-24} increased with dose for both males and females. Increases were roughly proportional to the increase in dose between 1 and 5 mg/kg/day and slightly less than proportional to the increase in dose between 5 and 15 mg/kg/day.
- AUC_{0-24} and C_{max} were higher in females than males treated with 5 mg/mg/day, and these differences were significant at the 6 month time point.
- AUC_{0-24} for the 5 mg/kg/day group increased approximately 1.5 fold form Day 1 to 6 months and 1.2 fold from Day 1 to 9 months, indicating drug accumulation following repeated administration.
- Terminal half-life was estimated to be approximately 15 hours after 9 months of daily dosing for the 20/15 mg/kg/day group, however, this estimate is based on a limited number of time-points.

Toxicokinetics for ZD6474 in dogs

Parameter	Sex	Day 1	Month 6	Month 9		
		5 mg/kg/day	5 mg/kg/day	1 mg/kg/day	5 mg/kg/day	20/15 mg/kg/day
AUC_{0-24} (ng.h/mL)	Male	716	745	222	846	2181
	Female	959	1681	214	1225	2366
	Mean	837	1213	218	1035	2273
C_{max} (ng/mL)	Male	42.5	44.1	11.7	53.8	160
	Female	63.1	111	11.7	69.1	160
	Mean	52.8	77.6	11.7	61.5	160
t_{max} (h)	Male	4-8	4-8	4-8	4	4
	Female	1-8	4	8	2-8	4-8

Histopathology inventory

Study	TPR2939	TPD1043
Species	Rat	Dog
Adrenals	X*	X*
Aorta	X	X
Bone Marrow smear	X	X
Bone (femur)	X	
Brain	X*	X*
Cecum	X	
Cervix	X	X
Colon	X	X
Duodenum	X	X
Epididymis	X	X*
Esophagus	X	X
Eye	X	X
Fallopian tube		
Gall bladder		X
Gross lesions		
Harderian gland	X	
Heart	X*	X*
Ileum	X	X
Injection site		
Jejunum	X	X
Kidneys	X*	X*
Lachrymal gland		X
Larynx		
Liver	X*	X*
Lungs	X*	X*
Lymph nodes,		X

Study	TPR2939	TPD1043
Species	Rat	Dog
cervical		
Lymph nodes mandibular	X	
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Nasal cavity		
Optic nerves		
Ovaries	X*	X*
Pancreas	X	X
Parathyroid	X	X
Peripheral nerve		
Pharynx		
Pituitary	X*	X*
Prostate	X*	X*
Rectum	X	X
Salivary gland	X	X
Sciatic nerve	X	X
Seminal vesicles	X	
Skeletal muscle	X	X
Skin	X	X
Spinal cord	X	X
Spleen	X*	X*
Sternum	X	X
Stomach	X	X
Testes	X*	X*
Thymus	X*	X
Thyroid	X	X*
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X*	X*
Vagina	X	X
Zymbal gland		

X, histopathology performed
*, organ weight obtained

7 Genetic Toxicology

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

Study title: **ZD6474: Bacterial Mutation Assay in *S.typhimurium* and *E.coli***

Key findings:
ZD6474 did not cause mutations in *Salmonella typhimurium* strains (TA1535, TA1537, TA98, TA100) or *E.coli* strains (WP2P and WP2P *urvA*) in the absence or presence of S9 mix under the conditions of this valid assay.

Study no.: TMV777

Study report location: M4.2.3.3.1
Conducting laboratory and location: (b) (4)

Date of study initiation: May 19, 1999
GLP compliance: Yes
QA reports: Yes (X) No ( )
Drug, Batch ref., % purity: Zeneca ZD6474, lot: C267/1, 98% w/w
(b) (4) **Test Substance Ref. #:** Y10483/001

Methods

Strains/species/cell line: *Salmonella typhimurium* (TA1535, TA1537, TA98, TA100)
E.coli (WP2P and WP2P *urvA*)

Doses used in definitive study:

	ZD6474 (µg/plate)					
- S9 mix	20	50	100	200	500	1000
+ S9 mix	20	50	100	200	500	1000

Basis of dose selection: Toxicity in the first experiment at 5000 µg/plate and low control values in the TA100 strain led to a reduction in the dose range (20 to 1000 µg/plate +/- S9 mix) in two subsequent experiments.
Negative controls: DMSO (100 µL)

Positive controls:

Positive control	Solvent	Strain	+/- S9
Acridine Mutagen ICR191	DMSO	TA1537	-
2-Aminoanhracene (2AA)	DMSO	WP2P	+
Benzo(a)pyrene (BP)	DMSO	WP2P *uvrA*	+
Daunomycin HCl (DR)	DMSO	TA98	-
N-Ethyl-N'-nitro-N-	DMSO	WP2P *uvrA*	-

Positive control	Solvent	Strain	+/- S9
nitrosoguanidine (ENNG)			
Mitomycin C (MMC)	H2O	WP2P	-
Sodium Azide (NaZ)	H2O	TA1535 and TA100	-

Incubation and sampling times: ZD6474 was pre-incubated with S9 mix for 3 days at 37^0C prior to the experiment.

Results

Control samples in Experiment #1 had baseline mutation rates below historical controls. Low control values may have contributed to statistically significant increases in mutations for doses ranging from 5 to 200 µg ZD6474/plate. New cells were tested in Experiments #2 and #3, resulting in a valid assay where control values were in agreement with historic control values.

Experiment#2

STRAIN	DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	STATS SIGNIF.	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 1535	+S9 1000	1.0	0.0	0.1		1	1	1
	500	2.0	1.7	0.3		1	1	4
	200	4.0	1.0	0.5		4	5	3
	100	8.0	4.6	1.0		4	7	13
	50	12.0	2.6	1.5		11	15	10
	20	6.0	3.6	0.8		5	3	10
TA 1535	-S9 1000	0.0	0.0	0.0		0	0	0
	500	1.0	1.7	0.2		0	3	0
	200	4.7	1.5	0.7		5	6	3
	100	6.7	3.5	1.0		7	3	10
	50	5.0	1.7	0.8		4	7	4
	20	8.7	0.6	1.4		9	9	8
TA 1537	+S9 1000	1.0	1.0	0.1		2	1	0
	500	3.3	0.6	0.5		3	4	3
	200	7.7	2.3	1.1		5	9	9
	100	13.3	5.9	2.0	*	9	20	11
	50	12.0	5.6	1.8		11	18	7
	20	8.7	3.5	1.3		12	9	5
TA 1537	-S9 1000	0.0	0.0	0.0		0	0	0
	500	1.0	1.0	0.1		2	0	1
	200	3.0	2.0	0.3		5	1	3
	100	6.3	1.2	0.6		7	5	7
	50	12.7	3.8	1.3		11	10	17
	20	11.3	9.1	1.2		10	3	21
TA 98	+S9 1000	1.0	1.0	0.0		2	1	0
	500	17.7	10.6	0.8		8	16	29
	200	22.3	1.5	1.0		21	24	22
	100	21.3	5.0	0.9		22	16	26
	50	25.0	4.4	1.1		28	20	27
	20	22.3	4.6	1.0		17	25	25
TA 98	-S9 1000	0.0	0.0	0.0		0	0	0
	500	5.3	4.6	0.3		8	8	0
	200	16.0	6.1	0.9		13	23	12
	100	16.0	4.6	0.9		17	20	11
	50	17.0	1.0	0.9		17	18	16
	20	18.0	4.6	1.0		23	14	17

STRAIN	DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	STATS SIGNIF.	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 100 +S9	1000	30.7	8.4	0.2		21	35	36
	500	43.0	10.8	0.3		40	34	55
	200	124.7	6.8	0.7		127	117	130
	100	156.3	23.4	0.9		131	161	177
	50	147.7	13.6	0.9		146	162	135
	20	162.7	3.5	1.0		166	163	159
WP2P +S9	1000	12.0	6.1	0.2		16	15	5
	500	40.3	2.1	0.7		41	38	42
	200	54.3	15.5	0.9		37	59	67
	100	56.3	8.7	1.0		49	66	54
	50	61.3	8.1	1.0		52	66	66
	20	62.3	12.6	1.1		74	64	49
WP2P -S9	1000	1.7	2.9	0.0		5	0	0
	500	26.0	1.0	0.6		25	27	26
	200	46.0	9.8	1.0		54	49	35
	100	42.3	1.5	0.9		42	41	44
	50	48.7	14.5	1.1		34	49	63
	20	53.7	2.3	1.2		55	51	55
WP2P uvrA+S9	1000	6.3	4.9	0.0		12	4	3
	500	44.7	9.1	0.2		38	41	55
	200	121.0	33.0	0.6		89	119	155
	100	137.0	27.8	0.7		123	119	169
	50	187.0	36.6	0.9		162	170	229
	20	172.0	25.9	0.9		164	151	201
WP2P uvrA-S9	1000	0.7	0.6	0.0		1	0	1
	500	49.3	12.9	0.3		35	53	60
	200	207.0	30.4	1.1		213	234	174
	100	212.7	31.4	1.2		202	248	188
	50	210.3	9.0	1.1		216	215	200
	20	219.7	10.6	1.2	*	218	231	210

Experiment #3

STRAIN	DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	STATS SIGNIF.	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 1535 +S9	1000	24.0	17.3	2.0		43	20	9
	500	8.0	6.9	0.7		4	4	16
	200	3.0	5.2	0.3		9	0	0
	100	37.0	54.7	3.1		100	9	2
	50	7.0	2.6	0.6		8	9	4
	20	12.0	2.0	1.0		14	10	12
TA 1537 +S9	1000	0.7	1.2	0.0		2	0	0
	500	1.3	1.5	0.1		0	3	1
	200	7.3	2.5	0.4		7	5	10
	100	8.3	5.0	0.5		3	9	13
	50	17.3	6.1	0.9		12	16	24
	20	14.0	0.0	0.8		14	14	14
TA 98 +S9	1000	7.7	13.3	0.2		23	0	0
	500	1.0	1.7	0.0		0	3	0
	200	29.0	10.6	0.8		37	17	33
	100	25.0	5.3	0.7		23	31	21
	50	43.3	5.9	1.2		39	50	41
	20	33.3	0.6	0.9		34	33	33
TA 100 +S9	1000	0.7	0.6	0.0		1	0	1
	500	28.3	3.1	0.3		29	25	31
	200	84.3	18.8	0.8		64	101	88
	100	77.3	48.6	0.8		43	56	133
	50	86.3	21.5	0.8		62	94	103
	20	117.0	11.8	1.2		104	127	120
WP2P +S9	1000	2.0	1.0	0.0		2	1	3
	500	23.3	14.2	0.3		7	33	30
	200	45.0	19.5	0.6		23	60	52
	100	61.7	11.4	0.8		49	65	71
	50	65.3	1.5	0.9		64	67	65
	20	58.7	6.5	0.8		52	65	59
WP2P uvrA+S9	1000	33.3	9.3	0.1		41	36	23
	500	41.7	13.2	0.2		56	39	30
	200	127.0	13.1	0.5		115	141	125
	100	217.0	10.0	0.9		227	217	207
	50	206.0	10.5	0.9		196	205	217
	20	224.7	32.6	0.9		232	189	253

STRAIN	DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/ CONTROL	STATS SIGNIF.	NO REVERTANTS/PLATE PLATE 1	PLATE 2	PLATE 3
TA 1535 -S9	1000	0.0	0.0	0.0		0	0	0
	500	2.7	1.5	0.3		1	3	4
	200	8.3	4.0	0.8		12	9	4
	100	6.7	2.1	0.7		9	5	6
	50	12.3	0.6	1.2		12	13	12
	20	12.7	3.1	1.3		10	12	16
TA 1537 -S9	1000	0.3	0.6	0.0		1	0	0
	500	0.3	0.6	0.0		0	0	1
	200	3.3	3.5	0.3		3	7	0
	100	9.7	4.5	0.9		14	10	5
	50	14.3	3.8	1.3		16	10	17
	20	6.3	4.9	0.6		3	12	4
TA 98 -S9	1000	5.7	2.3	0.4		7	3	7
	500	6.7	2.9	0.4		5	5	10
	200	15.0	2.0	0.9		15	17	13
	100	16.7	7.1	1.0		23	18	9
	50	23.3	6.0	1.5	*	29	24	17
	20	19.7	9.1	1.2		13	30	16
TA 100 -S9	1000	5.7	1.2	0.1		5	5	7
	500	37.0	9.6	0.5		44	41	26
	200	73.3	15.8	1.0		87	56	77
	100	105.3	13.6	1.4	**	118	107	91
	50	98.0	10.5	1.3	**	108	99	87
	20	91.3	12.7	1.2	*	84	106	84
WP2P -S9	1000	2.0	2.6	0.1		5	1	0
	500	22.3	8.3	0.7		29	25	13
	200	40.0	2.6	1.2		37	42	41
	100	41.7	8.6	1.3		51	40	34
	50	39.3	8.3	1.2		42	46	30
	20	48.0	7.2	1.5	*	42	46	56
WP2P uvrA-S9	1000	17.7	18.9	0.1		39	11	3
	500	61.0	12.3	0.4		52	56	75
	200	224.3	20.5	1.3	**	207	247	219
	100	242.7	1.5	1.5	**	241	243	244
	50	207.0	24.3	1.2	**	191	195	235
	20	188.0	11.1	1.1	*	198	176	190

Study validity:
Each dose of ZD6474 was tested in triplicate and each positive control was tested in duplicate. Samples were considered positive for genetic toxicity if they had a 2-fold or greater increase in colonies compared with controls and had statistical significance according to a one-tailed Student's t-test. Dose dependence and reproducibility between experiments are also criteria for a positive result in this assay.

ZD6474 was initially tested on TA100 strain at concentrations up to 5000 µg/plate. Experiment #1 had low negative control values compared to historic controls and may have contributed to statistically significant increases in mutation rates in ZD6474-treated groups. In addition, toxicity at the 5000 mg/plate dose led the investigators to change the dose to range between 20 and 1000 µg/plate ZD6474. The first experiment is not considered valid due to inconsistent control samples.

Experiments #2 and 3 were well-replicated and had valid negative and positive control results.

Study outcome:
A dose of 1000 µg/mL was toxic to most strains in experiments #2 and 3 demonstrating drug exposure. Though some ZD6474 doses produced statistically significant increases in mutant colonies, they were less than 2-fold increases, did not have dose dependence and were not

reproducible between experiments. ZD6474 was not genotoxic under the conditions of this assay.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: **Zeneca ZD6474: In Vitro Cytogenetic Study Using Cultured Human Lymphocytes**

Key findings:
ZD6474 did not cause an increase in the number of chromosomal aberrations at 3 or 20 hours in the absence of S9 mix or at 3 hours in the presence of S9 mix. A single sample (2 µg/mL) was statistically higher than control when including chromosomal gaps, but was not replicated and was not dose dependent. This test included a dose range which produced acceptable cell toxicity indicative of drug exposure. Positive control produced statistically significant increases in chromosomal aberrations in this valid assay.

Study no.: TYX103
Study report location: M4.2.3.3.1

Conducting laboratory and location: Zeneca Pharmaceuticals
Safety of Medicines Department
Cheshire SK10 4TG, England
Date of study initiation: April 13, 1999
GLP compliance: Yes
QA reports: Yes (X) No ( )
Drug, lot #, and % purity: ZD6474, C267/1, 98% w/w

Methods

Strains/species/cell line: Cultured human peripheral lymphocytes

Doses used in definitive study:

Cytotoxicity test

	ZD6474 (µg/mL)						Negative control	Positive control
- S9 mix (3 hr. treatment)	0.4	2	4	10	20	40	DMSO	Mitomycin C (0.5 µg/mL)
+ S9 mix (3 hr. treatment)	0.4	2	4	10	20	40	DMSO	Cyclophosphamide (30 and 50 µg/mL)

Clastogenicity tests

	ZD6474 (µg/mL)						Negative control	Positive control
- S9 mix (3 or 20 hours)	0.2	0.4	1	2	4	10	DMSO	Mitomycin C (0.5 µg/mL)
+ S9 mix (3 hours)	2	4	10	-	-	-	DMSO	Cyclophosphamide (30 and 50 µg/mL)

Basis of dose selection: The dosing for this clastogenicity assay was limited by the mitotic inhibition of 20 and 40 µg/mL ZD6474 for lymphocytes in the cytotoxicity assay.

Negative controls: Dimethyl sulfoxide (DMSO)

Positive controls: Cyclophosphamide as a compound requiring activation by S9 mix and mitomycin C as a direct-acting clastogen

Incubation and sampling times: Human lymphocytes were treated with ZD6474 for 3 hours or 20 hours in absence of S9 mix, and for 3 hours in the presence of S9 mix.

Results

In assay #1 and #2, ZD6474 did not produce statistically significant increases in cells with chromosomal aberrations after 3 hours (+/- S9) at any dose tested. All positive controls caused statistically significant increases in abberations, validating the assay.
(Representative table is excerpted from sponsor's study report)

Treatment	Culture	Number of cells	Mitotic index (%)	Number of cells containing aberrations						Total aberrations		Percentage cells with aberrations	
					Chromatid		Chromosome						
				Gap	Deletion	Exchange	Deletion	Exchange	Other	+ gaps	-gaps	+ gaps	- gaps
DMSO 10 µl/ml	1 2	100 100	7.4 8.4	0 2	0 1	0 0	2 0	0 0	0 0	2 4	2 1	2 3	2 1
ZD6474 10 µg/ml	1 2	100 100	3.6 3.8	1 1	1 1	0 0	0 0	0 0	0 0	2 1	1 1	2 1	1 1
ZD6474 4 µg/ml	1 2	100 100	3.9 4.4	2 2	0 1	0 0	0 1	0 0	0 0	2 4	0 2	2 4	0 2
ZD6474 2 µg/ml	1 2	100 100	5.1 5.9	2 2	2 2	0 0	0 2	0 0	0 0	4 6	2 4	4 6	2 4
Mitomycin C 0.5 µg/ml	1	25	2.9	2	8	3	7	0	0	21	18	56 ***	48 ***

*** Statistically significant increase compared with DMSO control : $p<0.001$

Cultured human lymphocytes that were incubated with 2 µg/mL ZD6474 for 20 hours had an increase ($p \leq 0.05$) in the percentage of cells with aberrations when including gaps with the number of chromosomal anomalies. The duplicate 2 µg/mL ZD6474 sample did not result in a statistically significant increase in cells with aberrations. In addition, there were not statistically significant differences between any ZD6474-treated group and control when excluding gaps. There were no differences among 0.4 or 4 µg/mL doses and control values.
(Table is excerpted from sponsor's study report)

Treatment	Culture	Number of cells	Mitotic index (%)	Number of cells containing aberrations						Total aberrations		Percentage cells with aberrations	
					Chromatid		Chromosome						
				Gap	Deletion	Exchange	Deletion	Exchange	Other	+ gaps	-gaps	+ gaps	- gaps
DMSO 10 µl/ml	1 2	100 100	13.6 8.4	1 0	0 0	0 0	0 0	0 0	0 0	1 0	0 0	1 0	0 0
ZD6474 4 µg/ml	1 2	100 100	10.4 7.7	1 0	0 0	0 0	0 0	0 0	0 0	1 0	0 0	1 0	0 0
ZD6474 2 µg/ml	1 2	100 100	6.6 14.4	1 3	1 2	0 0	1 0	0 0	0 0	4 5	3 2	3 5 *	2 2
ZD 6474 0.4 µg/ml	1 2	100 100	15.1 15.0	2 0	0 0	0 0	0 0	0 0	0 0	2 0	0 0	2 0	0 0
Mitomycin C 0.5 µg/ml	2	25	4.3	3	3	3	10	0	0	35	31	60 ***	56 ***

* Statistically significant increase compared with DMSO control : $p<0.05$
*** Statistically significant increase compared with DMSO control : $p<0.001$

Study validity
Cytotoxicity was defined as a reduction in mitosis as judged by counting the number of cells (per 1000 scored) that were in metaphase. Cytotoxic concentrations of ZD6474 (20 and 40 µg/mL) were not used in the clastogenicity assay. Doses used in the assay ranged from 0.2 to 10 µg/mL representing a mitotic index ranging from 80% to 47% of solvent control, respectively. This represents a valid dose range for this test.

One hundred cells per culture were microscopically evaluated for chromosomal aberrations. Each dose of ZD6474 in the clastogenicity assay was performed in duplicate. Positive controls had only single samples and were evaluated by counting the clastogenic effects per 25 cells. Positive results were identified as samples that reproducibly scored outside the range of negative control values with statistical significance and dose dependence.
Dosing solutions were not analyzed to confirm concentrations

Study outcome:
ZD6474 did not cause an increase in the number of deletions or exchanges in chromosomes or chromatid and does not appear to be clastogenic in this assay. Doses of ZD6474 were high enough to cause cell toxicity and represent an appropriate range for this study. Positive controls caused statistically significant increases in chromosomal aberrations.

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

Study title: Zeneca ZD6474: Micronucleus Test in the Rat: Oral Administration.

Key findings:
Male rats receiving a single dose of 100, 330 or 1000 mg/kg ZD6474 were evaluated for micronuclei in polychromatic erythrocytes at 24 and 48 hours post-dose. At 48 hours post-dose, ZD6474-treated groups had a 3.3 to 4.6-fold increase in MPE which was statistically significant for a positive trend. Blinded extended counting of the same samples resulted in a non-statistically significant increase in MPEs. The results of this study are negative, indicating that ZD6474 did not induce clastogenicity.

Study no.: TQR2942
Study report location: M4.2.3.3.2

Conducting laboratory and location: Zeneca Pharmaceuticals
Safety of Medicines Department
Cheshire SK10 4TG, England
Date of study initiation: May 11, 1999
GLP compliance: Yes
QA reports: Yes (X) No ( )
Drug, lot #, and % purity: ZD6474, C267/1, 98% w/w

Methods

Strains/ Species: Alderley Park (Wistar derived) male rats
14 rats/dose
6 weeks old
127 to 213 g body weight range

ZD6474 doses used in definitive study: 100, 330 and 1000 mg/kg body weight (600, 1980, and 6000 mg/m^2) formulated in HPMC (see vehicle below) and delivered as 20 mL/kg body weight.

Basis of dose selection: The high dose in this study (1000 mg/kg) was chosen based on data from the study "Acute toxicity (limit) study in rats: oral administration." A single dose of 2000 mg/kg ZD6474 caused extensive mortality in rats on the day of administration. The MTD approaches 1000 mg/kg ZD6474 for a single dose of ZD6474 in rats and is therefore an appropriate high-dose in this study.

Negative controls: Vehicle control is 0.5% w/v hydroxypropyl methylcellulose in 0.1% w/v aqueous polysorbate 80 (HPMC).

Positive controls: Cyclophosphamide (20 mg/kg) in HPMC in a volume of 20 mL/kg body weight.

Incubation and sampling times: Bone marrow smears were evaluated from 7 rats/group at 24 hours and 7 rats/group at 48 hours after the ZD6474 dose. Cyclophosphamide-treated groups were assessed only at 24 hours post-dose.

Results

The results of the study are represented in the tables excerpted from the sponsor's report**.**

Table 2 Study number TQR/2942. Mean incidence of micronucleated polychromatic erythrocytes in rat bone marrow and percentage of polychromatic erythrocytes 24 and 48 hours after dosing ZD6474, first sample

	Mean number of micronucleated polychromatic erythrocytes in 2000 polychromatic erythrocytes, first sample		Mean percentage of polychromatic erythrocytes	
Group and dose level	24 hour NS	48 hour[Δ]	24 hour	48 hour
I HPMC 20 ml/kg	0.6	0.3	76.2	70.2
II ZD6474 100 mg/kg	1.4	1.0	72.4	74.7
III ZD6474 330 mg/kg	1.9	0.4	77.7	76.7
IV ZD6474 1000 mg/kg	1.1	1.4	77.2	68.6
V Cyclophosphamide 20 mg/kg	35.0***	-	58.9	-

- Not tested *** Pairwise comparison p<0.001
[Δ] Statistically significant trend with dosing, Groups I to IV Cochran-Armitage
NS No statistically different trend with dosing, Cochran-Armitage

Table 3 Study number TQR/2942. Mean incidence of micronucleated polychromatic erythrocytes in rat bone marrow 24 and 48 hours after dosing ZD6474, first and second samples combined

	Mean number of micronucleated polychromatic erythrocytes in 2000 polychromatic erythrocytes first and second samples combined	
Group and dose level	24 hour NS	48 hour NS[1]
I HPMC 20 ml/kg	0.8	0.6
II ZD6474 100 mg/kg	1.6	1.5[a]
III ZD6474 330 mg/kg	1.9	0.7
IV ZD6474 1000 mg/kg	1.4	1.8[b]
V Cyclophosphamide 20 mg/kg	34.9***	-

- Not tested [a] 5/7 animals resampled [b] 6/7 animals resampled *** Pairwise comparison p<0.001
NS No statistically different trend with dosing, Cochran-Armitage
NS[1] No statistically different trend with dosing, ANOVA

Study validity:

Each animal had 2000 polychromatic erythrocytes (PE) scored for the occurrence of micronucleated polychromatic erythrocytes (MPE). Cytotoxicity was evaluated by the ratio of

polychromatic to normochromatic erythrocytes from 1000 cells. Positive results were defined as an increase in MPEs that are 3-fold higher than historic control values. Treatment groups scoring positive results that are less than a 3-fold higher than controls were subject to extended counting of 4000 cells to confirm the reproducibility of the result. In this study, scoring of 2000 cells in some samples led to a statistically significant and dose-related increase in MPE resulting in an additional scoring of 2000 cells for the presence of MPE.

Study outcome:
ZD6474 caused a 2 to 3-fold increase in MPE 24 hours post-dose which did not reach statistical significance. ZD6474-treated groups had increased MPEs at 48 hours showing a positive trend with statistical significance according to the Cochrane-Armitage test. After an extended counting (4000 total cells) of these samples, higher variability between groups may have contributing to a non-significant difference between groups (ANOVA). Based on the results of the extended counting, ZD6474 did not induce clastogenicity.

8 Carcinogenicity

Carcinogenicity studies have not been conducted.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: **Zeneca ZD6474 : Fertility Study in Male Rats: Oral Administration.**

Key study findings:
- Male treatment with 0, 1, 5, or 20 mg/kg ZD6474 had no effect on untreated female weight gain during pregnancy
- The incidence of copulation, time until copulation, and fertility rate were unaffected by the dosage of ZD6474 delivered to the male.
- Total resorptions occurred in 2 females who mated with males that received 20 mg/kg. Resorptions did not occur in groups that mated with males in lower dose groups.
- Females that mated with ZD6474 showed a slight decreases in the number of implants and number of live embryos, and an increase in pre-implantation loss.

Study no.:	TGR3138
Study report location:	M4.2.3.5.1
Conducting laboratory and location:	AstraZeneca UK Limited Safety Assessment UK Alderley Cheshire, England
Date of study initiation:	May 15, 2000
GLP compliance:	Yes
QA reports:	Yes (X) no ( )
Drug, lot #, and % purity:	ZD6474, C268/1, 99.9% pure,

Methods

Doses:	0, 1, 5, or 20 mg/kg ZD6474. Males are from the 6 month repeat-dose toxicology

study (Study TPR2939) and were dosed 8 weeks prior to pairing. Males were dosed during the 1 week mating period and females remained untreated.

Species/strain: Rat/ Alpk:APfSD strain, Wistar derived

Number/sex/group:

Group	Dose level (mg/kg/day)	Number of rats
1	0	19 F
2	1	19F
3	5	20F
4	20	20F

Route, formulation, volume, infusion rate: Oral gavage, 0.5% w/v HPMC in 0.1% w/v aqueous polysorbate 80, 0.1 mL per 100 g body weight

Satellite groups used for toxicokinetics: None

Study design: Males from the general toxicology study were dosed for 8 weeks with ZD6474 and were then mated with females that remained ZD6474-naive before and during the study. Females were sacrificed 13 days after the predicted pregnancy.

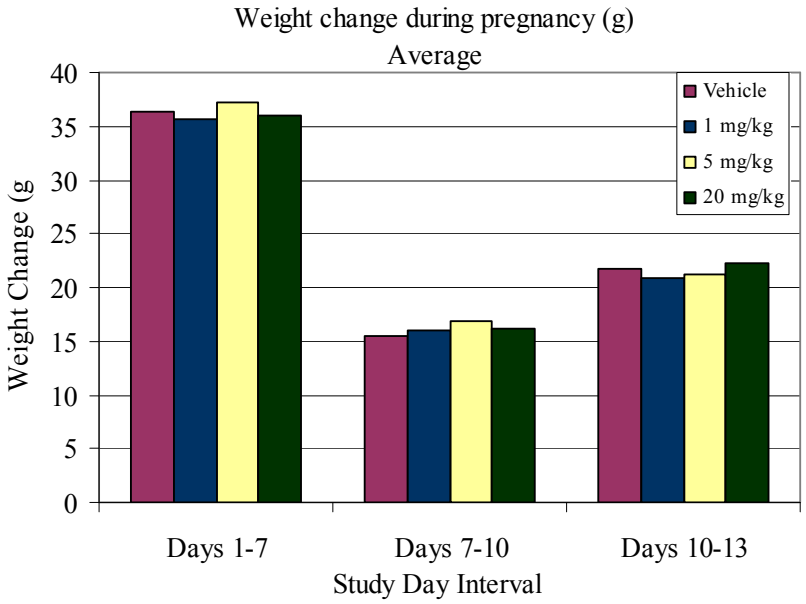
Parameters and endpoints evaluated: Females: In-life observations, detailed physical exams, body weight, vaginal smears, pre-coital interval, ovary and uterine examination, corpora lutea, implantations, resorptions, placental morphology, live and dead embryos
Male: Body weight, in-life observations

Results

Mortality:
No deaths occurred in the study

Clinical signs:
Male observations and body weights are in study TPR2939

Body weight:
Females were not treated with ZD6474, but were mated with males who received 0, 1, 5 or 20 mg/kg daily for 8 weeks prior to mating. All pregnant females gained similar weight during Days 1-7, Day 7-10, and Day 10-13, therefore weight gain was independent of the ZD6474 dose administered to their mates.



Males in the repeat dose toxicology study did not have any difference in body weight up to Week 7. In Week 8, the 20 mg/kg dose group had a 6% lower body weight ($p < 0.05$) than other animals in the study. Weight was similar among all other groups in this study.

Food consumption: Food consumption was not measured during this study.

Toxicokinetics:
Pharmacokinetic parameters were assessed only on the 5 mg/kg dose group on Day 1 of their 8 weeks of treatment. The males in this dose group were exposed to ZD6474. All other groups were evaluated on Week 26, long after this study was conducted. Pharmacokinetics were not evaluated in females of this study.

Mating and fertility:
Treatment of males with 0, 1, 5, or 20 mg/kg ZD6474 did not affect the incidence of copulation or the fertility rate in this study. The number of females with live embryos was similar among all groups, however, there were 2 females in the high dose group (20 mg/kg ZD6474) with total resorptions.

(the following table is excerpted from the sponsor's study report)

Table 3 Study number TGR3138. Summary of mating and fertility

	Group I (control)	Group II * 1 mg/kg/day	Group III * 5 mg/kg/day	Group IV * 20 mg/kg/day
MALES				
Number paired	19	19	20	20
Number mated	19	17	19	20
Copulation index (%)	100	89.5	95.0	100
Fertility Index (%)	94.7	100	100	95.0
FEMALES (undosed)				
Number paired	19	19	20	20
Number mated	19	17	19	20
Number pregnant at scheduled day 13 necropsy				
Number with live embryos -	18	17	19	17
Number with resorptions only -	0	0	0	2
Number not pregnant at scheduled necropsy	1	2	1	1
Copulation index (%)	100	89.5	95.0	100
Fertility index (%)	94.7	100	100	95.0

* The dose levels in this table refer to doses given to males on study TPR2939; the females on this study were not dosed.

The time until pregnancy was similar among all females in the study, therefore the dosage received by the male did not appear to affect pre-coital interval. (Table adapted from sponsor's report)

Pre-coital interval

Pre-coital interval (days)	ZD6474 dose (mg/kg)			
	0	1	5	20
	Rats pregnant per day (n)			
Day 1	5	7	10	3
Day 2	8	3	6	8
Day 3	4	6	2	3
Day 4	2	0	0	1
Day >4	0	1	0	1

For pregnancy parameters, the sponsor reported medians for each group and performed Cochrane-Armitage statistical test. This approach resulted in no statistically significant trend for an effect of ZD6474-treatment of males on any pregnancy parameters.

Pregnancy parameters
Median

ZD6474 Dose	Corpora lutea	Implants	No. of abortion scars	No. of early/mid resorptions	No. of late resorptions	Number of live embryos	Pre-implantation loss	Post-implantation loss
Control	17	16	0	1	0	14.5	1	1
1 mg/kg	18	15	0	0	0	15	2	0
5 mg/kg	18	16	0	0	0	16	2	0
20 mg/kg	17	15	0	1	0	15	1	1

The average values for each dose group indicate that there is a dose-dependent decrease in implants, reduction in the number of live embryos, and an increase in pre-implantation loss (Table below). The individual data were examined in order to determine that these findings were not due to a single litter. The number of rats showing a decrease in the number of implants and/or live embryos and/or an increase in pre-implantation loss was 2 in the control group and increased with dose to 7 rats in the 20 mg/kg group.

Pregnancy parameters
Average

ZD6474 Dose	No. of rats (n)	Corpora lutea	Implants	No. of abortion scars	No. of early/mid resorptions	No. of late resorptions	Number of live embryos	Pre-implantation loss	Post-implantation loss
Control	18	17.1	15.1	0	1.11	0	14	2	1.1
1 mg/kg	17	17.4	14.4	0	0.82	0	13.6	2.9	0.8
5 mg/kg	19	18.2	13.6	0	0.42	0	13.2	4.6	0.4
20 mg/kg	17	16.9	12.2	0	1.63	0	11.8	4.1	1.6

Study title: **Zeneca ZD6474 : Oral Fertility and Early Embryonic Development Study in the Female Rat**

Key study findings:

- Females receiving 10 and 25 mg/kg/day ZD6474 had an increased incidence of irregular cycle patterns, however, this did not affect their ability to mate. The copulation index and pre-coital intervals were approximately similar among all groups.
- Treatment with 25 mg/kg/day ZD6474 resulted in a lower fertility index for this group.
- Two females treated with 25 mg/kg/day had total intrauterine death.
- Females in the high dose group (25 mg/kg ZD6474) demonstrated increased pre-implantation loss, a significant increase in post-implantation loss, a markedly increased number of early embryo deaths, and a significant reduction in the number of live embryos per female

Study no.: TGR2940
Study report location: M4.2.3.5.1
Conducting laboratory and location: AstraZeneca UK Limited
Safety Assessment UK Alderley
Cheshire, England
Date of study initiation: August 2, 2001
GLP compliance: Yes
QA reports: Yes (X) No ( )
Drug, lot #, and % purity: ZD6474, Batch C268/1 also known as ADM65949A99, 99% pure

Methods

Doses: Females received 0, 1, 10, or 25 mg/kg
Species/strain: Wistar-derived (Alpk:APfSD) rats
Number/sex/group: 22 females/dose
Age, weight Female: 11 weeks old, weight range 248 to 310 grams
Male: 15 weeks old, weight range 423 to 618 grams

Route, formulation, volume	Oral, 0.5% w/v hydroxypropyl methylcellulose (HPMC) solution containing 0.1% w/v aqueous polysorbate 80, 0.5 mL/100 g body weight
Satellite groups used for toxicokinetics:	None
Study design:	F0 females were dosed for 14 days before housing with breeder males. Dosing was continued until gestational Day 6. Females were then euthanized on GD 12 for necropsy. Breeder males were not treated with drug. An additional 12 females were dosed with 0 or 25 mg/kg ZD6474 for 19 days. Four weeks after dosage (withdrawal), the females were paired with males to evaluate any effects on mating performance or fertility. These females were euthanized on gestational Day 12.

Table 2 Study number TGR2940. Dose levels and distribution of animals to groups

Group	Dose level (mg/kg/day)	Number of rats and sex
I Control	0	22 Main F + 12 Withdrawal F + 22 M
II	1	22 Main F + 22 M
III	10	22 Main F + 22 M
IV	25	22 Main + 12 Withdrawal F + 22 M

Males were not dosed during the study.

Parameters and endpoints evaluated:	Females: in-life observations, body weight, food consumption, estrous cycle, mating, fertility, necropsy, corpora lutea, counting intrauterine deaths, live embryos, and uterus implantations

Results

Mortality:
Animal #63, receiving 10 mg/kg/day, was found dead on Day 4. One control animal with respiratory distress was euthanized on Day 2. Both the control and 10 mg/kg/day animals had microscopic evidence of acute tracheitis.

Clinical Signs:
Broken and missing teeth, along with abnormal tooth growth and discoloration were noted in the high dose group (25 mg/kg). This was a frequent observation, present in 10 of 12 females in the high dose in the withdrawal segment of this study. Females in the high dose group (25 mg/kg/day) also had signs of hunched posture and piloerection through the study.

Observation	Incidence & Duration	ZD6474 dose (mg/kg/day)			
		0	1	10	25
	Total animals	22	22	21	22
Activity decreased	# of animals				2
	Duration (from - to)				10/11
Discharge eye/eyelid	Incidence				2
	Duration				53/55
Discharge red - nostrils	Incidence				1
	Duration				10
Discharge red - vagina	Incidence				1
	Duration				53/53
Eye half shut	Incidence				1
	Duration				10/10
Feces consistancy - Soft	Incidence				1
	Duration				11
Hunched posture	Incidence				5
	Duration				10/55
Pilo-erection	Incidence				4
	Duration				10/55
Skin lesion - muzzle	Incidence				1
	Duration				13/17
Teeth abnormal growth	Incidence				9
	Duration				39/64
Teeth broken	Incidence				6
	Duration				37/64
Teeth discoloration	Incidence				1
	Duration				64
Teeth missing	Incidence				5
	Duration				52/64
Thin	Incidence				3
	Duration				5/62

Body weight:
Body weights were measured weekly during the study prior to pairing. ZD6474 treatment did not alter female body weight in any dose group prior to pairing.

During the gestation period, body weights were measured on Days 0, 3, 6, 9, and 12. No ZD6474-related effect on body weight occurred throughout gestation for both the main study and withdrawal study females.

Food consumption:
Food consumption was measured on Days 0 and 7, during the dosing period but prior to pairing with mates. Food consumption was similar among all dose groups on both Days 0 and 7.

Toxicokinetics:
Not conducted

Estrous cycle

Main study females receiving 10 and 25 mg/kg/day ZD6474 had an increased incidence of irregular cycle patterns. Irregular cycle length was observed in females dosed with 25 mg/kg during the dosing phase of the withdrawal study. Females in this dose group continued to have irregular cycle length or cycle pattern during the non-dosing phase of the withdrawal study.

(the following was adapted from the sponsor's study report)

Cycle length	Cycle pattern	Control)	1 mg/kg/day	10 mg/kg/day	25 mg/kg/day
MAIN TEST					
Regular	Regular	6 (27%)	8 (36%)	2 (10%)	2 (9%)
Regular	Irregular	6 (27%)	5 (23%)	11 (52%)	10 (45%)
Irregular	Irregular	8 (36%)	7 (32%)	8 (38%)	10 (45%)
Irregular	Regular	2 (9%)	2 (9%)	0 (0%)	0 (0%)
WITHDRAWAL – during dosing period					
Regular	Regular	3 (27%)	N/A	N/A	3 (25%)
Regular	Irregular	8 (73%)	N/A	N/A	6 (50%)
Irregular	Irregular	0 (0%)	N/A	N/A	3 (25%)
WITHDRAWAL – during withdrawal period					
Regular	Regular	2 (22%)	N/A	N/A	3 (25%)
Regular	Irregular	6 (67%)	N/A	N/A	4 (33%)
Irregular	Irregular	1 (11%)	N/A	N/A	5 (42%)

Cycle Length - Regular - cycling with 4 day cycle; Irregular – one or more cycles lengthened/shortened
Cycle Pattern – Regular – cycling with stages in correct order; Irregular – one or more cycle patterns disturbed or stages extended

Mating and fertility

Less females were pregnant in the high dose group (25 mg/kg) resulting in a lower fertility index for this group. The high dose group (25 mg/kg) also had 2 females with total intrauterine death. Though one female did not copulate in the high dose, the copulation index and pre-coital intervals were approximately similar among all groups.

Study segment		0 mg/kg ZD6474	1 mg/kg ZD6474	10 mg/kg ZD6474	25 mg/kg ZD6474
Females -Main test	No. paired	22	22	21	22
	No. mated	22	22	21	21
	No. pregnant at necropsy	21	20	19	18
	No. with live embryos	21	20	19	16
	No. with total intrauterine death	0	0	0	2
	No. not pregnant at scheduled necropsy	1	2	2	4
	Copulation index (%)	100	100	100	95
	Fertility index (%)	95	91	90	86
	Mean pre-coital interval (days)	2	2.3	2	2.3
Females - Withdrawal study					
	No. paired	9	--	--	12

Study segment		0 mg/kg ZD6474	1 mg/kg ZD6474	10 mg/kg ZD6474	25 mg/kg ZD6474
	No. mated	9	--	--	12
	No. pregnant	8	--	--	10
	No. not pregnant	1	--	--	2
	Copulation index (%)	100	--	--	100
	Fertility index (%)	89	--	--	83
	Mean pre-coital interval (days)	2.1	--	--	2.3

Pregnancy parameters

Females in the high dose group (25 mg/kg ZD6474) demonstrated increased pre-implantation loss and a statistically significant increase in post-implantation loss. Females in the high dose group (25 mg/kg) also had a markedly increased number of early embryo deaths and a significant reduction in the number of live embryos per female.

Main test

Pregnancy parameters	ZD6474 dose (mg/kg)			
	0	1	10	25
Number of females with implantations at scheduled kill	21	20	19	18
Number of corpora lutea	311	300	291	336
Mean number corpora lutea per female	15.6	15.8	15.3	18.7
Std. dev.	2.5	2.7	2.8	6.1
Number of implantations	250	251	231	214
Mean number implantations per female	11.9	12.6	12.2	11.9
Std. dev.	5.7	4.6	4.7	4.3
Mean % pre-implantation loss	23.3	16.6	23.2	34.3
Number of early embryo deaths	10	10	20	101
Number of late embryo deaths	0	0	0	0
Mean % post implantation loss	5.1	4	9.8	42**
Number of live embryos	240	241	211	113
Mean number per female	11.4	12.1	11.1	6.3**
Std. dev.	5.7	4.6	4.9	4.5

**$p < 0.01$

Withdrawal study

Pregnancy parameters	ZD6474 dose (mg/kg)	
	0	25
Number of females with implantations at scheduled kill	8	10
Number of corpora lutea	137	166
Mean number corpora lutea per female	17.1	16.6
Std. dev.	1.1	2.1
Number of implantations	133	146
Mean number implantations per female	16.6	14.6

Pregnancy parameters	ZD6474 dose (mg/kg)	
	0	25
Std. dev.	1.4	4.6
Mean % pre-implantation loss	2.9	14.1
Number of early embryo deaths	5	12
Number of late embryo deaths	0	0
Mean % post implantation loss	3.8	7.6
Number of live embryos	128	134
Mean number per female	16	13.4
Std. dev.	1.8	4.5

Uterine assessment

The number of females with greater than 20% pre-or post-implantation loss was increased in the high dose group (25 mg/kg).

Main test	ZD6474 dose (mg/kg)			
	0	1	10	25
Pre-implantation loss (# greater than 20%)	7	7	8	12
Post-implantation loss (# greater than 20%)	1	1	2	11

Embryonic Fetal Development

Study title: Zeneca ZD6474: Teratology sighting study in rats: Oral administration

Key Study Findings:

- ZD6474 (20 mg/kg/day) caused an increase in post implantation loss when administered to pregnant rats either between gestation Days 1-7 or Days 7-16
- Fetal weight was decreased by administration of 10 and 20 mg/kg/day ZD6474 in females dosed between gestation Days 7-16
- A variety of major abnormalities were observed in a small number of fetuses across different dose groups, however, the findings were not consistent across groups

Study no.:	TRR3073
Study report location:	M4.2.3.5.2
Conducting laboratory and location:	Astrazeneca UK Limited Safety Assessment UK Alderley Alderley Park Macclesfield Cheshire SK10 4TG England
Date of study initiation:	June 21, 2000
GLP compliance:	Yes
QA statement:	No
Drug, lot #, and % purity:	ZD6474

Methods

Doses:	0, 0.5, 1, 5, 10, or 20 mg/kg/day
Frequency of dosing:	Daily Days 1-7 of gestation or Days 7-16 of gestation
Dose volume:	0.5 mL/100 g body weight
Route of administration:	Gastric intubation
Formulation/Vehicle:	0.5% w/v hydroxypropyl methylcellulose containing 0.1% w/v aqueous polysorbate 80
Species/Strain:	Rat/ Alpk:AP_fSD (Wistar derived)
Number/Sex/Group:	12 females/group (0 mg/kg/day group had only 11 females)
Satellite groups:	None
Study design:	6 females/group were dosed Days 1-7 of gestation and euthanized on Day 13 and 6 females/group (5 females in 0 mg/kg/day group) were dosed Days 7-16 of gestation and euthanized on Day 22
Parameters and endpoints evaluated:	Females: Body weight, food consumption, gross necropsy, number of implants, corpora lutea, number of live and dead fetuses, weight of live fetuses, weight of placentas, and uterus weights Fetuses: Fetal examinations (malformations)

Results

Mortality

No mortality was observed in the females dosed in this study.

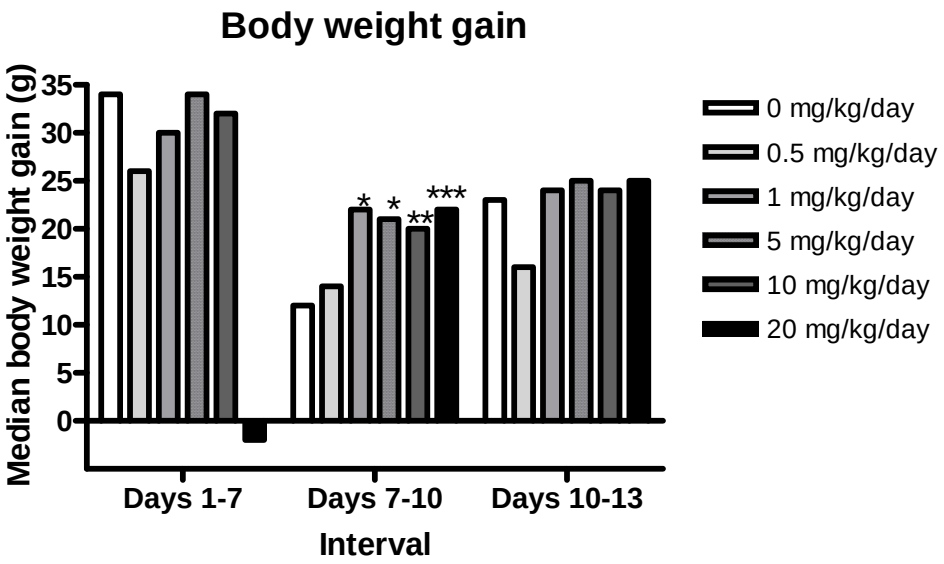
Clinical Signs

Unremarkable

Body Weight

- In females dosed with 20 mg/kg/day between gestation Days 1-7, body weight gain was decreased during the dosing period on Days 1-7. Weight gain was significantly increased in females dosed with 1, 5, 10, and 20 mg/kg/day following the dosing period on

gestation Days 7-10. The median body weight gains for each group are shown in the figure below.



*= significantly different from control (0 mg/kg/day), p <0.05
**= significantly different from control (0 mg/kg/day), p <0.01
***= significantly different from control (0 mg/kg/day), p <0.001

- There were no effects on maternal body weight gain in females dosed between gestation Days 7-16

Food Consumption

- In females dosed between gestation Days 1-7, food consumption was decreased in females treated with 20 mg/kg/day during the dosing period on Days 1-7. The median food consumption for each group is listed in the table below.

	Median food consumption (g/animal/day)					
Dose (mg/kg/day)	0	0.5	1	5	10	20
Food consumption Days 1-7	23	22	22	24	24	12

- There were no effects on food consumption in females dosed between gestation Days 7-16

Toxicokinetics

Not conducted

Necropsy

Unremarkable

Cesarean Section Data

Results of the uterine examinations are presented in the tables below. Two females, one treated with 10 mg/kg/day on gestation Days 7-16 and one treated with 20 mg/kg/day on gestation Days 1-7 were not pregnant, therefore, the data are not reported for these animals.

Females dosed between gestation Days 1-7: Day 13

	Median					
Dose (mg/kg/day)	0	0.5	1	5	10	20
Number of females with data	6	6	6	6	6	5
Number of live fetuses/litter	12	14	12	10	12	10
Number of implants/dam	12	14	12	11	13	14
Number of Corpora lutea/dam	14	14	14	14	14	15
Pre-implantation loss/dam	1	0	1	2	1	2
Post-implantation loss/dam	0	0	0	1	0	3*

* p< 0.05

Females dosed between gestation Days 7-16: Day 22

	Median					
Dose (mg/kg/day)	0	0.5	1	5	10	20
Number of females with data	5	6	6	6	5	6
Number of live fetuses/litter	13	14	12	14	15	12
Number of implants/dam	13	14	14	14	15	14
Number of Corpora lutea/dam	15	14	15	14	16	14
Pre-implantation loss/dam	0	1	1	2	1	0
Post-implantation loss/dam	0	0	1	0	0	2
Mean fetal body weight/ litter (g)	5.1	5.1	5.3	5.0	4.7	4.5**
Mean placental weight/litter (g)	0.6	0.5	0.6	0.5	0.5	0.5
Male proportion/litter	0.6	0.4	0.6	0.5	0.5	0.5
Empty uterus weight (g)	4.6	5.8	5.6	5.4	5.5	4.6

** p< 0.01

Offspring

External and visceral fetal defects

Dose (mg/kg/day)		Incidence by fetus (litter)					
		0	0.5	1	5	10	20
Defect							
Brain, lateral ventricles dilated		-	-	-	2 (1)	-	-
Diaphragm, incompletely formed		-	-	-	-	-	2 (1)
Kidney, pelvis dilated	Slight	-	1 (1)	-	-	-	1 (1)
	Moderate	-	-	-	-	1 (1)	1 (1)
	Extreme	-	-	-	-	-	1 (1)
Ureter, dilated	Slight	3 (2)	5 (1)	2 (2)	2 (2)	-	8 (4)
	Moderate	-	5 (2)	1 (1)	-	-	-
	Extreme	-	-	-	-	1 (1)	3 (3)
Bladder, left umbilical artery		9 (4)	12 (6)	7 (5)	6 (2)	14 (5)	25* (6)

* $p < 0.05$
- = defect not observed in this group

Study title: Astrazeneca ZD6474: Oral embryofetal development study in the rat

Key Study Findings:

- Post implantation loss was increased and the number of live fetuses was decreased in females treated with 25 mg/kg/day ZD6474
- Fetal weight and mean placental weight were decreased with treatment of ZD6474 at both 10 and 25 mg/kg/day
- There was an increase in fetuses and litters with visceral abnormalities/variations at 10 and 25 mg/kg/day
- Treatment with ZD6474 caused a number of different heart vessel abnormalities at 10 and 25 mg/kg/day. Heart vessel abnormalities were observed in one fetus at 1 mg/kg/day
- Treatment with ZD6474 also reduced ossification of skull bones, vertebrae and sternebrae indicating delayed fetal development
- There was not a NOEL for embryofetal developmental toxicity in this study based on delayed ossification and heart vessel abnormalities. These findings occurred in the absence of maternal toxicity.

Study no.:	TTR2938
Study report location:	M4.2.3.5.2
Conducting laboratory and location:	Astrazeneca UK Limited Safety Assessment UK Alderley Alderley Park Macclesfield Cheshire SK10 4TG England
Date of study initiation:	May 4, 2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ZD6474, batch # C268/1, Purity: 99.0%

Methods

Doses:	0, 1, 10, or 25 mg/kg/day
Frequency of dosing:	Daily from gestation Days 6-15
Dose volume:	0.5 mL/100 g (0.1 mL/100 g for 1 mg/kg/day group)
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% w/v hydroxypropyl methylcellulose containing 0.1% w/v aqueous polysorbate 80
Species/Strain:	Rat/Alpk:AP_fSD (Wistar derived)
Number/Sex/Group:	22/females/group
Satellite groups:	None
Study design:	22 females/group were dosed from gestation Days 6-15 with the day of mating as gestation Day 0 and euthanized on gestation Day 21
Parameters and endpoints evaluated:	Females: Body weight, food consumption, clinical observations, toxicokinetics, gross necropsy, number of implants, corpora lutea, number of live and dead fetuses, weight of live fetuses, weight of placentas, and uterus weights Fetuses: Fetal examinations (malformations and abnormalities)

Results

Mortality

No mortality was observed in the females dosed in this study.

Clinical Signs

Clinical sign		Dose (mg/kg/day)			
		0	1	10	25
Soft feces	No. of animals	0	2	1	3
	No. of observations	0	3	1	4
	Days observed	-	14-18	12	9-16

Body Weight

Body weight gain was decreased compared to controls (0 mg/kg/day) in females treated with 25 mg/kg/day from the start of the dosing period to the end of the study. There was no effect on body weight after adjustment for gravid uterus weight on Day 21 of gestation.

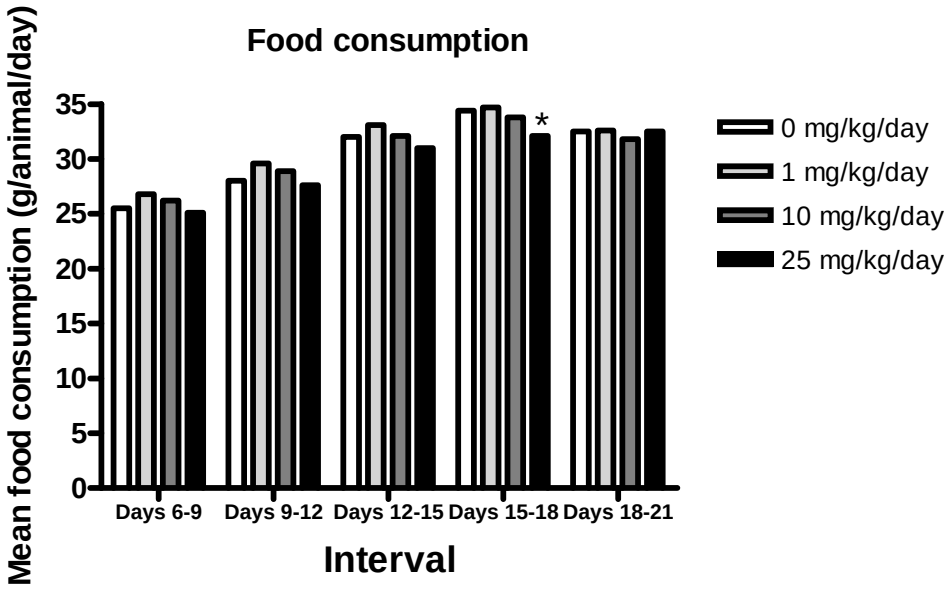
Mean body weight gain (g)				
Dose (mg/kg/day)	0	1	10	25
Day 6-15	45.7	49.0	47.3	36.6**
Day 6-21	127.0	132.1	131.0	101.4***

** $p<0.01$ *** $p<0.001$

Body weight on Day 21 (g)				
Dose (mg/kg/day)	0	1	10	25
Body weight	413.2	420.6	419.3	393.2
Body weight adjusted for gravid uterus weight	325.8	325	332.8	336.0

Food Consumption

Food consumption was slightly decreased compared to controls in females treated with 25 mg/kg/day just after the dosing period between gestation Days 15-18.



*= significantly different from control (0 mg/kg/day), p <0.05

Toxicokinetics

Blood samples were collected after 10 days of dosing on gestation Day 15 at 2, 4, and 8 hours after dosing.

- C_{max} values increased with an increase in dose. The increase in the mean C_{max} values was greater than proportional between 1 and 10 mg/kg/day and proportional between 10 and 25 mg/kg/day

Maternal plasma concentrations of ZD6474 (excerpted from sponsor's submission)

Group (dose level)	Time (hours) after dosing on day 15	Mean concentration of ZD6474 ± SE (ng/ml)	C_{max} (ng/mL)	t_{max} (hours)
II	2	21.5 ± 0.49		
(1 mg/kg/day)	4	18.5 ± 0.81	21.5	2
	8	18.7 ± 1.73		
III	2	256 ± 24.0		
(10 mg/kg/day)	4	347 ± 41.5	347	4
	8	282 ± 40.3		
IV	2	813 ± 50.3		
(25 mg/kg/day)	4	867 ± 153	867	4
	8	701 ± 23.2		

SE standard error

Necropsy

Necropsy observations		No. of animals affected			
Dose (mg/kg/day)		0	1	10	25
Kidneys	Renal pelvic dilatation, left	0	1	1	0
	renal pelvic dilatation, right	0	1	1	3

Cesarean Section Data

Results of the uterine examinations are presented in the table below. All females survived to the scheduled C-section, however, one female in the 10 mg/kg/day group was not pregnant.

Dose (mg/kg/day)	0	1	10	25
Number of females mated	22	22	22	22
Number of pregnant females (Pregnancy rate)	22	22	21	22
Mean gravid uterus weight (g)	87.4	95.6	88.3	57.1***
Number of corpora lutea				
Total	331	343	332	329
Mean number per female	15.0	15.6	15.8	15.0
Number of implantations				
Total	293	314	297	296
mean number per female	13.3	14.3	14.1	13.5
Mean percent pre-implantation loss	12.7	8.2	11.3	11.0
Intrauterine deaths				
Early embryo/fetal deaths	18	12	13	87
Late embryo/fetal deaths	2	2	8	9
Dead fetuses	0	0	0	0
Total	20	14	21	96
Mean number of deaths per female	0.91	0.64	1.0	4.36***
Mean percent post-implantation loss	7.1	4.4	7.7	33.6***
Number of live fetuses				
Total	273	300	276	200
Mean number per female	12.4	13.6	13.1	9.1**

** $p<0.01$ *** $p<0.001$

Offspring

Fetal observations are presented in the tables below.

Number of fetuses and weights

Dose (mg/kg/day)	0	1	10	25
Number of live fetuses				
Total	273	300	276	200
Mean number per female	12.4	13.6	13.1	9.1**
Sex distribution				
Males	135	160	136	103
Females	138	140	140	97
Mean % male fetuses	48.8	53.1	49.7	53.0
Mean litter weight	63.3	69.8	63.3	38.2***
Mean fetal weight				
Males only	5.25	5.28	4.98***	4.29***
Females only	5.02	4.95	4.71***	4.07***
Combined	5.14	5.13	4.84***	4.20***
Mean placental weight	0.53	0.51	0.47*	0.46**

* p<0.05 ** p<0.01 *** p<0.001

Total number of malformations observed

		No. of fetuses affected (No. of litters affected)			
Dose (mg/kg/day)		0	1	10	25
Total number of litters examined		22	22	21	22
Total number of fetuses examined		273	300	276	200
External examination	Major abnormalities	0 (0)	1 (1)	1 (1)	0 (0)
	Minor abnormalities	11 (4)	13 (6)	13 (2)	5 (3)
	Variations	0 (0)	0 (0)	0 (0)	0 (0)
Fresh visceral examination	Major abnormalities	0 (0)	1 (1)	2 (2)	30 (16)
	Minor abnormalities	21 (11)	26 (11)	21 (11)	41 (22)
	Variations	40 (17)	51 (15)	73 (21)	96 (22)
Skeletal examination	Major abnormalities	0 (0)	1 (1)	1 (1)	0 (0)
	Minor abnormalities	255 (22)	284 (22)	272 (21)	200 (22)
	Variations	263 (22)	289 (22)	273 (21)	199 (22)

Malformations

Visceral abnormalities

			No. of fetuses affected (Group mean percent)			
Dose (mg/kg/day)			0	1	10	25
Total number of litters examined			22	22	21	22
Total number of fetuses examined			273	300	276	200
Cavity	**Malformation**	**Type**				
Thoracic	Subclavian artery, bifurcated	Major	0 (0)	1 (0.3)	0 (0)	0 (0)
	Subclavian artery, retro-esophageal	Minor	0 (0)	0 (0)	0 (0)	4 (2.4)
	Subclavian artery, arising from pulmonary arch	Major	0 (0)	0 (0)	0 (0)	8 (4.9)**

			No. of fetuses affected (Group mean percent)			
Dose (mg/kg/day)			0	1	10	25
Total number of litters examined			22	22	21	22
Total number of fetuses examined			273	300	276	200
Cavity	**Malformation**	**Type**				
	Subclavian artery, arising from ductus arteriosus	Major	0 (0)	0 (0)	0 (0)	7 (4.5)**
	Common carotid artery, bifurcated	Major	0 (0)	1 (0.3)	1 (0.4)	2 (1.1)
	Innominate artery, absent	Minor	0 (0)	0 (0)	0 (0)	12 (7.6)***
	Aortic arch, interrupted	Major	0 (0)	0 (0)	0 (0)	10 (7.9)***
	Aortic arch, right sided	Major	0 (0)	0 (0)	0 (0)	11 (6.8)***
	Aortic arch, pre-ductal constriction	Major	0 (0)	0 (0)	1 (0.3)	0 (0)
Abdominal	Kidney, increased pelvic cavitation	Minor	0 (0)	1 (0.3)	0 (0)	5 (3.7)*
	Kidney, increased pelvic cavitation	Variant	0 (0)	0 (0)	2 (0.9)	14 (5.7)***
	Ureter, dilated	Minor	0 (0)	1 (0.3)	0 (0)	10 (6.3)***
	Ureter, dilated	Variant	17 (7.3)	22 (7.2)	23 (8.5)	41 (22.4)***
	Umbilical artery, left of bladder	Variant	28 (12.0)	35 (11.5)	58 (21.1)***	75 (36.4)***

* p<0.05 ** p<0.01 *** p<0.001

Skeletal abnormalities

			No. of fetuses affected (Group mean percent)			
Dose (mg/kg/day)			0	1	10	25
Total number of litters examined			22	22	21	22
Total number of fetuses examined			273	300	276	200
Bone	**Malformation**	**Type**				
Skull	Parietal, additional ossification center	Minor	0 (0)	1 (0.3)	3 (9.8)	8 (4.7)***
	Parietal, unossified areas	Minor	12 (3.6)	39 (13.1)***	22 (11.6)	5 (2.8)
	Occipital, incompletely ossified	Minor	91 (31.4)	88 (28.9)	190 (68.3)***	199 (99.4)***
	Occipital, bipartite	Minor	0 (0)	0 (0)	1 (0.3)	10 (5.4)***
	Occipital, unossified areas	Minor	67 (23.4)	76 (24.8)	137 (50.3)***	127 (67.5)***
	Zygomatic process of maxilla, fused to jugal	Minor	2 (0.6)	3 (1.1)	11 (3.6)*	60 (38.2)***
Cervical vertebra	One or more centra, not ossified	Minor	41 (17.9)	48 (16.0)	119 (44.2)***	176 (89.7)***
	Odontoid process, not ossified	Minor	17 (7.0)	15 (4.7)	109 (40.5)***	160 (80.8)***
	Ventral arch of vertebra 1, not ossified	Minor	4 (1.4)	4 (1.3)	13 (4.7)*	64 (34.2)***
	Ventral arch of	Minor	56 (20.1)	78 (25.3)	98 (36.1)***	28 (14.1)*

			No. of fetuses affected (Group mean percent)			
Dose (mg/kg/day)			0	1	10	25
Total number of litters examined			22	22	21	22
Total number of fetuses examined			273	300	276	200
Bone	**Malformation**	**Type**				
	vertebra 1, bipartite					
	One or more centra, hemicentric	Minor	0 (0)	1 (0.3)	0 (0)	20 (13.8)***
Thoracic vertebra	One or more centra, bipartite	Minor	10 (3.9)	7 (2.4)	18 (6.5)	91 (44.2)***
	One or more centra, asymmetrically ossified	Minor	42 (15.7)	66 (21.9)	110 (41.3)***	181 (91.2)***
	One or more centra, incompletely ossified	Variant	63 (24.9)	48 (16.1)	100 (37.7)***	180 (90.9)***
Lumbar vertebra	One or more centra, incompletely ossified	Minor	0 (0)	1 (0.4)	3 (1.0)	13 (7.7)***
	One or more centra, asymmetrically ossified	Minor	0 (0)	1 (0.4)	7 (2.5)**	20 (9.8)***
Sternum	One or more sternebrae, incompletely ossified	Minor	2 (0.6)	12 (3.9)	31 (10.9)***	62 (30.0)***
	5th sternebra, not ossified	Variant	0 (0)	0 (0)	12 (3.9)***	29 (11.8)***

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

Prenatal and Postnatal Development

Study title: ZD6474 (Zactima)- Dose range-finding pre- and post-natal development study by the oral route (gavage) in the rat

Key Study Findings:

- All females dosed with 25 mg/kg/day ZD6474 were euthanized on Day 24 of gestation due to the absence of parturition. At necropsy, all of these dams were found to have undergone total resorptions early in gestation, indicating that 25 mg/kg/day ZD6474 was embryotoxic in this study.
- Lower pup weight was observed in the 10 mg/kg/day group dosed from Day 6 of gestation.
- Toxicokinetics measured in dams and pups indicated the transfer of ZD6474 from the dams to the pups by milk secretion. Transfer through milk resulted in relative constant exposure as indicated by predose exposure in pups. Predose exposure in pups was approximately equivalent to the 8 hour time point.
- The mid dose of ZD6474 (10 mg/kg/day) was considered to be an appropriate high dose level for a subsequent pre- and post-natal development study in the rat.

Study no.:	AA29682
Study report location:	M4.2.3.5.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 19, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ZD6474, Batch C357/3, Purity: 99.8% w/w

Methods

Doses:	0, 1.0, 10, or 25 mg/kg/day (0, 2.1, 21, or 53 µmol/kg/day)
Frequency of dosing:	Daily from Day 6 of gestation to Day 8 of lactation or from Day 16 of gestation to Day 8 of lactation
Dose volume:	5 mL/kg/day (1 mL/kg/day for 1 mg/kg/day group)
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% w/v hydroxypropyl methylcellulose containing 0.1% w/v polysorbate 80
Species/Strain:	Rat/ Wistar
Number/Sex/Group:	6/females/group
Satellite groups:	None
Study design:	F0 females dosed from Day 6 of gestation to Day 8 of lactation, with the day of mating as Day 0 of gestation and the day of parturition as Day 0 of lactation. Additional group treated with 10 mg/kg/day dosed from Day 16 of gestation to Day 8 of lactation. Females gave birth naturally. Surviving dams and pups were necropsied on Day 8 of lactation (*post-partum*).
Deviation from study protocol:	All females administered 25 mg/kg/day were necropsied on Day 24 of gestation due to the absence of parturition in this group.
Parameters and endpoints evaluated:	**F0 females**: Clinical signs, body weight, food consumption, toxicokinetics, necropsy, duration of gestation, number of implantation sites, and live pups counted; pregnancy status, number of corpora lutea and numbers and types of uterine implantations were determined for females administered 25 mg/kg/day **F1 rats**: Number of male and female pups born (live and dead), weight, external abnormalities, necropsy, and toxicokinetics

Results

F_0 Dams:

Mortality

- All females dosed with 25 mg/kg/day (53 µmol/kg/day) were euthanized on Day 24 of gestation due to the absence of parturition. At necropsy, all of these dams were found to have undergone total resorptions early in gestation.
- Two females, one dosed with 1 mg/kg/day and one dosed with 10 mg/kg/day from Day 16 of gestation, that were found not pregnant were euthanized and necropsied on Day 27
- One female (#21) administered 10 mg/kg/day (21 µmol/kg/day) from Day 16 of gestation was euthanized with its litter on Day 2 of lactation due to abnormal nursing behavior.

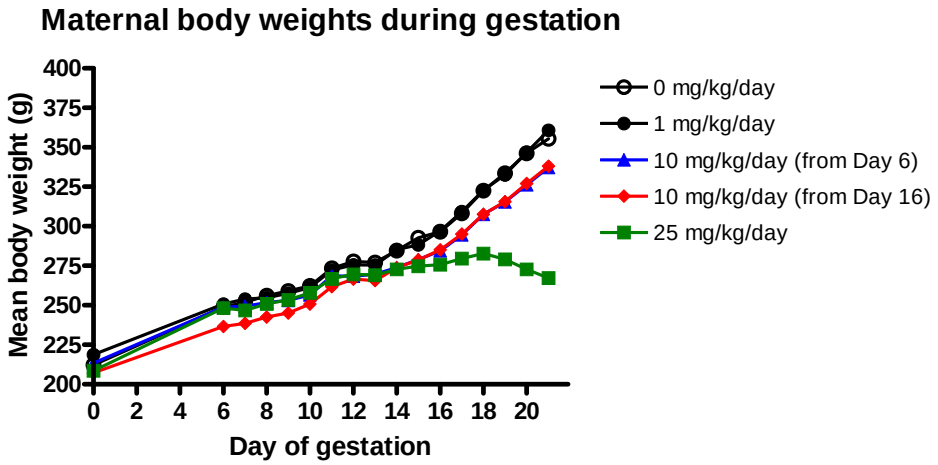
Clinical signs

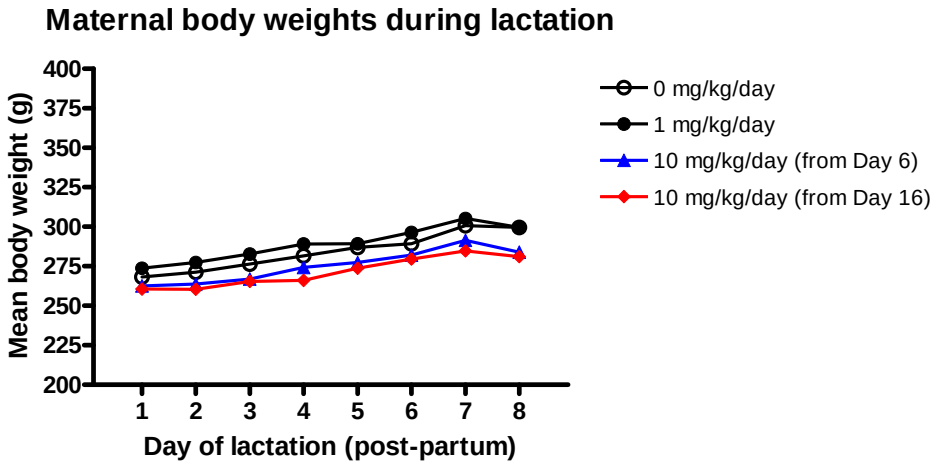
Clinical signs for the female (#21) administered 10 mg/kg/day (21 µmol/kg/day) from Day 16 of gestation euthanized with its litter on Day 2 of lactation are listed in the table below. There were no remarkable clinical signs in other females.

Clinical sign	Day of lactation
Pale and red/black vaginal discharge	1
Raised hair and subdued behavior	1-2
Pups cold to touch	2
Dam not attending to litter	2

Body weight

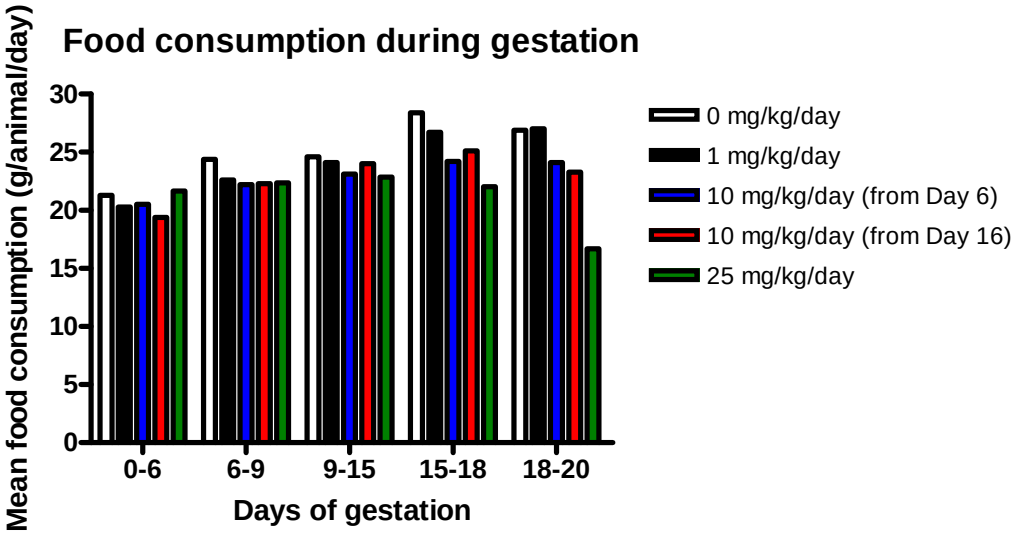
The maternal body weights of the females during gestation and lactation are shown in the figures below. Females treated with 25 mg/kg/day showed a marked reduction in weight gain compared to controls during gestation, when they all underwent total litter resorption. Both groups dosed with 10 mg/kg/day showed slightly lower body weights compared to controls during gestation and lactation.

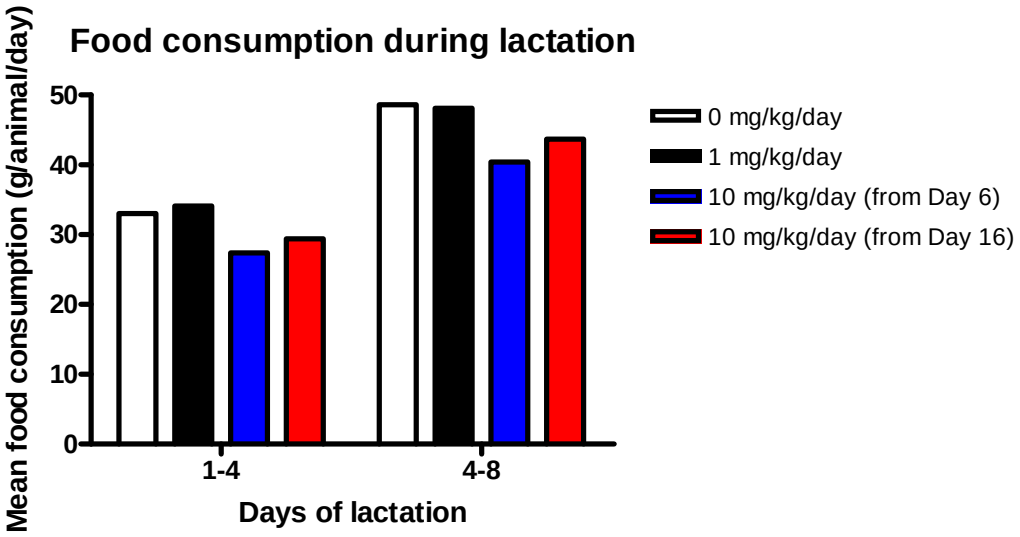




Food consumption

There was a reduction in mean food consumption compared to controls between Days 9 and 20 in females treated with 10 and 25 mg/kg/day from gestation Day 6. In females treated with 10 mg/kg/day from Day 16, mean food consumption was decreased towards the end of gestation. Food consumption was also decreased in both groups treated with 10 mg/kg/day during lactation.





Delivery, litter data, and uterine content

Results of the delivery and uterine examinations are presented in the table below. Two females, one dosed with 1 mg/kg/day and one dosed with 10 mg/kg/day from Day 16 of gestation, were not pregnant. All females dosed with 25 mg/kg/day had total resorptions early in gestation.

Dose (mg/kg/day)	0	1	10	10	25
Dosing period (Day of gestation dosing started)	Day 6	Day 6	Day 6	Day 16	Day 6
Number of females mated	6	6	6	6	6
Number of pregnant females	6	5	6	5	6
Females completing delivery	6	5	6	5	0
Duration of gestation (days)	22	22	22	22.4	NA
Number of implantations					
Total	75	59	74	60	62
mean number per female	12.5	11.8	12.3	12.0	10.3
Stillborn pups	0	0	0	0	NA
Number of live pups					
Total	70	56	63	52	NA
Mean number per female (litter)	11.7	11.2	10.5	10.4	

NA= not applicable

Necropsy observations

- Two females, one dosed with 1 mg/kg/day and one dosed with 10 mg/kg/day from Day 16 of gestation, were confirmed to not be pregnant at necropsy
- Dark fluid in the urinary bladder and dilatation was observed in the female (#21) administered 10 mg/kg/day (21 µmol/kg/day) from Day 16 of gestation euthanized with its litter on Day 2 of lactation.

Toxicokinetics

Blood samples were collected from dams on Day 7 of lactation at 8 hours after dosing in the control (0 mg/kg/day; 0 µmol/kg/day) group. Samples were collected at 2, 8, 12, and 24 hours after dosing in the 1 mg/kg/day (2.1 µmol/kg/day), 10 mg/kg/day (21 µmol/kg/day; Day 6 of gestation to Day 8 of lactation; Group 3), and 10 mg/kg/day (21 µmol/kg/day; Day 16 of gestation to Day 8 of lactation; Group 4) groups. No plasma samples could be collected from the group dosed with 25 mg/kg/day, since they were necropsied on Day 24 of gestation. Samples were also collected from suckling pups in all groups with pups on Day 8 of lactation. Half of the pups in each litter were pooled to provide a sample before dosing of the dams on Day 8 and half were pooled to provide a sample at 8 hours post-dose.

- Systemic exposure to ZD6474 was demonstrated in all female rats dosed with ZD6474 on Day 7 of lactation and in all corresponding pooled pup samples on Day 8 of lactation
- C_{max} and $AUC_{(0-24)}$ values increased proportionally with an increase in dose from 1 to 10 mg/kg/day (2.1 to 21 µmol/kg/day)
- Mean plasma concentrations from pooled samples in suckling pups 8 hours after dosing on Day 8 of lactation were 12.0, 15.5, and 17.8% of the C_{max} levels observed in dams dosed with 1 mg/kg/day, 10 mg/kg/day (Day 6 of gestation to Day 8 of lactation), and 10 mg/kg/day (Day 16 of gestation to Day 8 of lactation) respectively and indicate the transfer of ZD6474 from the dams to the pups by milk secretion.

Mean ± SE plasma concentrations (µmol/L) of ZD6474 obtained in females rats on Day 7 of lactation and pups on Day 8 of lactation (excerpted from sponsor's submission)

Day	Time after dose (h)	Group 1 0 µmol/kg/day	Group 2 2.1 µmol/kg/day	Group 3 21 µmol/kg/day	Group 4 21 µmol/kg/day
Day 7 Dams	2	NS	0.099 ± 0.010	1.20 ± 0.187	1.14 ± NC
	8	NQ	0.159 ± NC	1.56 ± 0.052	1.38 ± NC
	12	NS	0.110 ± 0.010	1.19 ± 0.190	0.968 ± NC
	24	NS	0.086 ± NC	1.10 ± 0.051	0.796 ± NC
Day 8 Pups	Pre-dose	NQ	0.017 ± 0.002	0.241 ± 0.024	0.199 ± 0.029
	8	NQ	0.019 ± 0.002	0.241 ± 0.024	0.246 ± 0.032

NQ Non-quantifiable ie, below the limit of quantification of 0.011 umol/L
NC Not calculable
NS No sample
Please note that for the dams the mean concentrations are based on 3 animals per time-point. Where there were only 2 animals per time-point the SE could not be calculated.
All pup samples were pooled per litter at each time-point.

A summary of the toxicokinetic parameters for ZD6474 in female rats sampled on Day 7 of lactation (excerpted from sponsor's submission)

Parameter	Group 2 2.1µmol/kg/day	Group 3 21 µmol/kg/day	Group 4 21 µmol/kg/day
C_{max} (µmol/L)	0.159	1.56	1.38
t_{max} (h)	8	8	8
$AUC_{(0-24)}$ (µmol.h/L)	2.67	29.8	24.8

C_{max} Maximum plasma concentration
t_{max} Time of maximum plasma concentration
$AUC_{(0-24)}$ Area under the plasma concentration time curve up to 24 hours
1 µmol/L = 475.36 ng/mL

F1 pups:

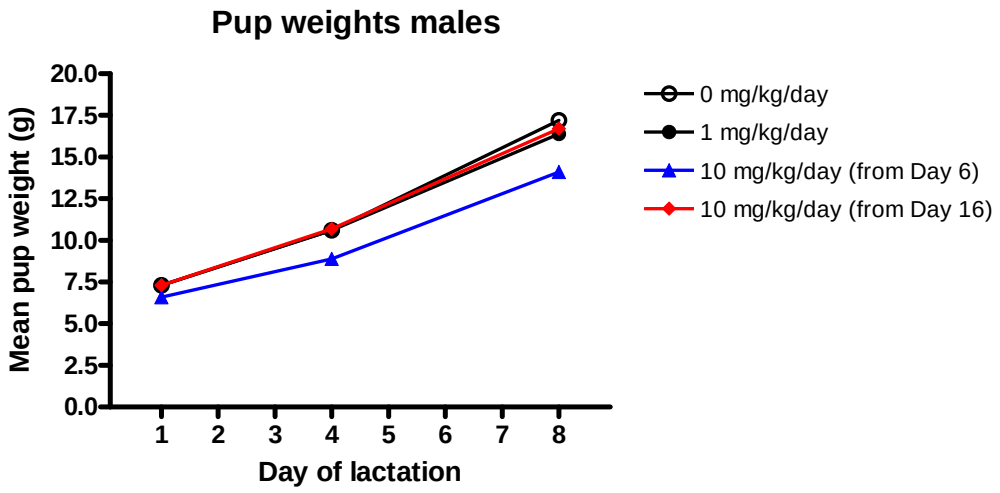
Survival

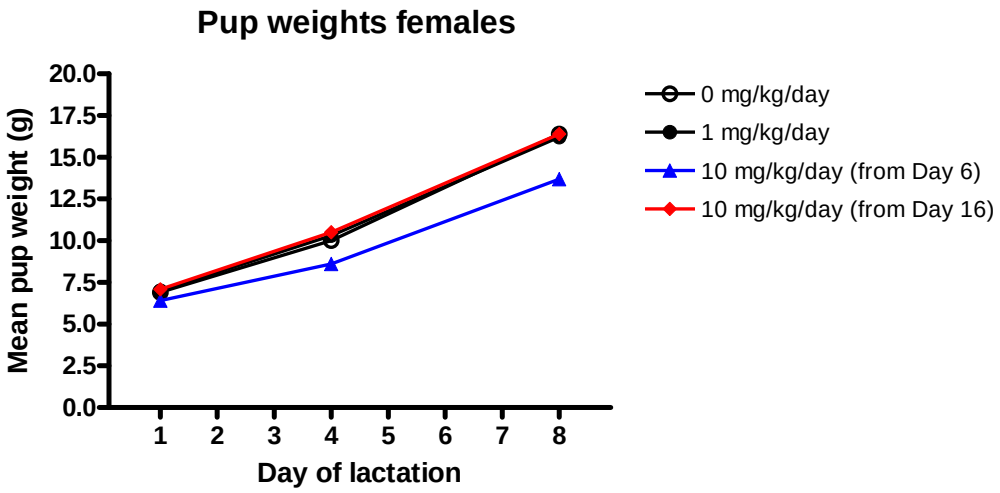
Dose (mg/kg/day)	0	1	10	10
Dosing period (Day of gestation dosing started)	Day 6	Day 6	Day 6	Day 16
Number of live pups				
Total	70	56	63	52
Mean number per female (litter)	11.7	11.2	10.5	10.4
Pups dying, missing and/or cannibalized Days 1-4	2	0	1	0
Euthanized for ethical reasons	0	0	0	11*
Pups surviving until Day 8 of lactation				
Total	68	56	62	41*
Mean number per female (litter)	11.3	11.2	10.3	10.25*
Sex distribution Day 0				
Males	33	26	24	28
Females	37	30	39	24
% male pups	47.1	46.4	38.1	53.8
Sex distribution Day 8				
Males	32	26	24	21*
Females	36	30	38	20*
% male pups	47.1	46.4	38.7	51.2*

* The entire litter for female #21 was euthanized on Day 2 along with the female due to abnormal nursing behavior

Body weight

The mean pup weight for both males and females was lower in pups in the 10 mg/kg/day group dosed from Day 6 of gestation compared to controls.





Necropsy

Unremarkable

Study title: ZD6474 (Zactima)- Pre- and post-natal development study by the oral route (gavage) in the rat

Key Study Findings:

- ZD6474 was maternally toxic with lower mean body weight gain during gestation and decreased food consumption at both 1 and 10 mg/kg/day
- Increased percentage of pre-birth loss in 10 mg/kg/day group treated from Day 6 of gestation
- Decreased number of pups with treatment of 1 and 10 mg/kg/day ZD6474
- Decreased pup survival at Day 4 *post-partum* in all groups treated with ZD6474; pup mortality was highest in the 10 mg/kg/day group treated from Day 6 of gestation

- Reduced post-natal pup growth was associated with a delay in physical development in pups in the 10 mg/kg/day groups
- No effects of ZD6474 were observed in the behavioral tests, mating performance, fertility, and caesarian (uterus content) for the F_1 generation

Study no.:	AA32728
Study report location:	M4.2.3.5.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 13, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ZD6474, Batch C357/6, Purity: 99.3% w/w

Methods

Doses:	0, 1.0, or 10 mg/kg/day (0, 2.1, or 21 µmol/kg/day)
Frequency of dosing:	Daily from Day 6 or Day 16 of gestation until weaning at Day 21 *post-partum*
Dose volume:	5 mL/kg/day (1 mL/kg/day for 1 mg/kg/day group)
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% w/v hydroxypropyl methylcellulose containing 0.1% w/v polysorbate 80
Species/Strain:	Rat/ Wistar
Number/Sex/Group:	25/females/group
Satellite groups:	None
Study design:	F0 females dosed with ZD6474 (0, 1.0, or 10 mg/kg/day) from Day 6 of gestation to Day 21 *post-partum*, with the day of mating as Day 0 of gestation and the day of parturition as Day 0 of lactation or *post-partum*. Additional group treated with 10 mg/kg/day dosed from Day 16 of gestation to Day 21 *post-partum*. Females gave birth naturally. Litters were culled to 4 males and females per litter on Day 4 *post-partum*. F0 females were necropsied after the weaning of F1 pups. After weaning of the F1 pups on Day 21 *post-partum*, one male and female pup per litter were selected to yield 25 males and females in each group for the post-weaning behavioral tests and mating. F1 animals were maintained for approximately 8 weeks after weaning and at 11 weeks of age were mated for up to 3 weeks (one male and one female from the same group). F1 females were necropsied on Day 13 of gestation in mated females or 3 weeks after the first day of the mating period in females with undetected mating. F1 males were necropsied after the F1 females.

Deviation from study protocol:

Parameters and endpoints evaluated: **F0 females**: Clinical signs, body weight, food consumption, necropsy, duration of gestation, number of implantation sites, and live pups counted
F1 rats: Number of male and female pups born (live and dead), weight, external abnormalities, physical development (onset of pinna unfolding, incisor eruption and eye opening), behavioral and functional tests (surface righting reflex, gripping reflex, papillary reflex, and auditory reflex), post-weaning developmental and behavioral tests (day of vaginal opening in females, day of balano preputial skinfold cleavage, water maze test, and open field test), ophthalmology, mating, necropsy, and uterine examinations in females (pregnancy status, number of corpora lutea, and number and distribution of intrauterine implantations and intrauterine deaths)

Results

F_0 females:

F_0 Mortality

The following animals were euthanized early, including those females that were not pregnant and those with total litter death.

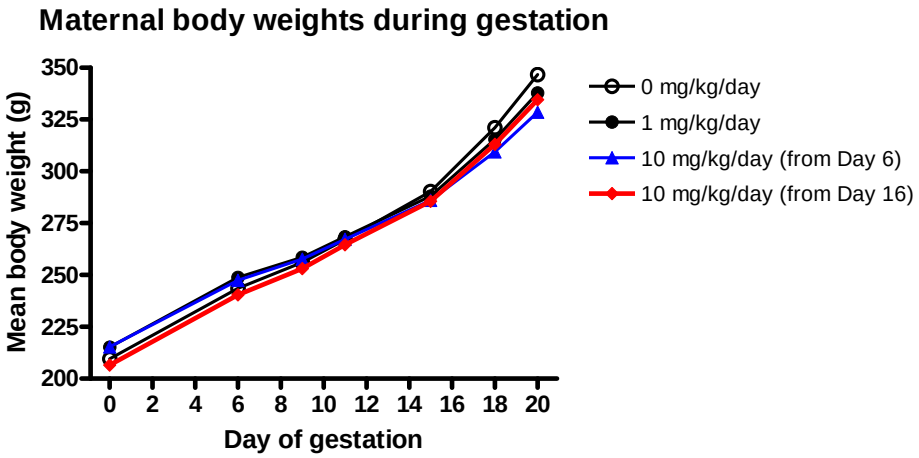
Group	Animal #	Day euthanized	Reason for euthanization
Control (0 mg/kg/day)	2	Day 26 of gestation	No viable fetuses
	17	Day 26 of gestation	Not pregnant
1 mg/kg/day	35	Day 26 of gestation	Not pregnant
	49	Day 8 *post-partum*	No visible milk, total litter death (only had 1 pup)
10 mg/kg/day from Day 6 of gestation	76	Day 26 of gestation	Not pregnant
	83	Day 1 *post-partum*	Total litter death
	89	Day 26 of gestation	No viable fetuses
	92	Day 26 of gestation	Not pregnant
10 mg/kg/day from Day 16 of gestation	52	Day 26 of gestation	Not pregnant

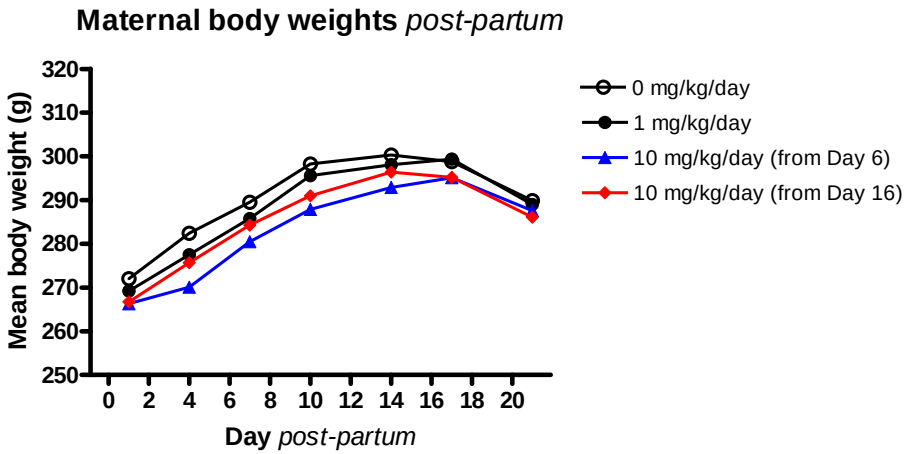
F_0 Clinical signs

- Three females administered 10 mg/kg/day from Day 6 of gestation (# 91, 93, and 97) and one female administered 10 mg/kg/day from Day 16 of gestation (# 59) had lame limbs on occasion from Day 9-19 of lactation

F_0 Body weight

The mean maternal body weights during gestation and *post-partum* are shown in the figures below and the mean body weight gains from Days 6-20 of gestation and Days 1-4 *post-partum* for each group are listed in the table below. The overall mean body weight gain during gestation was significantly lower in females treated with 1 and 10 mg/kg/day ZD6474 than controls. Days 1-4 *post-partum*, weight gain was decreased in the 10 mg/kg/day group treated from Day 6 compared to controls.





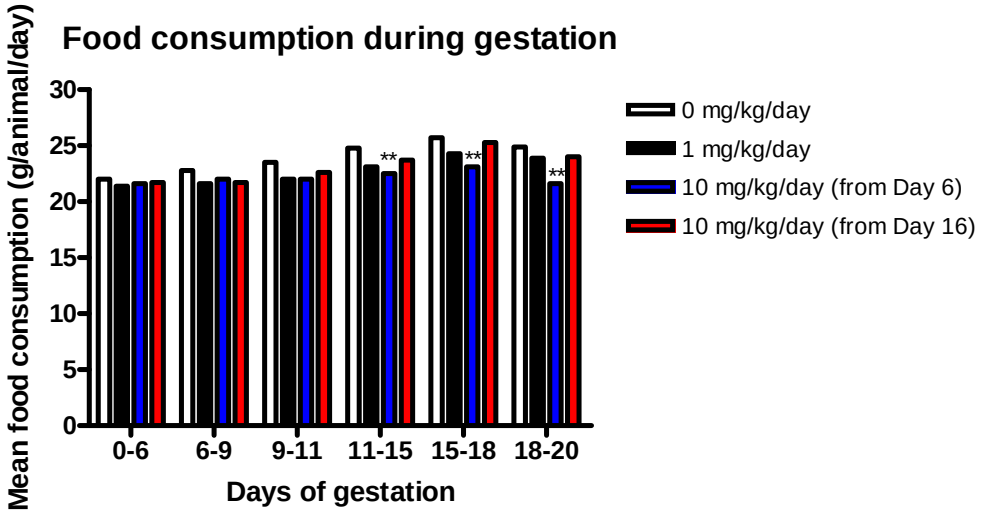
Body weight gain

Mean body weight gain (g)				
Dose (mg/kg/day)	0	1	10	10
Dosing period (Day of gestation dosing started)	Day 6	Day 6	Day 6	Day 16
Days 6-20 of gestation	103.2	89.0**	94.2*	81.3**
Day 1-4 *post-partum*	10.3	8.3	9.0	4.6

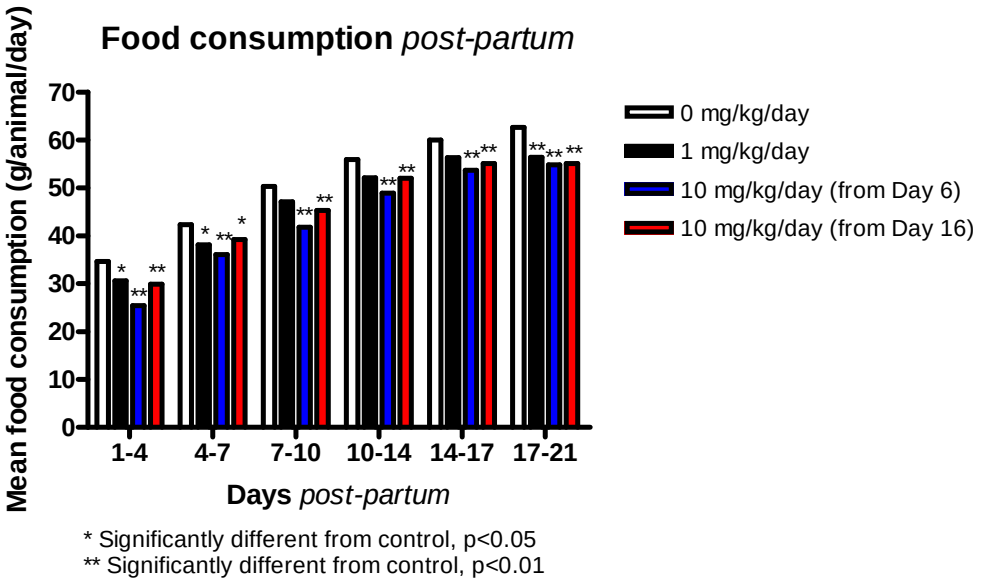
* p<0.05 ** p<0.01

F_0 Food consumption

The mean food consumption was significantly lower in the 10 mg/kg/day group treated from Day 6 of gestation than the control group from Day 11 of gestation until weaning. In the 1 mg/kg/day group and the 10 mg/kg/day group treated from Day 16 of gestation, food consumption was not affected during gestation but was lower than the control group *post-partum* during lactation. Mean food consumption during gestation and *post-partum* for each group is shown in the figures below.



** Significantly different from control, p<0.01



F_0 Delivery, litter data, and uterine content

Results of the delivery and uterine examinations are presented in the table below. One female in each of the control, 1 mg/kg/day, and 10 mg/kg/day treated from Day 16 groups and two females in the 10 mg/kg/day group treated from Day 6 were not pregnant. One female in the control group and one female in the 10 mg/kg/day group treated from Day 6 of gestation had a single uterine implantation site with no viable fetuses.

Dose (mg/kg/day)	0	1	10	10
Dosing period (Day of gestation dosing started)	Day 6	Day 6	Day 6	Day 16
Number of females mated	25	25	25	25
Number of pregnant females	24	24	23	24
Females completing delivery	23	24	22	24
Females with stillborn pups	0	0	3	1
Duration of gestation (days)	22	22	22.1	21.9
Number of implantations				
Total	287	246	245	265
mean number per litter	12.5	10.7	11.1	11.0
Mean prebirth loss (%)	8%	10%	15%	10%
Number of pups				
Total	265	234	208	238
Mean number per female (litter)	11.5	9.8*	9.5*	9.9
Liveborn	265	234	205	237
Stillborn	0	0	3	1
Livebirth index	100%	100%	98.6%	99.6%

* p<0.05 ** p<0.01

F_0 Necropsy observations

Unremarkable

F_1 pups/rats

F_1 Pup survival

Dose (mg/kg/day)	0	1	10	10
Dosing period (Day of gestation dosing started)	Day 6	Day 6	Day 6	Day 16
Number of live pups				
Total	265	234	205	237
Mean number per female (litter) at birth	11.5	9.8*	9.3*	9.9
Mean number per female (litter) Day 4	11.5	9.7*	9.2**	9.8*
Culled Day 4	81	56	37	49
Not culled	184	178	168	188
Pups dying, missing and/or cannibalized				
Days 1-4	0	1	12**	3
Days 5-21	0	1	0	0
Pups surviving until Day 21				
Total	184	176	156	185
Mean number per female (litter)	8.0	7.3	7.4	7.7
Sex distribution Day 0				
Males	124	112	106	97
% male pups	46.8%	47.9%	53.5%	41.1%
Sex distribution Day 21				
Males	89	90	85	84
% male pups	48.4%	51.1%	54.5%	45.4%

* $p<0.05$ ** $p<0.01$

F_1 Pup clinical signs

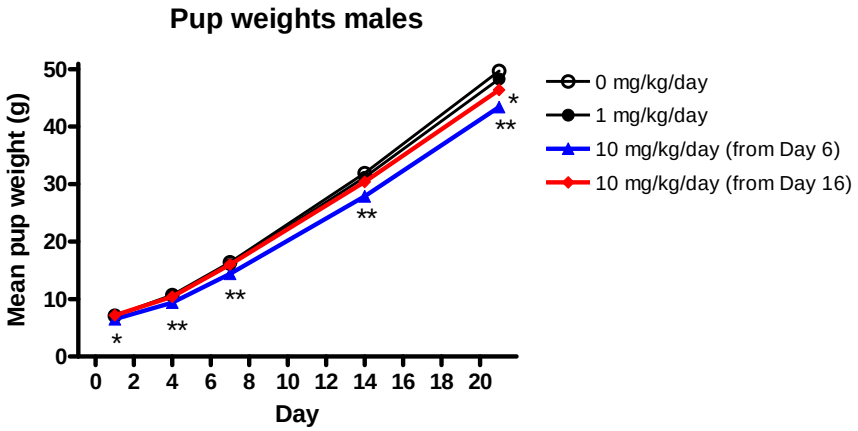
Clinical signs observed in pups

Clinical signs		No. of pups affected (No. of litters affected)			
Dose (mg/kg/day)		0	1	10	10
Dosing period (Day of gestation dosing started)		Day 6	Day 6	Day 6	Day 16
Clinical sign	Days observed				
Thin	1-7	-	2 (2)	14 (2)	3 (3)
Cold to touch	1, 6-7	-	1 (1)	13 (1)	-
Weak and paralyzed hindlimbs	14-21	-	1 (1)	-	-
Slight palor	5-8	-	-	8 (1)	-
Pale	9-10	-	-	7 (1)	-

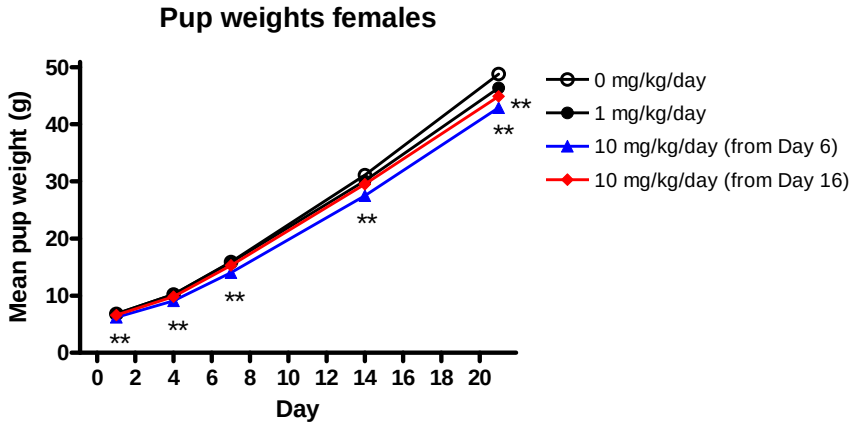
- = clinical sign not observed in this group

F_1 Pup body weight

The mean pup weight for both males and females was significantly lower in pups in the 10 mg/kg/day group dosed from Day 6 of gestation compared to controls throughout the lactation period (Days 1-21). Pup weights were also significantly lower in both males and females in the 10 mg/kg/day group dosed from Day 16 of gestation compared to controls at weaning on Day 21. The mean pup weights for male and female pups are shown if the figures below.



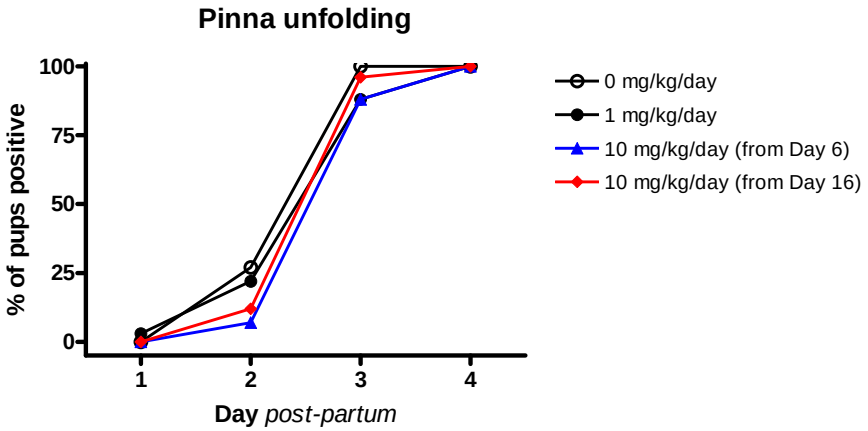
* Significantly different from control, p<0.05
** Significantly different from control, p<0.01

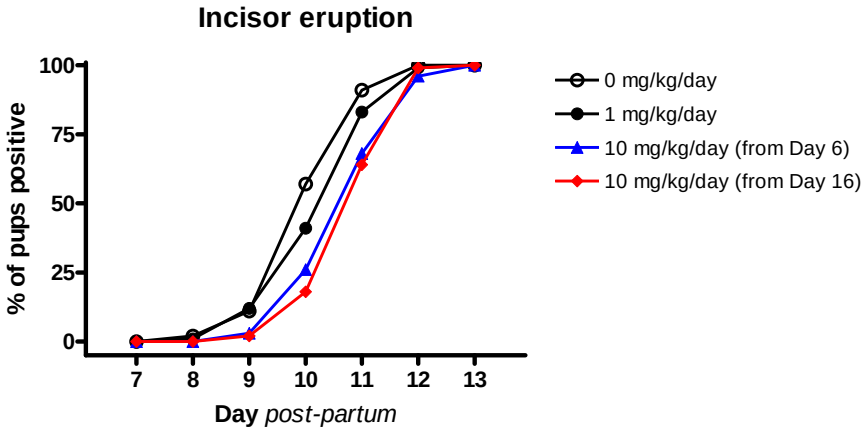


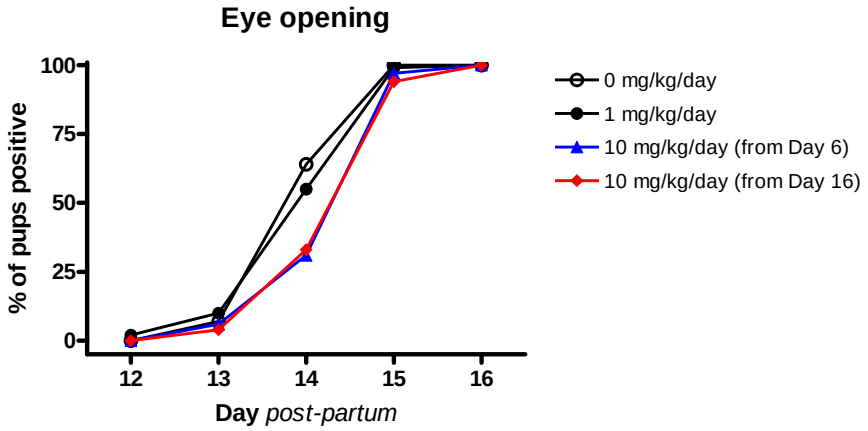
* Significantly different from control, p<0.05
** Significantly different from control, p<0.01

F_1 Pup physical and functional development

Pinna unfolding, incisor eruption, and eye opening were slightly delayed in pups in both 10 mg/kg/day groups. Pup reflexes (surface righting on Day 8, gripping on Day 17, and the pupil and auditory responses on Day 21) were not affected by treatment of ZD6474.







F_1 Generation

According to the study report, the start of the F1 generation, nominally Day 0, was equivalent to animals at approximately 3 weeks of age. The following data are from those F_1 pups that were selected to yield 25 males and females in each group for the post-weaning behavioral tests and mating.

F_1 Mortality

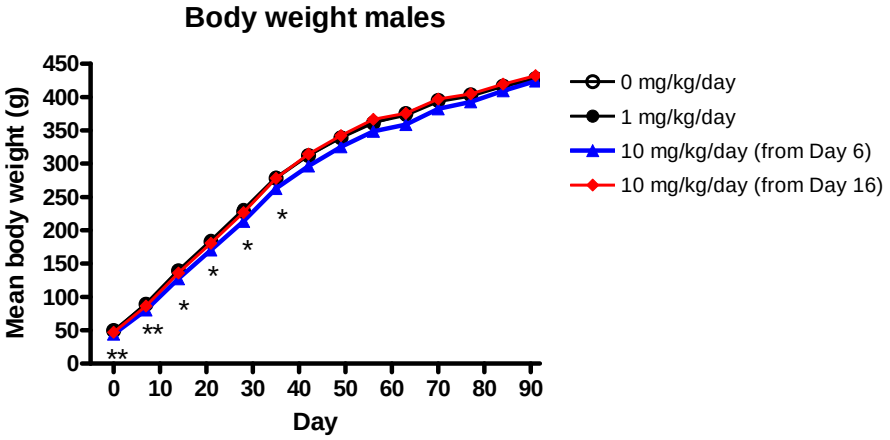
- One female from the 10 mg/kg/day group treated from Day 6 of gestation was euthanized moribund on Day 19. Histopathological examinations indicated hydrocephaly, which most likely contributed to the poor health of this animal.

F_1 Clinical signs

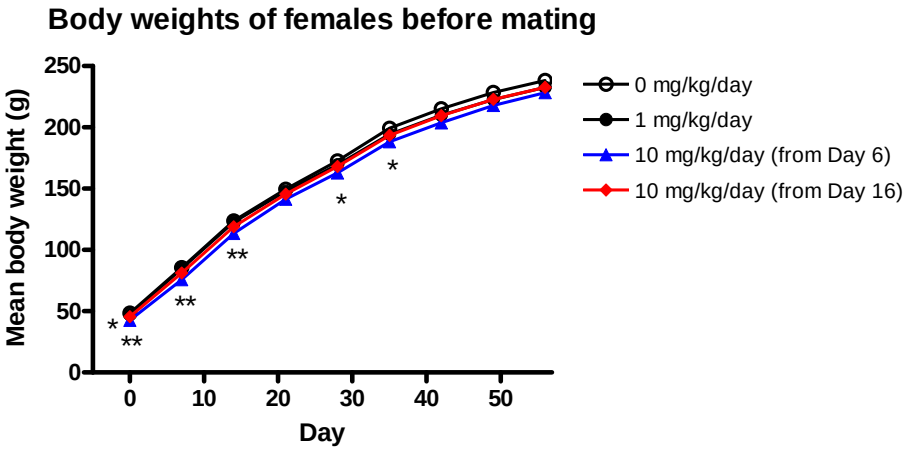
Unremarkable

F_1 Body weights

Body weights continued to be significantly lower in males and females in the 10 mg/kg/day group treated from Day 6 of gestation compared to controls up to Day 35 (56 days of age). Mean body weights for males and for females prior to mating are shown in the figures below, with day numbers refering to days after start of F1 period at 3 weeks of age. During gestation, the weight gain was comparable for all groups.



* Significantly different from control, p<0.05
** Significantly different from control, p<0.01



F_1 Sexual maturation

There was slight but significant delay in vaginal opening in females in the 10 mg/kg/day group treated from Day 6 of gestation.

	Mean *post-partum* day of occurrence			
Dose (mg/kg/day)	0	1	10	10
Dosing period (Day of gestation dosing started)	Day 6	Day 6	Day 6	Day 16
Vaginal opening	32	33	35**	33
Balano preputial skinfold cleavage	44	44	45	45

** p<0.01

F_1 Behavioral tests

Learning and memory (Water maze test)

The water maze test (E-shaped maze) was performed at approximately 6 and 7 weeks of age. Three trials were conducted with an exit ramp placed at the end of the right-hand channel at 6 weeks of age. Time taken to find the exit and the distance swum in each channel were recorded. One trial was repeated under the same conditions one week later. The exit ramp was then moved to the left hand channel and another three trials were performed in the same day. There were no differences between groups in these tasks, indicating that swimming ability and learning and memory were not affected by treatment.

Locomotor activity (Open field)

The open field test was performed at 7 weeks of age using the animals that had already experienced the water maze test. Motor activity was divided into 3 categories, ambulatory activity, small movements, and inactivity. The proportion of time spent engaged in each type of activity and the total distance travelled by the rat were calculated. There were no differences between groups in these tasks, indicating that motor activity and exploratory behavior were not affected by treatment.

F_1 Ophthalmology

Unremarkable

F_1 Reproduction

Mating and fertility

Dose (mg/kg/day)	0	1	10	10
Dosing period (Day of gestation dosing started)	Day 6	Day 6	Day 6	Day 16
Number of females:				
Paired	25	25	24	25
Inseminated	25	25	24	25
Pregnant	25	24	22	25
Pre-coital interval-days				
Mean	2.28	2.35	3.22	2.44
Number mated	25	23[(2)]	23[(1)]	25
Copulation index (%)	100	100	100	100
Fertility index (%)	100	96	92	100

[(1)] Mating not detected for one pair of rats
[(2)] Mating not detected for two pairs of rats

Caesarean data

Dose (mg/kg/day)	0	1	10	10
Dosing period (Day of gestation dosing started)	Day 6	Day 6	Day 6	Day 16
Number of pregnant females	25	24	22	25
Number of corpora lutea				
Total	364	362	315	366
Mean per female	15	15	14	15
Number of implantations				
Total	329	317	277	327
% of corpora lutea	90.4	87.6	87.9	89.3
mean number per female	13	13	13	13
Viable embryos				
Total	315	299	259	316
Mean per female	13	12	12	13
% of implantations				
Total	95.7	94.3	93.5	96.6
Mean per female	96	95	93	97
Preimplantation loss				
Total	35	45	38	39
% of corpora lutea	9.6	12.4	12.1	10.7
Mean per female	1	2	2	2
Number of females affected	17	16	16	16
Postimplantation loss				
Total	14	18	18	11
% of implantations	4.3	5.7	6.5	3.4
Mean per female	1	1	1	0
Number of females affected	12	4	11	9

10 Special Toxicology Studies

Study title: Cytotoxicity assay *in vitro* with BALB/C3T3 cells: Neutral red (NR) assay with ZD6474 at simultaneous irradiation with artificial sunlight

Key study findings: **Phototoxic potential was observed after treatment of BALB/C3T3 cells with ZD6474**

Study no.: TKU9
Study report location: M4.2.3.7.7
Conducting laboratory and location: (b) (4)

Analytical test site:
Formulation and Analytical Support Group
AstraZeneca UK Limited
Safety Assessment UK Alderley
Alderley Park
Macclesfield
Cheshire SK10 4TG
U.K.

Date of study initiation: August 10, 2001 (Date of protocol and German translation)
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: ZD6474, batch # ADM65949A99, purity: 99%
Formulation/vehicle: DMSO, final concentration of 1% (v/v) in culture medium

Methods

Cells: BALB/C3T3 c31 cell line
Concentrations: Without irradiation: 0.46, 1.37, 4.12, 12.35, 37, 111, 333, and 1000 µg/mL
With irradiation: 0.046, 0.147, 0.41, 1.23, 3.70, 11.1, 33.3, and 100 µg/mL
Negative controls: Cultures treated with EBSS containing 1% DMSO without ZD6474, either stored in the dark during exposure or irradiated for 50 minutes during exposure
Positive control: Chlorpromazine, 6.25-200 µg/mL without irradiation and 0.125-4.0 µg/mL with irradiation

To test the phototoxicity potential of ZD6474, the neutral red assay was conducted with BALB/C3T3 cells, in which cells were treated with various concentrations of ZD6474 in the absence and presence of artificial sunlight (wave-length >320 nm). After one hour of pre-incubation with one of the concentrations of ZD6474 or the positive control, the cells were irradiated with artificial sunlight for 50 minutes with 1.7 mW/cm^2 UVA, resulting in a radiation

dose of J/cm^2 UVA. Parallel cultures were kept in the dark for 50 minutes. The cytotoxicity response curve of the test groups were compared, and the EC_{50} values were determined to calculate a photo-irritancy factor (PIF) to determine any phototoxicity. Evaluation criteria used: If PIF <5: No phototoxic potential predicted. If PIF $\geq$5: Phototoxic potential predicted.

$$PIF = \frac{EC_{50}\text{ (without UV)}}{EC_{50}\text{ (with UV)}}$$

Results:

Drug	EC_{50} Without UV (µg/mL)	EC_{50} With UV (µg/mL)	PIF
ZD6474	9.29	0.47	19.9
Chlorpromazine (positive control)	10.99	0.35	31.5

The PIF of ZD6474 was 19.9 and based on the evaluation criteria (PIF $\geq$5), ZD6474 is determined to have phototoxic potential

11 Integrated Summary and Safety Evaluation

Pharmacology

In vitro and *in vivo* studies were conducted to investigate the pharmacologic and anti-tumor activity of vandetanib. Vandetanib was tested in multiple *in vitro* recombinant enzyme assays to evaluate the potency and selectivity of the compound by determining the IC_{50} values for various protein kinases. In assays containing 96 protein kinases, the IC_{50} values ranged from 3.6 x 10^{-8} M to >10^{-4} M. The IC_{50} values for many kinases were lower than 1.8 µM, the C_{max} value in the pivotal clinical study (Study 58), with the strongest inhibitions for BRK (IC_{50}: 36 nM), VEGF-R2 (KDR; IC_{50}: 38 nM), and EGFR (IC_{50}: 43 nM). Multiple studies were conducted for RET protein kinase and the IC_{50} values ranged from 2.0 x 10^{-8} M to 5.20 x 10^{-7} M. Based on these assays vandetanib is a kinase inhibitor with activity at multiple kinases. While vandetanib does inhibit vascular endothelial growth factor receptors (VEGFR), epidermal growth factor receptor (EGFR), and RET kinase, other kinases are also being inhibited. The pharmacologic effect of vandetanib may not be solely attributed to these targets. Other possible targets for vandetanib that were indentified in high-throughput screens include BRK, TIE2, and members of the EPH kinase and Src family tyrosine kinase families. Given the steady state levels achieved in the clinical trial the role of these kinases in the pharmacodynamic effects of vandetanib should be considered. In kinase assays, the N-desmethyl metabolite of vandetanib was found to have very similar inhibitory activity to vandetanib for inhibition of VEGF, EGF, and bFGF. Additionally, the N-desmethyl metabolite of ZD6474 demonstrated similar inhibitory activity to vandetanib for inhibition of KDR (VEGF-2), Flt-1 (VEGF-1), and EGFR tyrosine kinases. The N-oxide metabolite of vandetanib was less potent than vandetanib and the N-desmethyl metabolite for KDR tyrosine kinase, but had similar inhibitory activity to vandetanib for Flt-1 and EGFR tyrosine kinases. Based on this data, the pharmacological classification of vandetanib is a kinase inhibitor.

In published *in vitro* studies (Wu et al., 2007), vandetanib dose-dependently inhibited phosphorylation of VEGFR and EGFR in lung adenocarcinoma cells (H441) and endothelial cells (MLEC). Vandetanib (1.25µM) induced apoptosis in MLEC and H441 cells and blocked the protective effects of EGF and VEGF. The direct effect of ZD6474 on migration, invasion, and proliferation of endothelial cells and tumor cells (H441) was studied *in vitro* by treating cells with ZD6474. Results indicate that ZD6474 dose-dependently inhibited migration, invasion, and proliferation of HUVEC, HPAEC, MLEC, and H441 cells, however, it is important to note that these effects on migration and proliferation are an effect of ZD6474 since the assay was conducted without VEGF or EGF stimulation.

In vivo effects of vandetanib were demonstrated using angiogenesis assays and in human tumor xenograft models in nude mice. In an *in vivo* study, the effect of vandetanib on VEGF165-induced angiogenesis was studied with matrigel plugs in athymic nude mice. Female athymic nude mice were injected subcutaneously with control preparations of matrigel or matrigel containing VEGF165 (150 ng/mL). Mice receiving the matrigel containing VEGF165 were administered vehicle or 12.5, 25, or 50 mg/kg vandetanib by oral gavage daily. VEGF165 induced vascularization, and mice treated with vandetanib showed reduced vascularization. Treatment with vandetanib decreased the number of vessel nodes and vessel length compared to vehicle in mice. Therefore, treatment with vandetanib showed a dose dependent inhibition of VEGF-induced angiogenesis. In a published study (Wu et al., 2007), a Miles assay of vascular permeability was conducted in which PBS, H441 cell conditioned medium, VEGF (10 ng/mL), or EGF (10 ng/mL) were injected into the skin of mice treated with ZD6474 for 14 days following intravenous injection of 150 µL of 0.5% Evens blue. Treatment with vandetanib decreased vascular permeability induced by H441 cell conditioned medium, VEGF, or EGF.

Vandetanib has also been shown to inhibit tumor growth in a variety of human cancer xenografts including Calu-6 human lung and PC-3 prostate cancer xenografts of various sizes. One study assessed the effects of vandetanib on the expression of pVEGFR-2 and pEGFR levels in paraffin-embedded sections of human lung or colon tumor xenografts from mice treated with vehicle or 12.5 and 50 mg/kg (37.5, or 150 mg/m^2) vandetanib for 1, 2, 3, and 5 doses. A dose of 150 mg/m^2 vandetanib inhibited VEGFR2 phosphorylation in the Calu-6 lung xenograft model following 1, 2, 3, and 5 doses (4, 28, 52, and 100 hours after the first dose, respectively). Vandetanib also inhibited pEGFR staining in the LoVo human colon tumor xenograft model at 150 mg/m^2 and the decrease was observed after 1 dose and was maintained throughout the study. These studies provide some evidence that vandetanib has *in vivo* activity against VEGF and EGFR, however, vandetanib was not tested in a model with thyroid cancer cells.

The effects of vandetanib (0, 50, or 100 mg/kg/day; 0, 150, or 300 mg/m^2) on wound healing in mice was determined by measuring breaking strength in a murine model of cutaneous wound healing in a published study (Ko et al., 2005). Results indicated that wound breaking strength was dose-dependently decreased in mice treated with vandetanib compared to controls at both 7 and 28 days after wounding. This suggests that vandetanib slows but does not prevent wound healing. No wound healing complications have been reported in patients, therefore, this non-clinical finding has been reported in the label.

Safety Pharmacology

Both *in vivo* and *in vitro* safety pharmacology studies were conducted to assess the effects of vandetanib on neurological, cardiovascular, pulmonary, renal, and gastrointestinal functioning. Neurological functioning was assessed in both rats and mice. A functional battery was assessed 4 hours after single doses of vandetanib (0, 40, 200, or 1000 mg/kg; 0, 240, 1200, or 6000 mg/m^2) in male rats. The mid and high doses (1200 and 6000 mg/m^2) resulted in a lowered body weight gain, slow pupil response, and reduced activity in open field assessments. Decreased landing foot splay, reduced grip strength, and piloerection were also observed at 6000 mg/m^2. Vandetanib reduced approach response in rats at all doses. A multi-observation test conducted in male mice following a single dose of vandetanib (0 or 50 mg/kg; 0 or 300 mg/m^2) resulted in no changes in behavior. Based on the findings observed in the rat, vandetanib appears to impair aspects of autonomic and neuromuscular function. Although adrenergic and histaminergic receptors were preliminarily identified as targets when the radioligand receptor and enzyme screening assay was conducted, further testing indicated that there was not vandetanib activity at relevant concentrations. It is not clear through what mechanism the autonomic and neuromuscular toxicity is occurring, however, it does not appear to be through primary receptor binding of vandetanib.

The effects of vandetanib on cardiovascular functioning were assessed using multiple *in vitro* and *in vivo* and studies including hERG channel and Purkinje fiber assays and telemetry studies in rats and anesthetized dogs. The potential for vandetanib or its N-desmethyl or N-oxide metabolites to delay repolarization and prolong the QT interval was evaluated in hERG-expressing HEK cells. Vandetanib inhibited the hERG channel with a $-p[IC]_{50} = 6.4 \pm 0.1$, which equates to 0.4 µM or 190 ng/mL. The N-desmethyl metabolite inhibited the hERG channel with a $-p[IC]_{50} = 5.9 \pm 0.1$, which equates to 1.3 µM or 600 ng/mL, and the N-oxide metabolite inhibited the hERG channel with a $-p[IC]_{50} = 5.4 \pm 0.1$, which equates to 4.0 µM or 2038 ng/mL. The inhibition produced by the N-desmethyl and N-oxide metabolites is 3- and 10-fold less than vandetanib, respectively, in this assay. The effect of vandetanib on transmembrane action potential in isolated canine purkinje cells was also tested. Concentrations of 1 and 10 µM lengthened the action potential duration at 50, 70 and 90% of repolarization in canine isolated cardiac Purkinje cells at stimulation frequencies of 0.33 and 1 Hz under normal and low potassium Tyrode's buffers. The prologation was greater at 0.33 Hz stimulation and in low-potassium buffer. Vandetanib did not affect the resting potential, action potential amplitude or maximal rate of depolarization (Vmax) at any dose tested. Vandetanib appeared to have affected the cardiac potassium channels under the conditions of this assay. Interestingly, this was not predicted by radioligand binding assays. An *in vivo* telemetry study was conducted in rats to evaluate the effects on systolic and diastolic blood pressure. Mean systolic and diastolic blood pressures were dose-dependently increased with single oral doses of 12.5 and 50 mg/kg (75 or 300 mg/m^2) vandetanib. Seven daily treatments with 75 mg/m^2 caused greater increases than a single dose, suggesting that daily dosing had a cumulative effect on blood pressure in rats. Changes in blood pressure with vandetanib may be linked to effects of VEGF inhibition on vasculature. In a hemodynamic study in dogs, blood pressure, heart rate, left ventricular pressure, lead II ECG, and blood flows were monitiored in anesthetized dogs after intravenous administration of ascending doses of vandetanib (0.2, 0.67, 2.0, 6.7, and 13.4 mg/kg; 4, 13.4, 40, 134, and 238 mg/m^2). Vandetanib caused both QTcV prolongation at doses $\geq$ 40 mg/m^2 and dose-dependent increases in T wave amplitude and polarity at doses $\geq$ 134 mg/m^2. In another

study in anesthetized dogs, similar findings on QTcV interval and T wave amplitude were shown along with increases in blood pressure at doses of 5.19, 20.75, and 27.5 mg/kg (103.8, 415, and 550 mg/m^2). Based on these results, vandetanib produces QT prolongation in anesthetized dogs at doses below the proposed clinical dose of 300 mg (185 mg/m^2).

Since vandetanib may be co-administered with anti-emetics in the clinic, safety pharmacology studies were conducted to determine if QT prolongation is exacerbated with the combination of vandetanib and ondansetron. A combination of vandetanib and ondansetron at their respective IC_{50} values resulted in 70% inhibition of the hERG channel, indicating that co-administration was inhibited the hERG channel than either compound alone, but was sub-additive. In a telemetry study in anesthetized dogs, ondansetron caused a dose-related increase in QTcV with intravenous administration of ascending doses (1, 1.5, and 3 mg/kg; 20, 30, and 60 mg/m^2), however, vandetanib (20.75 mg/kg; 415 mg/m^2) did not further increase the QTcV interval following ondansetron treatment.

The effects of vandetanib on pulmonary, renal, and gastrointestinal function were evaluated in *in vivo* rat studies. Respiratory parameters were monitored for 4 hours after a single oral administration of vandetanib (0, 40, 200, or 1000 mg/kg; 0, 240, 1200, or 6000 mg/m^2). Peak expiratory flow, respiratory rate, tidal volume, and minute volume were not affected by treatment with vandetanib. The highest dose (6000 mg/m^2) increased peak inspiratory flow and reduced inspiratory time. In a diuretic test in rats, a dose of 50 mg/kg (300 mg/m^2) vandetanib reduced sodium, potassium, and chloride ions and increased total protein content in the urine by 24 hours after treatment. Urine volume, specific gravity, creatinine, and glucose levels were unaffected. These results suggest that vandetanib is mildly toxic to the kidneys and are consistent with repeat-dose toxicology findings of renal toxicity in the rat. Intestinal transit and gastric emptying were evaluated in male rats treated with a single dose of vandetanib (0, 40, 200, or 1000 mg/kg; 0, 240, 1200, or 6000 mg/m^2). Vandetanib dose dependently inhibited both intestinal transit and gastric emptying beginning at the lowest dose (240 mg/m^2). These results indicate impairment of gastrointestinal function and are consistent with findings of gastrointestinal toxicity observed in the repeat-dose toxicology studies in the rat and dog.

Phamacokinetics

Pharmacokinetics of vandetanib were studied in mice, rats, and dogs, the non-clinical species tested for toxicity, and for both intravenous and oral administration. Vandetanib was well absorbed in the rat and dog. Oral bioavailability of >90% was achieved in rat at 5 mg/kg (30 mg/m^2), however, slightly lower bioavailability (72-78%) was observed at a higher dose of 30 mg/kg (180 mg/m^2). Following an oral dose of 20 mg/kg to dogs, bioavailability was 56%. Absorption was long with T_{max} typically 2-8 hours in the rat and dog. Exposure increased with an increase in dose for both males and females. Increases in exposure were dose proportional at lower doses, but less than proportional at higher doses. Exposure (AUC) of vandetanib was higher in females than males in both rats and dogs. The half-life of vandetanib is relatively long and ranged from 15-55.2 hours in rats and dog, with no dose-related pattern. This range for half-life in animals is distinctly different from the clinical data showing that the plasma half-life is 19 days in humans. Distribution studies in the show that high concentrations of vandetanib were observed in the gastrointestinal tract, lungs, liver, and kidneys, which are all target organs of toxicity. This finding suggests that vandetanib produces toxicity in organs that contain have high

concentrations of the drug. Disposition studies show that with oral administration, vandetanib is primarily eliminated in the feces (55-85%), with some excretion in the urine (5-10%). The two major metabolites of vandetanib are N-desmethyl and N-oxide. The N-desmethyl metabolite has shown similar pharmacological activity to vandetanib. Vandetanib has been shown to affect the activity of human liver P450 isozymes. Vandetanib clearly inhibited CYP2D6 activity, with only 14% of vehicle control activity remaining in the presence of 100 µg/mL vandetanib. CYP1A2, CYP2C9, CYP2C19, and CYP3A4 (testosterone 6β-hydroxylase) activities were inhibited to a lesser extent with 55-89% of vehicle control activity remaining in the presence of 100 µg/mL vandetanib. Therefore, vandetanib may inhibit the clearance of compounds which are metabolized by CYP2D6.

General toxicology

Single dose studies in mice and rats as well as the one month repeat dose toxicology studies in rats and dogs were previously reviewed under IND 60042 (Wendelyn Schmidt, Ph.D.; 2000). In the one month studies vandetanib was administered daily for 29 days in rats (0, 5, 25, or 75 mg/kg/day; 0, 30, 150 or 450 mg/m^2/day) and dogs (0, 5, 15, or 40 mg/kg/day; 0, 100, 300, or 800 mg/m^2/day). In both studies dosing was stopped early in the high dose group, on Day 25 in rats and Day 14 in dogs. Mortality was also observed in both studies, with 9 deaths (4 males and 5 females) in the high dose group in the rat and 6 dogs (3 males and 3 females) in the high dose group euthanized for poor condition. Decreased body weight and food consumption compared to controls and clinical signs of loose feces, thinness, loss of skin tone, and subdued behavior were observed in both species. Target organs of toxicity were the liver, kidney, ovaries/uterus, bone, and teeth in the rats, and the liver, gastrointestinal tract, and bone in the dogs.

The major difference between on the one month studies and the pivotal studies is the clear observance of liver toxicity in the one month studies and not the pivotal studies. In the one month studies, liver toxicities included increases in ALT in both the rat and dog, decreases in liver weight in the rat, and histopathology findings in the liver including reduced hepatocyte glycogen vacuolation, centrilobular hepatocyte vacuolation, foamy macrophages, and necrosis. In the 6 month rat study, increases in AST and ALT were observed in both males and females at all doses of vandetanib, however, no histopathology findings were observed in the liver in this study. Liver toxicity was not observed in the 9 month dog study. Since liver histopathology findings were observed at the high doses only in the one month studies (450 mg/m^2/day in rat; 800 mg/m^2/day in dog) and lower doses were used in the pivotal studies (120/60 mg/m^2/day in rat; 400/300 mg/m^2/day in dog), results suggest that vandetanib may only produce liver toxicity at these higher doses, even with a longer treatment time. Toxicity in the ovaries and uterus were also observed in the one month rat study and not the 6 month rat study.

The pivotal rat study was a six-month oral study in which vandetanib (0, 1, 5, or 20/10 mg/kg/day; 0, 6, 30, or 120/60 mg/m^2/day) was administered daily for 26 weeks with a 13 week recovery period. The highest dose was initially 120 mg/m^2/day, however, due to significant toxicity (mortalities and significant reductions in food consumption and body weight gain), this dose was reduced to 60 mg/m^2/day starting at Week 13 of the study. While mortality was observed at all doses of vandetanib and in the control group, the majority (10 total; 2 males and 8 females) were observed in the high dose group. Probable causes of mortality include toxicities of the lung (pleuritis, abcess, foreign body pneumonia, and alveolar congestion and/or edema),

esophagitis in the esophagus, pancreatitis in the pancreas, and cholangitis in the bile duct. Target organs of toxicity include the adrenal gland, bile duct, heart, kidney, lungs, pancreas, mesenteric lymph node, skin, spleen, teeth, and thymus. Teeth abnormalities (broken, missing, loose, or discolored) and histopathology findings of dental dysplasia and adjacent tissue abscess were observed in the high dose group. Teeth abnormalities have been observed in other studies including the one month repeat-dose rat study and the female fertility study in rats. Masses were observed as clinical signs in one mid dose male and high males and females, and masses were also observed in multiple organs (bile duct, esophagus, intestine, liver, mesenteric lymph node, pancreas, and thymus) primarily in the high dose group in the gross pathology. Histopathology was not conducted on the masses observed in gross pathology. Increases in WBC and neutrophils and histopathology findings of inflammatory cell infiltration in multiple organs indicate inflammation. Renal toxicity was observed based on histopathology changes in the kidney, and increases in urea and creatinine in the blood. Pericarditis and ventricular myocardial fibrosis of the heart were observed in the high dose groups. Increases in ALT and/or AST were observed in both males and females at all doses of vandetanib, however, no histopathology findings were observed in the liver in this study. This is in contrast to findings in the liver observed in the one month rat study.

The pivotal dog repeat-dose study was a 9 month oral study in which vandetanib (0, 1, 5, or 20/15 mg/kg/day; 0, 20, 100, or 400/300 mg/m^2/day) was administered daily for 40 weeks with a 13 week recovery period. The highest dose was initially 400 mg/m^2/day, however, due to a high incidence of abnormal feces and adverse effects on body weight, this dose was reduced to 300 mg/m^2/day starting at Week 17 for main study animals and Week 18 for recovery animals. Abnormal feces was the dose limiting toxicity, and as a result of the dose reduction other possible toxicities like liver toxicity were not observed. Recovery of the abnormal feces occurred rapidly during the recovery period. Target organs of toxicity include the intestine (jejunum, rectum, and ileo-caecal junction), kidney, spleen, stomach, and thymus. Gastrointestinal toxicities include abnormal feces and gross pathology and histopathology findings in the intestine and stomach. Two dogs in the high dose group received enteral anti-microbial therapy due to clinical signs related to abnormal feces. Clinical signs observed and the response to the enteral anti-microbial therapy suggest opportunistic intestinal infection, secondary to the effects of vandetanib on gastrointestinal function in the high dose group.

Genetic Toxicology

Vandetanib was tested for mutagenicity in an *in vitro* reverse mutation (Ames) assay and tested for clastogenicity in an *in vitro* cytogenetic assay using human lymphocytes and an *in vivo* rat micronucleus assay. At the doses tested in these valid studies, with and without metabolic activation, vandetanib was not mutagenic or clastogenic.

Carcinogenicity

Carcinogenicity studies have not been conducted.

Reproductive and Developmental Toxicology

Fertility studies in the rat were conducted with vandetanib with treated males bred to untreated females and treated females bred to untreated males. In the male fertility study, males from the 6 month repeat-dose toxicology study treated daily with vandetanib (0, 1, 5, or 20 mg/kg/day; 0, 6, 30, or 120 mg/m^2/day) were dosed for 8 weeks prior to pairing with untreated females. Vandetanib had no effect on copulation, time to copulation, or fertility rate, however, in females mated with males treated with vandetanib, there was a slight decrease in the number of implants and live embryos at 120 mg/m^2/day and an increase in preimplantation loss at $\geq$30 mg/m^2/day. Additionally, two females mated with males treated with 120 mg/m^2/day vandetanib had total resorptions. In the female fertility study, females were dosed daily with vandetanib (0, 1, 10, or 25 mg/kg; 0, 6, 60, or 150 mg/m^2) for 14 days before pairing with untreated males, and dosing was continued until gestational Day 6. Females treated with 60 or 150 mg/m^2 vandetanib had an increased incidence of irregular cycle patterns, however, this did not affect their ability to mate. Treatment with 150 mg/m^2 vandetanib decreased the pregnancy incidence and the number of live embryos per female, and increased preimplantation loss, postimplantation loss, and the number of early embryo deaths. Two females treated with 150 mg/m^2 had total intrauterine death.
The embryo-fetal development effects of vandetanib were studied in the rat. In the range finding study, females were administered vandetanib (0, 0.5, 1, 5, 10, or 20 mg/kg/day; 0, 3, 6, 30, 60, or 120 mg/m^2/day) daily on Days 1-7 of gestation and euthanized on Day 13 or on Days 7-16 of gestation and euthanized on Day 22. Treatment with 120 mg/m^2/day vandetanib caused an increase in postimplantation loss when administered to pregnant females either between gestation Day 1-7 or Days 7-16. Fetal weight was decreased by administration of 60 and 120 mg/m^2/day vandetanib in females dosed between gestation Days 7-16.
In the pivotal embryo-fetal development in the rat, females were dosed daily with vandetanib (0, 1, 10, or 25 mg/kg/day; 0, 6, 60, or 150 mg/m^2/day) from gestation Days 6-15 and were euthanized on gestation Day 21. Postimplantation loss was increased and the number of live fetuses was decreased in females treated with 150 mg/m^2/day vandetanib during organogenisis. Fetal weight and mean placental weight were decreased with treatment of vandetanib at both 60 and 150 mg/m^2/day. Treatment with vandetanib caused a number of different heart vessel abnormalities at 60 and 150 mg/m^2/day. Heart vessel abnormalites were also observed in one fetus at 6 mg/m^2/day. Treatment with vandetanib was teratogenic at all doses tested, therefore, a no effect level (NOEL) for teratogenicity was not identified in this study. Developmental delays were also observed; vandetanib reduced ossification of skull bones, vertebrae and sternebrae. These findings occurred in the absence of maternal toxicity.

The effects of vandetanib on pre- and post-natal development were also studied in the rat. In a dose-range finding study, females were dosed treated daily with vandetanib (0, 1, 10, or 25 mg/kg/day; 0, 6, 60, or 150 mg/m^2/day) from Day 6 of gestation to Day 8 of lactation. An additional group treated with 60 mg/m^2/day was dosed from Day 16 of gestation to Day 8 of lactation. All females dosed with 150 mg/m^2/day were euthanized on Day 24 of gestation due to the absence of parturition. At necropsy, all of these dams were found to have undergone total resorptions early in gestation, indicating that 150 mg/m^2/day was embryotoxic in this study. Treatment of 60 mg/m^2/day vandetanib from Day 6 of gestation resulted in lower pup weight. In this study, toxicokinetics measured in dams and pups indicated the transfer of vandetanib from the dams to the pups by milk secretion. Transfer through milk resulted in relative constant exposure as indicated by predose exposure in pups. Predose exposure in the pups was

approximately equivalent to pup exposure 8 hours after dosing to the dams. These findings were consistent with those of an excretion study investigating the transfer of vandetanib into milk following oral administration of [^{14}C]-ZD6474 to lactating rats. Vandetanib and trace amounts of the N-desmethyl and N-oxide metabolites of vandetanib were excreted in milk with peak concentrations observed at 8 hours after dosing. Concentrations of vandetanib were higher in milk than blood at all time points except 1 hour after dosing.

In the pivotal pre- and post-natal development study in the rat, females were treated with vandetanib (0, 1, or 10 mg/kg/day; 0, 6, or 60 mg/m^2/day) from Day 6 of gestation until weaning at Day 21 *post-partum*. An additional group treated with 60 mg/m^2/day was dosed from Day 16 of gestation. After weaning, some pups (F_1 generation) from each group were selected for post-weaning behavioral tests and mating. In this study, vandetanib was maternally toxic with lower mean body weight gain during gestation and decreased food consumption at both 6 and 60 mg/m^2/day. There was a decrease in number of live pups per litter at birth and a decrease in pup survival at Day 4 *post-partum* with treatment of 6 and 60 mg/m^2/day. Both doses reduced post-natal pup growth, which was associated with a delay in physical development at 60 mg/m^2/day. No effects of vandetanib were observed in the behavioral tests, mating performance, fertility, and caesarian (uterus content) for the F_1 generation. The reproductive and developmental toxicology studies suggest that administration of vandetanib may impair fertility and pose a risk for fetal toxicity. Pregnancy category D is recommended.

Summary of Pharmacology Studies

Kinases and targets with IC_{50} values lower than 1.8 µM, the C_{max} value in the pivotal clinical study (Study 58), are listed in the tables below. For the kinases assessed in the primary pharmacology studies, the lowest value reported in the review is listed along with the study number in which the value was reported. Bolded kinases are potential targets of vandetanib. The cutoff for potential targets was set at 5 x 10^{-7} M because RET inhibition was shown to vary through this range and physiologic inhibition at this level is plausible.

Primary Pharmacology			
Kinase	**IC_{50} (M)***	**IC_{50} (µM)**	**Study**
EGFR (L858R)	**3 x 10^{-9}**	**0.003**	**ASZ128ABC**
VEGF-R2	**1.84 x 10^{-8}**	**0.0184**	**2501**
RET	**2.0 x 10^{-8}**	**0.02**	**ASZ128ABC**
EGF-R	**2.1 x 10^{-8}**	**0.021**	**ASZ128ABC**
BRK	**3.6 x 10^{-8}**	**0.036**	**17702**
EGFR (T790M)	**1.08 x 10^{-7}**	**0.108**	**ASZ128ABC**
EPHB2	**1.10 x 10^{-7}**	**0.11**	**17702**
VEGF-R1	**1.50 x 10^{-7}**	**0.15**	**17702**
EPHA1	**1.7 x 10^{-7}**	**0.17**	**17702**
LCK	**2.00 x 10^{-7}**	**0.2**	**3179**
YES	**2.40 x 10^{-7}**	**0.24**	**17702**
VEGF-R3	**2.60 x 10^{-7}**	**0.26**	**17702 and 3179**
EPHA2	**3.10 x 10^{-7}**	**0.31**	**17702**

Primary Pharmacology			
Kinase	**IC_{50} (M)***	**IC_{50} (µM)**	**Study**
EPHB4	**3.20×10^{-7}**	**0.32**	**17702**
EPHA4	**3.70×10^{-7}**	**0.37**	**17702**
TIE2	**3.70×10^{-7}**	**0.37**	**3179**
ERBB2	**3.90×10^{-7}**	**0.39**	**17702**
LYN	**4.00×10^{-7}**	**0.4**	**17702**
SRC	**4.20×10^{-7}**	**0.42**	**17702**
EPHB1	**4.30×10^{-7}**	**0.43**	**17702**
IRAK4	5.00×10^{-7}	0.5	17702
FGF-R1	5.80×10^{-7}	0.58	17702
ERBB4	7.00×10^{-7}	0.7	17702
FGF-R3	7.50×10^{-7}	0.75	17702
FGR	1.10×10^{-6}	1.1	17702
PDGFRα	1.10×10^{-6}	1.1	17702
ABL1	1.30×10^{-6}	1.3	17702
KIT	1.40×10^{-6}	1.4	17702
CSK	1.60×10^{-6}	1.6	17702

Secondary Pharmacology (Study 0393SY)	
Target	IC_{50} (µM)
Adrenergic α_{2A}	0.239
Adrenergic α_{2B}	0.219
Dopamine D_1	1.51
Histamine H_1	0.142
Histamine H_2	0.733
Imidazoline I_2, central	0.847
Serotonin transporter	1.51

Summary of Safety Pharmacology Studies

Study #/Organ System	Method of Administration	Species/cells	Doses/ concentrations	Gender/n	Findings
1218SR/ CNS	Oral	Rat	0, 40, 200, or 1000 mg/kg (0, 240, 1200, or 6000 mg/m^2) ZD6474	Male/n=5-7	<u>All doses:</u> ↓ approach response <u>1200 and 6000 mg/m^2</u>: ↓ body weight, slow pupil response, ↓ activity levels in open field test <u>6000 mg/m^2</u>: ↓ landing foot splay, reduced grip strength, and piloerection
TSM1124/ CNS	Oral	Mouse	0 or 50 mg/kg (0 or 300 mg/m^2) ZD6474	Male/n=5	No effects on behavior
TSZ36/	*In vitro*	hERG-	1×10^{-7} to 1×10^{-5}	NA/n=5	hERG channel inhibited

Study #/Organ System	Method of Administration	Species/cells	Doses/ concentrations	Gender/n	Findings
Cardiovascular		expressing HEK cells	M ZD6474 in half-log increments		with a $-p[IC]_{50}$ of 6.4± 0.1 (0.4 µM or 190 ng/mL)
0048SZ/ Cardiovascular	*In vitro*	hERG-expressing HEK cells	3.16×10^{-8} to 1×10^{-5} M M382558 and M447882 in half-log increments	NA/n=4-5	N-desmethyl metabolite of ZD6474 inhibited hERG channel with an $-p[IC]_{50}$ of 5.9 ± 0.1 (1.3 µM or 600 ng/mL) N-oxide metabolite of ZD6474 inhibited hERG channel with an $-p[IC]_{50}$ of 5.4 ± 0.1 (4.0 µM or 2038 ng/mL)
0102SZ/ Cardiovascular	*In vitro*	hERG-expressing CHO cells	ZD6474: 4×10^{-7} M Ondansetron: 1 $\times 10^{-6}$ M	NA	Combination of ZD6474 and Ondansetron (at their IC_{50} values) resulted in 70% inhibition of hERG channel; effects of combination on hERG not additive or synergistic
TSD1293/ Cardiovascular	*In vitro*	Purkinje fibers from 5 male Beagle dogs	$.1 \times 10^{-6}$ to 10×10^{-6} M ZD6474	NA/n=6	1 and 10 µM lengthened the action potential duration at 50, 70, and 90% of repolarization in canine isolated cardiac purkinje cells at stimulation frequencies of 0.3 and 1 Hz under normal and low potassium Tyrode's buffers
Internal report #10/ Cardiovascular	Oral	Rat	12.5 or 50 mg/kg (75 or 300 mg/m^2) single dose or 12.5 mg/kg (300 mg/m^2) daily x 7 ZD6474	Male/n=3	Mean systolic and diastolic blood pressures were dose-dependently ↑ with single doses of 75 and 300 mg/m^2; 7 daily doses of 75 mg/m^2 caused greater ↑ compared to single dose
0276SD/ Cardiovascular	Intravenous	Dog (anesthetized)	Escalating doses of 0.2, 0.67, 2.0, 6.7, and 13.4 mg/kg (4, 13.4, 40, 134, and 268 mg/m^2) ZD6474	Male/n=4	↑ QTcV interval at ≥ 40 mg/m^2 and dose-dependent ↑ in T wave amplitude and polarity at ≥ 134 mg/m^2 with slight changes occasionally at 13.4 and 40 mg/m^2; ↑ in PR intervals compared to controls at all doses
0257SD/ Cardiovascular	Intravenous	Dog (anesthetized)	ZD6474: Escalating doses of 5.19, 20.75, and 27.5 mg/kg (103.8,	Male/n=2	ZD6474: Dose-related ↑ QTcV interval, ↑ in blood pressure, and ↑ in T wave amplitude

Study #/Organ System	Method of Administration	Species/cells	Doses/ concentrations	Gender/n	Findings
			415, and 550 mg/m^2) Ondansetron: Escalating doses of 1, 1.5, and 3 mg/kg (20, 30, and 60 mg/m^2)		Ondansetron: Dose-related ↑ QTcV interval
0258SD/ Cardiovascular	Intravenous	Dog (anesthetized)	Ondansetron: Escalating doses of 1, 1.5, and 3 mg/kg (20, 30, and 60 mg/m^2) ZD6474: 0 or 20.75 mg/kg (0 or 415 mg/m^2)	Male/n=4	Ondansetron caused a dose-related ↑ QTcV interval; ZD6474 did not further ↑ QTcV interval following Ondansetron treatment ZD6474: ↑ in diastolic blood pressure and ↑ in T wave amplitude and polarity
20060012PCR/ Pulmonary	Oral	Rat	0, 40, 200, or 1000 mg/kg (0, 240, 1200, or 6000 mg/m^2) ZD6474	Male/n=8	6000 mg/m^2: ↑ peak inspiratory flow and ↓ inspiratory time
TSR2922/ Renal	Oral	Rat	0 or 50 mg/kg (0 or 300 mg/m^2) ZD6474	Female/n=5	300 mg/m^2: ↓ Na, K, and Cl ions and ↑ total protein content in urine by 24 hrs after dosing
1290SR/ gastrointestinal	Oral	Rat	0, 40, 200 or 1000 mg/kg (0, 240, 1200, or 6000 mg/m^2) ZD6474	Male/n=10	Dose dependently inhibited both intestinal transit and gastric emptying starting at the lowest dose of 240 mg/m^2

General Toxicology Summary:

Repeat Dose Toxicity Studies				
Species	**Route Duration**	**N/sex/ dose**	**mg/kg/day (mg/m^2/day)**	**Significant findings**
Rat	Oral Daily x 29 days (1 month)	10 (MS) 10 (Recov., control and HD)	5 (30) 25 (150) 75 (450)	Treatment stopped at Day 25 for 450 mg/m^2/day group 30 mg/m^2/day: Loose feces, ↑ WBC in males 150 mg/m^2/day: Loose feces, ↑ WBC, ↑ ALT, ↓ liver weight, ↓ ovary weight, femur epiphyseal growth plate dysplasia, papillary necrosis of kidney, foamy macrophages of lungs, lymph nodes and spleen, epidermal microabscesses and acute folliculitis of muzzle skin, 450 mg/m^2/day: Mortality in 4 males and 5 females, loose feces, thin, teeth broken, trembling, ↓ body weight and food consumption, ↑ RBC, WBC, and platelets, ↑ urea in males, ↑ creatinine, ↑ ALT, ↓ total protein and albumin, ↓ urine volume, ↑ urinary sodium, potassium, and creatinine, ↓ liver weight, ↓ overy and uterus weights in females, ↓ thymus weight, adrenal vacuolation and hemorrhage, severe acute cholangitis of bile duct in females, femur epiphyseal growth plate dysplasia, papillary necrosis and cortical tubular vacuolization of kidney, reduced hepatocyte glycogen vacuolation, centrilobular

Repeat Dose Toxicity Studies				
Species	**Route Duration**	**N/sex/ dose**	**mg/kg/day ($mg/m^2/day$)**	**Significant findings**
				hepatocyte vacuolation, foamy macrophages, and acute necrosis of liver. foamy macrophages of lungs, lymph nodes, and spleen, decreased corpora lutea in ovaries, acinar epithelial apoptosis of pancreas, epidermal microabscesses and acute folliculitis of muzzle skin, incisor dysplasia, uterus atrophy
Rat	Oral Daily x 26 weeks (6 months)	20 (MS) 10 (Recov.)	1 (6) 5 (30) 20/10 (120/60)	Highest dose reduced from 60 to 120 $mg/m^2/day$ starting Week 13 due to mortality and ↓ in body weight gain and food consumption 6 $mg/m^2/day$: Mortality in 5 males, ↑ urea and creatinine in males, ↑ AST and ALT, alveolar edema in males with mortality, tubular basophilia of kidney 30 $mg/m^2/day$: Mortality in 1 female, ↓ body weight gain in males, ↑ WBC and neutrophils in females, ↑ creatinine, ↑ AST and ALT, masses in liver, lungs, and mesenteric lymph node, tubular basophilia of kidney, foamy alveolar macrophages of lungs, alveolar edema in female with mortality, sinusoidal hemorrhagic cystic degeneration of mesenteric lymph node in males, folliculitis and inflammatory cell infiltration of skin 120/60 $mg/m^2/day$: Mortality in 2/30 males and 8/30 females, teeth abnormalities and masses, ↓ body weight gain, ↑ WBC and neutrophils, ↑ urea in females, ↑ creatinine, ↑ AST and ALT, ↑ urine volume and protein levels in urine, masses in multiple organs, adrenal hemorrhage in females, bile duct cholangitis in females with mortality, pericarditis of heart in females and ventricular myocardial fibrosis of heart in males, tubular basophilia of kidney, foamy alveolar macrophages of lungs, sinusoidal hemorrhagic cystic degeneration of mesenteric lymph node, pancreatitis, dental dysplasia in females and adjacent tissue abscess of teeth in males, folliculitis and inflammatory cell infiltration of skin, extramedullary hematopoiesis of spleen in females, thymus atrophy
Dog	Oral Daily x 29 days	3 (MS) 3 (Recov., control and HD only)	5 (100) 15 (300) 40 (800)	Treatment stopped at Day 25 for 800 $mg/m^2/day$ group 300 $mg/m^2/day$: Loose feces, ↑ ALT in males 800 $mg/m^2/day$: Mortality in 3 males and 3 females, poor general condition, loose feces, ↓ body weight and food consumption, ↑ platelets and WBC, ↑ uterus weight, femur epiphyseal growth plate dysplasia, reduced glycogenation of liver, lymphocytolysis of bronchial cervical, and mesenteric lymph nodes, reduced zymogen granules in pancreas, multifocal white pulp vacuolation in females, involution of thymus
Dog	Oral Daily x 40 weeks (9 months)	4 (MS) 3 (Recov.)	1 (20) 5 (100) 20/15 (400/300)	Highest dose reduced from 400 to 300 $mg/m^2/day$ starting Week 17 or 18 due to high incidence of abnormal feces 20 $mg/m^2/day$: ↑ in ALT, AST, ALP, and GLDH without corresponding change in histopathology, multifocal nerve root sheath mineralization in spinal cord in 1 male 100 $mg/m^2/day$: Abnormal feces, ↑ WBC and neutrophils, epithelial brown pigment in kidney in males, lamina propria mononuclear cell infiltration in stomach, multifocal nerve root sheath mineralization in spinal cord in 1 male and 1 female 400/300 $mg/m^2/day$: Abnormal feces (DLT), ↓ body weight gain, QT prolongation in 1 male, ↑ platelets, epithelial brown pigment in kidney in 1 male, mucosal congestion in intestine in

Repeat Dose Toxicity Studies				
Species	**Route Duration**	**N/sex/ dose**	**mg/kg/day ($mg/m^2/day$)**	**Significant findings**
				1 female, discoloration of intestine, lamina propria mononuclear cell infiltration in stomach, multifocal nerve root sheath mineralization in spinal cord in 1 male

MS=Main study
Recov.=Recovery groups, control and high dose only

Genetic Toxicology Summary

Title	Study #	Without Metabolic Activation	With Metabolic Activation
In vitro reverse mutation assay in bacterial cells (Ames)	TMV777	Negative for all strains at concentrations of 20-1000 µg/plate	Negative for all strains at concentrations of 20-1000 µg/plate
Zeneca ZD6474: *In vitro* cytogenetic study using cultured human lymphocytes	TYX103	Negative for clastogenicity at concentrations of 0.2-10 µg/mL	Negative for clastogenicity at concentrations of 2-10 µg/mL
Zeneca ZD6474: Micronucleus test in the rat: Oral administration	TQR2942	Negative for clastogenicity in the rat at doses of 100, 330 or 1000 mg/kg (600, 1980, or 6000 mg/m^2) at 24 and 48 hrs after treatment following extended counting	

Reproductive and Developmental Toxicity Summary

	Fertility and Early Embryonic Development		Embryonic Fetal Development	
Study #	**TGR3138**	**TGR2940**	**TRR3073**	**TTR2938**
Title	Zeneca ZD6474: Fertility study in male rats: Oral Administration	Zeneca ZD6474: Oral fertility and early embryonic development study in the female rat	Zeneca ZD6474: Teratology sighting study in rats: Oral administration	Astrazeneca ZD6474: Oral embryofetal development study in the rat
Methods	Males from 6 month repeat-dose study dosed daily starting 8 weeks prior to pairing with untreated females. Untreated females euthanized on GD 13	Females dosed daily for 14 days before pairing with untreated males, dosing continued until GD 6 Euthanized on GD 12	Females dosed daily on GD 1-7 and euthanized on GD 13 or dosed on GD 7-16 and euthanized on GD 22	Females dosed daily from GD 6-15 and euthanized on GD 21
Key Findings	No treatment related effect on copulation or fertility rate In untreated females : $\geq$ 30 mg/m^2/day: ↑ in pre-implantation loss 120 mg/m^2/day: ↓ in number of implants and live embryos; total resorptions in 2 females	↑ incidence of irregular cycle patterns at $\geq$ 60 mg/m^2/day; did not effect ability to mate 150 mg/m^2/day: ↓ fertility index (fewer rats pregnant) and ↓ number of live embryos per female, ↑ pre- and post-implantation loss and number of early embryo deaths; 2 females had total intrauterine death	↑ post implantation loss at 120 mg/m^2/day regardless of timing of administration Fetal weight ↓ at 60 and 120 mg/m^2/day in females dosed GD 7-16 A variety of major abnormalities were observed in a small number of fetuses across different groups, however, the findings were not consistent across groups	↑ post implantation loss and ↓ number of live fetuses at 150 mg/m^2/day Fetal weight and mean placental weight ↓ at 60 and 150 mg/m^2/day Malformations of the heart vessels observed at $\geq$ 6 mg/m^2/day and reduced ossification of skull bones, vertebrae, and sternebrae No NOEL for embryofetal developmental toxicity including teratogenicity
Species	Alpk:AP$_f$SD strain (Wistar derived) Rat	Alpk:AP$_f$SD strain (Wistar derived) Rat	Alpk:AP$_f$SD strain (Wistar derived) Rat	Alpk:AP$_f$SD strain (Wistar derived) Rat
Doses	0, 1, 5, 20 mg/kg/day (0, 6, 30, 120 mg/m^2/day)	0, 1, 10, 25 mg/kg/day (0, 6, 60, 150 mg/m^2/day)	0, 0.5, 1, 5, 10, 20 mg/kg/day (0, 3, 6, 30, 60, 120 mg/m^2/day)	0, 1, 10, 25 mg/kg/day (0, 6, 60, 150 mg/m^2/day)
Mortality and Clinical Signs	No mortalities	Mortality: No treatment related mortality Clinical signs: Teeth abnormalities, piloerection, and hunched posture at 150 mg/m^2/day	No maternal deaths during the study	No maternal deaths during the study

Body Weight/Food Consumption	Male treatment had no effect on untreated female weight gain during pregnancy	No treatment related effects on body weight or food consumption	↓ body weight and food consumption during dosing at 120 mg/m^2/day in females dosed GD 1-7	Body weight: No effect on maternal body weight after adjustment for gravid uterus weight on GD 21

GD= Gestational day

Study #	**Prenatal and Postnatal Development**	
	AA29682	**AA32728**
Title	ZD6474 (Zactima)- Dose range-finding pre- and post-natal development study by the oral route (gavage) in the rat.	ZD6474 (Zactima)- Pre- and post-natal development study by the oral route (gavage) in the rat.
Methods	Females dosed daily from GD 6 to Day 8 of lactation; additional 60 mg/m^2/day group dosed from GD 16 Dams and pups euthanized on Day 8 of lactation; all females dosed with 150 mg/m^2/day were euthanized on GD 24 due to absence of partition	Females dosed daily from GD 6 until weaning at Day 21 *post-partum*; additional 60 mg/m^2/day group dosed from GD 16 After weaning, F_1 generation pups from each group selected for post-weaning behavioral tests and mating
Key Findings	All females dosed with 150 mg/m^2/day had total resorptions early in gestation ↓ pup weight in 60 mg/m^2/day group dosed from GD 6 Toxicokinetics measured in dams and pups indicated the transfer of vandetanib from the dams to the pups by milk secretion; transfer through milk resulted in relative constant exposure in pups Mid dose (60 mg/m^2/day) considered appropriate high dose for subsequent study	Treatment with both 6 and 60 mg/m^2/day was maternally toxic, resulting in lower mean body weight gain during gestation and decreased food consumption ↓ number of pups per litter at birth and ↓ in pup survival at Day 4 *post-partum* at both 6 and 60 mg/m^2/day ↓ post-natal pup growth at both doses, associated with a delay in physical development at 60 mg/m^2/day No effects on behavioral tests, mating performance, fertility, and caesarian (uterus content) for F_1 generation
Species	Wistar Rat	Wistar Rat
Doses	0, 1, 10, 25 mg/kg/day (0, 6, 60, 150 mg/m^2/day)	0, 1, 10 mg/kg/day (0, 6, 60 mg/m^2/day)
Mortality and Clinical Signs	Mortality: All females dosed with 150 mg/m^2/day were euthanized early on GD 24 due to absence of partition One female treated with 60 mg/m^2/day from GD 16 was euthanized with its litter on Day 2 of lactation due to abnormal nursing behavior Clinical signs in female euthanized early with litter: Pale and red/black discharge, raised hair and subdued behavior, pups cold to touch, and dam not attending to litter	Mortality:One female treated with 6 mg/kg/day (Day 8 *post-partum*) and one female treated with 60 mg/m^2/day from GD 6 (Day 1 *post-partum*) euthanized due to total litter death One control and one female treated with 60 mg/m^2/day from GD 6 euthanized on GD 26 due to no viable fetuses Clinical signs: Lame limbs at 60 mg/m^2/day

Body Weight/Food Consumption	Females treated with 150 mg/m^2/day showed ↓ weight gain and food consumption than controls during gestation, when they had total litter resorption Body weight and food consumption were slightly ↓ at 60 mg/m^2/day during gestation and lactation	↓ mean body weight gain during gestation and ↓ food consumption at both 6 and 60 mg/m^2/day
Necropsy	All females dosed with 150 mg/m^2/day had total resorptions early in gestation	One control and one female treated with 60 mg/m^2/day from GD 6 euthanized on GD 26 due to no viable fetuses
F_1 generation findings	↓ pup weight in 60 mg/m^2/day group dosed from GD 6	↓ number of pups per litter at birth and ↓ in pup survival at Day 4 *post-partum* at both 6 and 60 mg/m^2/day ↓ post-natal pup growth at both doses, associated with a delay in physical development (pinna unfolding, incisor eruption, and eye opening) at 60 mg/m^2/day No effects on behavioral tests, mating performance, fertility, and caesarian (uterus content)

GD= Gestational day

References

Ko, J., Ross, J., Awad, H., Hurwitz, H., & Klitzman, B. (2005). The effects of ZD6474, an inhibitor of VEGF signaling, on cutaneous wound healing in mice. *Journal of Surgical Research, 129* (2):251-259.

Wu, W., Onn, A., Isobe, T., Itasaka, S., Langley, R.R., Shitani, T., Shibuya, K., Komaki, R., Ryan, A.J., Fidler, I.J., Herbst, R.S., & O'Reilly, M.S. (2007). Targeted therapy of orthotopic human lung cancer by combined vascular endothelial growth factor and epidermal growth factor receptor signaling blockade. *Mol Cancer Ther, 6* (2): 471-483.

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/

BRENDA J GEHRKE
12/10/2010

ROBERT T DORSAM
12/10/2010

SANDI L VERBOIS
12/10/2010

MEMORANDUM

Date: December 9, 2010
From: S. Leigh Verbois, Ph.D.
Supervisory Pharmacologist
Division of Drug Oncology Products
To: File for NDA #022405
Vandetinib
Re: Approvability of Pharmacology and Toxicology

Non-clinical studies that investigated the pharmacology and toxicology of vandetinib provided to support NDA 022405 for the treatment of patients with unresectable locally advanced or metastatic medullary thyroid cancer were reviewed in detail by Brenda Gehrke, Ph.D., and Robert Dorsam, Ph.D. The supporting information included studies of orally administered vandetinib that investigated the drug's pharmacology, toxicokinetics and ADME, safety pharmacology, general toxicology (rat and dog), and genetic toxicity (*in vivo* and *in vitro*). Reproductive and developmental toxicology studies were conducted in rats to assess the effects of vandetanib on fertility, embryofetal development, and pre- and post-natal development. The studies cited in the review consist primarily of original research conducted by the applicant.

The pharmacology studies submitted to the NDA demonstrate that vandetinib is a tyrosine kinase inhibitor which binds multiple receptor tyrosine kinases including VEGF, EGF, RET, BRK, TIE2, and members of EPH and SRC family of kinases. These kinases were inhibited at concentration lower than levels than that achieved in the plasma clinically. Based on this, the pharmacological classification of vandetinib is a kinase inhibitor. Drug induced toxicity, including gastrointestinal, cardiovascular, toxicity, skin, spleen, and teeth and bone (in rats) toxicity were observed non-clinically. These findings were well characterized non-clinically. Cardiovascular toxicity was highly discussed throughout the review process and manifested both in toxicology studies as well as safety pharmacology studies as prolongation of QT interval.

Vandetinib was not mutagenic or clastogenic when tested in the Ames assay or the *in vitro* cytogenetic assay using human lymphocytes and an *in vivo* rat micronucleus assay, respectively. Although carcinogencity studies have not been initiated to date, evidence of masses was detected in the repeat dose rat study in multiple organs. Masses were detected at both clinical observation and gross pathology. Given the proposed patient indication which has an extended life expectancy, studies are required to assess the risk of carcinogenicity.

Vandetinib may impair fertility in males and females. In a fertility study in male rats, vandetanib had no effect on copulation or fertility rate at doses approximately 0.03 to 0.38 times the AUC in patients with cancer at the recommended human dose of 300 mg/day. However, there was a slight decrease in the number of live embryos at 20

mg/kg/day (120 mg/m^2) and an increase in preimplantation loss at $\geq$5 mg/kg/day (30 mg/m^2). In a female fertility study, there was a trend towards increased estrus cycle irregularity, a slight reduction in pregnancy incidence and an increase in implantation loss. In a repeat-dose toxicity study in rats, there was a decrease in the number of corpora lutea in the ovaries of rats administered 75 mg/kg/day vandetanib (450 mg/m^2; approximately 1.8 times the AUC in patients with cancer at the recommended human dose) for 1 month.

Vandetanib is embryotoxic, fetotoxic, and teratogenic to rats, at exposures equivalent to or lower than those expected at the recommended human dose of 300 mg/day. When vandetanib was administered to female rats prior to mating and through the first week of pregnancy, there were increases in pre-implantation loss, post-implantation loss and early embryolethality resulting in a significant reduction in the number of live embryos. This dose administered to rats during organogenesis, caused an increase in post-implantation loss including embryofetal death. Vandetanib caused total litter loss when administered at a dose of 25 mg/kg/day (150 mg/m^2/day) during organogenesis until expected parturition. When administered during organogenesis, vandetanib doses of greater than 1 mg/kg/day (greater than 0.03 the C_{max} in patients with cancer at the recommended human dose), caused malformations of the heart vessels and delayed ossification of the skull, vertebrae and sternum, indicating delayed fetal development. A no effect level for these malformations was not identified in this study.

In a rat pre- and post-natal development study, at doses producing maternal toxicity (1 and 10 mg/kg/day; 6 and 60 mg/m^2/day) during gestation and/or lactation, vandetanib, decreased pup survival, and/or reduced post-natal pup growth. Reduced post-natal pup growth was associated with a delay in physical development. In the pre- and post-natal development study, it was determined that vandetinib was excreted in rat milk and found in the plasma of pups. Vandetinib was found in the plasma of pups pre-dosing and eight hours post-dosing indicating the persistence of vandetinib in plasma of dams, persistant excretion into breast milk or residence in breast milk or slow excretion of pups. Given the long half life of the drug it likely the first and last. Because the potential benefit from the use of the vandetinib in pregnant women in this patient population may outweigh the potential risk to the developing fetus, Pregnancy Category D is recommended for this patient population.

Recommendations: I concur with Drs. Gehrke's and Dorsam's conclusion that pharmacology and toxicology data support the approval of NDA 022405 for vandetinib. There are no outstanding nonclinical issues related to the approval of trabectedin for the proposed indication.

The following information should be conveyed in the CR letter regarding the PMRs:

1. To evaluate the potential for a serious risk of carcinogenicity, it is necessary to assess the potential for carcinogenicity by conducting a rodent carcinogenicity study in the mouse. Submit the carcinogenicity protocol for a Special Protocol Assessment (SPA) prior to initiating the study. Notify the Agency in writing at least 30 days prior to

submission of the study that a carcinogenicity protocol will be arriving. Submit the carcinogenicity protocol and questions regarding the protocol with sufficient time prior to the anticipated initiation of the study to allow for meaningful discourse with the agency and resolution of any issues before study initiation. Clearly mark in bold black letters as a REQUEST FOR SPECIAL PROTOCOL ASSESSMENT. Once the study results have been finalized, update package inserts to reflect study findings.

2. To evaluate the potential for a serious risk of carcinogenicity, it is necessary to assess the potential for carcinogenicity by conducting a long-term rodent carcinogenicity study in the rat. Submit the carcinogenicity protocol for a Special Protocol Assessment (SPA) prior to initiating the study. Notify the Agency in writing at least 30 days prior to submission of the study that a carcinogenicity protocol will be arriving. Submit the carcinogenicity protocol and questions regarding the protocol with sufficient time prior to the anticipated initiation of the study to allow for meaningful discourse with the agency and resolution of any issues before study initiation. Clearly mark in bold black letters as a REQUEST FOR SPECIAL PROTOCOL ASSESSMENT. Once the study results have been finalized, update package inserts to reflect study findings.

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/s/

SANDI L VERBOIS
12/13/2010

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 022405 **Applicant: IPR Pharmaceuticals Inc C/O AstraZeneca Pharmaceuticals LP** **Stamp Date: 7/7/2010**

Drug Name: Vandetanib (Zictifa) **NDA/BLA Type: NME**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	✓		NDA is submitted in the eCTD format
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	✓		Electronic submission
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	✓		
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)?	✓		• Carcinogenicity: Not done and not required • Mutagenicity: Done • Teratogenicity: Rat only, acceptable due to teratogenic effects in the rat • Effects on fertility: Done • Juvenile studies: Not conducted and not required • Acute dose animal studies: Done • Repeat dose animal studies: Done, studies include 6 month rat and 9 month dog • ADME: Done • Safety Pharmacology: Done
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).	✓		Oral formulations were used in pivotal clinical and nonclinical studies
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?	✓		Same route of administration

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
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?	✓		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	✓		
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?	✓		A substantive labeling review will be conducted after review of the submitted nonclinical program
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	✓		Applicant identified 8 impurities with potential for genotoxicity Genotoxicity studies were conducted on 4 impurities: (b) (4)
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.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

Brenda J. Gehrke, Ph.D.	August 20, 2010
Reviewing Pharmacologist	Date
S. Leigh Verbois, Ph.D.	August 20, 2010
Team Leader/Supervisor	Date

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22405	ORIG-1	IPR PHARMACEUTICALS INC	Zictifa (Vandetanib)

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

/s/

BRENDA J GEHRKE
08/20/2010

SANDI L VERBOIS
08/20/2010