CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208065Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Tagrisso (osimertinib)

Date: November 4, 2015
To: File for NDA 208065
From: John K. Leighton, PhD, DABT
Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting and labeling reviews for Tagrisso conducted by Dr. Weis, and secondary memorandum and labeling provided by Dr. Helms. I concur with Dr. Helms' conclusion that Tagrisso may be approved for the proposed indication.

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/s/

JOHN K LEIGHTON
11/04/2015

MEMORANDUM

Date: October 8, 2015
From: Whitney S. Helms, Ph.D.
Supervisory Pharmacologist
Division of Hematology Oncology Toxicology for Division of Oncology Products 2
To: File for NDA #208065
Osimertinib (TAGRISSO)
Re: Approvability of Pharmacology and Toxicology

The non-clinical pharmacology and toxicology data provided to support NDA 208065 for the use of TAGRISSO in the treatment of patients (b) (4) metastatic epidermal growth factor receptor (EGFR) T790M mutation-positive non-small cell lung cancer (NSCLC) as detected by an FDA approved test, who have progressed on or after EGFR tyrosine kinase therapy were reviewed in detail by Shawna L. Weis, Ph.D. Osimertinib received breakthrough designation for this population on April 16, 2014. The NDA submission included studies of orally administered osimertinib in mice, rats, and dogs that investigated the drug's pharmacology, pharmacokinetics, safety pharmacology, general toxicology, genetic toxicity (in vivo and in vitro), and reproductive toxicity.

The pharmacology studies submitted to this NDA demonstrate that the mechanism of action of osimertinib is consistent with an established pharmacological class of kinase inhibitor. (b) (4) of EGFR and demonstrates increased activity against EGFR proteins containing the T790M mutation as well as the L858R mutation and exon 19 deletion mutations. Osimertinib and/or its active metabolites (AZ5104 and AZ7550) also inhibit wild type EGFR as well as HER2, HER4, ACK1, and BLK, with IC_{50}S that are clinically achievable at the recommended clinical dose of 80 mg daily. Because it is an irreversible inhibitor, osimertinib binding results in prolonged inhibition of downstream signaling from EGFR and its mutants. Osimertinib also inhibited downstream signaling from HER2 and HER3 overexpressing cell lines.

The major route of elimination for osimertinib and its metabolites in animals and humans is in the feces and the drug appears to be highly protein bound. Osimertinib was negative in all *in vitro* and *in vivo* assays for genotoxicity. There was a single impurity, (b) (4) identified as positive in an in vitro bacterial reverse mutation assay. While the specification of (b) (4)% for this impurity is (b) (4) for a genotoxic impurity, it is below the ICH Q3A limit of 0.15%, and would result in a daily intake of no more than (b) (4). As this drug is intended for the treatment of patients with (b) (4) cancer, the proposed specification is acceptable at this time, (b) (4)
Carcinogenicity studies were not conducted for this drug and, consistent with the ICH S9 guidance, are not warranted for a drug for the treatment of patients with advanced cancer.

The Sprague Dawley rat and the Beagle dog were the primary models used to investigate the safety of osimertinib. The major targets for toxicity in both rats and dogs were the gastrointestinal tract, skin, and eyes. In rats the lungs and the kidney were additional target

organs, though GI effects were the most significant findings. In the dog, ocular lesions were dose-limiting. Effects on male and female reproductive organs were also noted in both 1-month and 13-week toxicology studies, including decreased corpora lutea and decreased signs of estrus in females and spermatid atrophy and retention in males. While some potential for inhibition of the hERG potassium channel was observed, the IC_{50} was above the expected free drug concentration of osimertinib determined in clinical studies using the 80 mg dose and data from the in vivo cardiovascular safety pharmacology study did not suggest clear effects on QTc prolongation at tolerable doses. QTc prolongation has occurred at a low frequency in clinical trials. In vivo cardiac safety pharmacology studies did suggest equivocal findings of decreased contractility dogs and guinea pigs. Overall, the toxicities seen with osimertinib were consistent with toxicities observed with other approved EGFR inhibitors.

The 2 active metabolites of osimertinib (AZ7550 and AZ5104) are present in humans at levels approximately 10% those of the parent. Both metabolites were present at sufficient levels in animals used for the toxicological assessment of osimertinib. In addition, due to problems with the detection method for AZ5104, the Applicant conducted a separate toxicology study to investigate the safety of this metabolite. The study confirmed no unique toxicities resulting from exposure to AZ5104, even at levels that exceeded its exposure in humans.

The Applicant included an assessment of osimertinib-mediated effects on male fertility in the 13 week toxicology study. Osimertinib treatment for 65 days was associated with decreases in male fertility, demonstrated by decreased numbers of live fetal implants due primarily to increases in pre-implantation loss in untreated females crossed with males treated at the osimertinib high dose level of 10 mg/kg. These effects occurred at osimertinib exposures of approximately 0.5X that observed in humans at the recommended dose of 80 mg. While the mechanism for this apparent effect on male fertility has not been fully elucidated, based on these findings the pharmacology/toxicology team recommends that males with female partners of reproductive potential use contraception during treatment with TAGRISSO and for 4 months following the final dose of the drug.

A multi-phase GLP-compliant pilot study in rats was conducted to assess the reproductive toxicity of osimertinib. Pregnant dams were administered osimertinib daily from gestation day (GD) 2 to 20, GD6-20, or GD6-lactation day (LD6). Early administration of osimertinib at the 20 mg/kg dose level (resulting in exposures approximately 1.5 times the exposure at Cmax in patients treated at the recommended dose of 80 mg) resulted in increased post-implantation loss and early embryonic death. While embryofetal loss did not occur in dams treated from GD6-20, there were equivocal findings of teratogenicity (craniofacial and lung malformations) that occurred in fetuses from treated animals at doses as low as 1 mg/kg (approximately 0.1 times the AUC in patients at the 80 mg dose). While these findings were rare, because the study was a pilot study with a low number of animals and there were no findings in concurrent controls, a treatment related effect could not be ruled out, particularly considering the pharmacology of the drug, and malformations described in EGFR knockout models. Finally, when osimertinib was administered at dose levels greater than or equal to 20 mg/kg from GD6-LD6, there were increases in embryolethality including postnatal death, as well as mild decreases in fetal birth weight; decreases in weight were enhanced between LDs 4 and 6. Osimertinib and its metabolites were detectable in suckling pups within 2 hours of osimertinib administration to the

dams suggesting that osimertinib is present in milk. Based on this study and its mechanism of action, a warning for embryofetal toxicity is recommended. In addition women are advised not to breastfeed while taking TAGRISSO or, based on the drug's plasma half-life, for 2 weeks after the final dose. Due to histopathological effects on the female reproduction system demonstrated in the 1-month rat study that did not fully recover by the end of the study, the Applicant's proposed recommendation of the use of contraception in females of reproductive potential during and for 6 weeks following the final dose of TAGRISSO is reasonable.

Recommendations: I concur with the conclusion of Dr. Weis that the pharmacology and toxicology data support the approval of NDA 208065 for the use of TAGRISSO the treatment of patients with (b) (4) metastatic epidermal growth factor receptor (EGFR) T790M mutation-positive-non-small-cell lung cancer (NSCLC), as detected by an FDA-approved test, who have progressed on or after EGFR TKI therapy. There are no outstanding nonclinical issues that would prevent the approval of osimertinib for the proposed indication.

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/s/

WHITNEY S HELMS
10/08/2015

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:	NDA 208,065
Supporting document/s:	001
Applicant's letter date:	05 June 2015
CDER stamp date:	05 June 2015
Product:	TAGRISSO (osimertinib)
Indication:	Non-Small Cell Lung Cancer (NSCLC)
Applicant:	AstraZeneca Pharmaceuticals LP 1800 Concord Pike Wilmington, DE 19803 UNITED STATES
Review Division:	DHOT/DOP2
Reviewer:	Shawna L. Weis, PhD
Supervisor/Team Leader:	Whitney S. Helms, PhD
Division Director:	John K. Leighton, PhD, DABT / Patricia Keegan, MD
Project Manager:	Ingrid Y. Fan

Disclaimer

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1 Executive Summary

1.1 Introduction

AstraZeneca (the Applicant) has submitted NDA 208065 to support the approval of TAGRISSO (osimertinib) for the treatment patients with (b) (4) metastatic epidermal growth factor receptor (EGFR) T790M mutation-positive-non-small-cell lung cancer (NSCLC), as detected by an FDA-approved test, who have progressed on or after EGFR TKI therapy. Osimertinib is an irreversible inhibitor of EGFR that shows greater inhibitory activity against mutant EGFR isoforms, including the T790M and L858R point-mutants, or exon 19 deletion mutants, than against wild-type EGFR. The product received breakthrough designation for treatment of the proposed patient population on 16 April 2014.

1.2 Brief Discussion of Nonclinical Findings

The Applicant conducted a series of pharmacology studies to assess the relative inhibitory activity of osimertinib against mutant forms of EGFR (EGFRm) relative to wild-type (wt) EGFR. Osimertinib exhibited greater anti-tumor activity in murine tumor models that are predominantly driven by mutant EGFR isoforms, including T790M, L858R, or exon 19 deletion mutants, than in those that express wild-type EGFR, a finding that correlated with the increased biochemical activity of osimertinib against EGFR mutants relative to wild type EGFR. In vitro, osimertinib also exhibited the potential to inhibit other members of the EGFR family (HER2, HER3, and HER4) as well as ACK1 and BLK at clinically relevant concentrations. While some potential for inhibition of cardiac ion channels, including hERG, and the L-type calcium channels, was observed, data from the in vivo cardiovascular safety pharmacology study did not suggest notable electrocardiology effects at tolerable doses, though equivocal findings of decreased contractility occurred in dogs and guinea pigs.

AZD9291 was evaluated in 13-week repeat-dose toxicology studies in the rat and the dog. Consistent with its pharmacologic mechanism of action as an inhibitor of EGFR, administration, administration of AZD9291 to dogs and rats was associated with adverse gastrointestinal (GI) clinical symptoms (loose feces, and/or inappetence), skin lesions (ulceration), and ocular lesions (corneal atrophy). Other histological target organs included the lungs (rats and dogs; macrophage infiltration) and kidney (rat). In the dog, ocular lesions were dose-limiting, whereas in the rat, GI effects were dose-limiting.

The highest doses of osimertinib administered to rats in the 13-week toxicology studies produced plasma exposures that were similar to the clinical Cmax of 501 nM and AUC of 11258 nM*h at the recommended dose of 80 mg daily. Plasma AUCs achieved in the dog study at the highest-tolerated dose of 6 mg/kg/day were approximately 0.48X those observed in patients who received the 80 mg daily oral dose.

Two major pharmacologically-active metabolites were identified in humans and animals: AZ13575104 and AZ13597550. The Applicant demonstrated that both metabolites exhibit comparable pharmacodynamic and target-inhibitory activities as osimertinib. In humans, plasma exposure to each metabolite is approximately 10% of those of osimertinib on the basis of both AUC and $C_{max.}$ While exposure to both metabolites was demonstrated in the rat and the dog, the Applicant only calculated TK parameters for AZ1397550 due to problems with bioanalytical reproducibility for AZ13575104. In the rat, exposure to AZ13597550 was approximately 0.8X those observed in humans on an AUC basis. In the dog, exposure to AZ13597550 (AZ7550) was approximately 0.51X that of humans on an AUC basis. Due to difficulties with the detection assay for AZ13575104 (AZ5104) in animals, the Applicant performed an additional 1-month metabolite-characterization study of AZ5104 in the rat. This study revealed no new toxicities at doses that exceeded the clinical exposure of this metabolite in patients treated with osimertinib. Thus, the Applicant has adequately characterized the toxicity of osimertinib and its two major metabolites in the overall toxicological assessment for osimertinib.

Osimertinib was non-mutagenic in bacterial and mammalian cell assays when tested in the presence and absence of metabolic activation by Aroclor-induced rat S9 liver fractions. Osimertinib was also and negative for induction of structural chromosome aberrations in primary human peripheral blood mononuclear cells both in the presence and absence of metabolic activation by Aroclor-induced rat S9 liver fractions and the in vivo rat micronucleus assay.

The Applicant assessed the potential for reproductive toxicity in a variety of studies. In an assessment of male fertility that was conducted in the context of the 13 week rat study, males were treated with osimertinib for 65 days prior to mating with untreated females. Osimertinib treatment was associated with decreases in male fertility, as demonstrated by decreased numbers of live fetal implants. These reductions were primarily due to increases in pre-implantation loss in naïve females crossed with males treated at the high dose level of 20 mg/kg and occurred at osimertinib exposures of approximately 0.5X those observed in humans at the recommended dose of 80 mg. While the mechanism for this apparent effect on male fertility has not been fully elucidated, a recommendation for male contraception for at least 4 months during treatment with TAGRISSO is warranted.

The Applicant conducted a GLP-compliant dose range-finding study of AZD9291 in pregnant dams. When administered to dams between gestation days (GDs) 2-20 (i.e. prior to implantation through the end of organogenesis), osimertinib exposure led to increased post-implantation loss and early embryonic death. The adverse effects on reproduction occurred at maternal exposures of approximately 1.5-times the clinical C_{max} observed in patients who receive the 80 mg oral dose. No clear adverse effects on pregnancy maintenance were noted when osimertinib was administered between GD6 and GD20 at doses up to 30 mg/kg; however, an equivocal increase in the rate of fetal malformations (anencephaly and missing lung lobe) and variations was observed in treated litters relative to those of concurrent controls at doses greater than or equal to 1 mg/kg (approximately 0.1 times the AUC in patients at the 80 mg dose). Given the

small number of dams included in the study and the mechanism of action of osimertinib, the relationship of these findings to osimertinib treatment cannot be excluded.

When osimertinib was administered to pregnant dams during organogenesis through lactation day 6 (GDs 6-LD6), an increase in total litter loss including in postnatal death occurred at 30 mg/kg/Day. At 20 mg/kg, there was an increase in postnatal death as well as a slight reduction in mean pup weight at birth that increased in magnitude between lactation Days 4 and 6.

Toxicokinetic exposures were also measured in fetuses and/or nursing pups. Fetal exposure to osimertinib at the end of gestation (GD20) was approximately 36% of that observed in dams on GD16. Fetal exposure to AZ13597550 was also demonstrated; however, fetal metabolite levels were relatively low - less than 7% of maternal levels. A low level of exposure to osimertinib and its metabolite was demonstrated in nursing pups, suggesting that osimertinib and/or AZ13597550 may be excreted in milk. At the maternal C_{max} (2 hours post-dose), neonatal exposure to osimertinib was approximately 2% of the maternal exposure levels. Peak neonatal exposure to AZ13597550, however, was approximately 12% of maternal levels at 2 hours post-maternal-dose.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical perspective, TAGRISSO is approvable for the treatment of patients with (b) (4) metastatic epidermal growth factor receptor (EGFR) T790M mutation-positive-non-small-cell lung cancer (NSCLC), as detected by an FDA-approved test, who have progressed on or after EGFR TKI therapy.

1.3.3 Labeling

A separate labeling review will be performed if warranted.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)
1421373-65-0

Generic Name
Osimertinib

Code Name
AZD9291, (b) (4)

Chemical Name

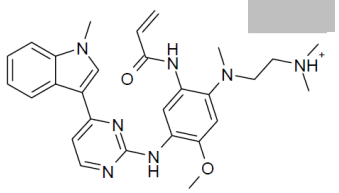
(b) (4)

Molecular Formula/Molecular Weight

$C_{28}H_{33}N_7O_2.CH_4O_3S$ / AZD9291 mesylate: (b) (4) (AZD9291 free base: (b) (4)

Structure or Biochemical Description

(b) (4)



Excerpted from the applicant's submission

Pharmacologic Class

kinase inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 117,879

2.3 Drug Formulation

Table 1: Composition of AZD9291 film-coated tablets

Components	Quantity (mg per unit)		Function	Standard
	40 mg	80 mg		
Tablet core				
AZD9291 mesylate[a]	47.7	95.4	Active	AstraZeneca
Mannitol	(b) (4)			NF
Microcrystalline cellulose				NF
Low-substituted hydroxypropyl cellulose				NF
Sodium stearyl fumarate				NF
Nominal core tablet weight				
Tablet coating[b, c]				
Polyvinyl alcohol				USP
Titanium dioxide				USP
(b) (4)				NF
Talc				USP
Yellow ferric oxide				NF
Red ferric oxide				NF
Black ferric oxide				NF
(b) (4)				USP
(b) (4)				
[illegible] The tablet coating ingredients listed may be included as a propriety composite, eg				(b) (4)
(b) (4)				

Excerpted from the applicant's submission

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None. Based on the Applicant's batch analysis of Batch 02-kcwg806-87, all specified impurities that exceed the ICH limits have been qualified in the 3-month rat toxicology study. While the [redacted] (b) (4) specification of (b) (4) % is high for a genotoxic impurity, it is below the ICH Q3A limit of 0.15%, and would result in a daily intake of no more than (b) (4) µg. As this drug is intended for the treatment of patients with advanced cancer, the proposed specification is acceptable at this time.

2.6 Proposed Clinical Population and Dosing Regimen

Patients with advanced NSCLC who have progressed following prior therapy with an epidermal growth factor receptor tyrosine kinase inhibitor agent.

2.7 Regulatory Background

Date	Event
11 June 2013	IND Submission
06 November 2013	Fast Track Designation Granted
14 January 2014	Type C Meeting
16 April 2014	Breakthrough Designation (BTD) Granted
12 August 2014	CMC Teleconference (Informal)
04 September 2014	Orphan Drug Designation Granted
02 October 2014	BTD Type B Meeting
07 October 2014	BTD Type B Meeting CMC
09 December 2014	Pre-NDA Meeting
26 January 2015	Initiate Rolling NDA Submission
05 June 2015	Completed NDA Submission

Excerpted from the applicant's submission

3 Studies Submitted

3.1 Studies Reviewed

PHARMACOLOGY

- AZD9291, AZ13575104, and A13597550 secondary kinase selectivity (Pharmacology Report 01)
- Effect of AZD9291 on In Vivo Tumour Growth of EGFR Single Mutant, EGFR Double Mutant and EGFR Wild Type Xenograft Models (Pharmacology Report 05, Amendment 1)
- Effect of AZ13575104, an Active Metabolite of AZD9291, on In Vivo Tumour Growth of EGFR Single Mutant, EGFR Double Mutant and EGFR Wild Type Xenograft Models (Pharmacology Report 06, Amendment 1)
- Effect of AZD9291 on phosphorylated biomarkers from in vivo xenografts representing EGFR wild type (A431), activating (PC9) and resistant (H1975) NSCLC disease settings (Pharmacology Report 07, Amendment 1)
- Effect of AZD9291 on In Vivo Tumour Growth of EGFR Double Mutant bi-transgenic NSCLC model (Pharmacology Report 10)
- Mass Spectrometric Analysis of Recombinant EGFRm (T790M) to Determine the Binding Site of (b) (4) (Pharmacology Report 11)
- In Vitro Enzyme and Cellular primary pharmacology for AZD9291 and metabolites AZ13575104 and AZ13597550 (Pharmacology Report 12, Amendment 1)
- To test the ability of AZD9291, AZ13575104 and AZ13597550 to inhibit in vitro proliferation of a panel of NSCLC cell lines expressing either mutant or wild type EGFR (Pharmacology Report 13, Amendment 1)
- AZD9291 Mechanism of action (Pharmacology Report 14, Amendment 1)
- Long Term Administration of AZD9291 in the NCI-H1975 NSCLC Xenograft Model (Pharmacology Report 16, Amendment 1)
- Long Term Administration of AZD9291 and Gefitinib in a PC9 NSCLC Xenograft Model (Study 2) (Pharmacology Report 17, Amendment 1)
- Long Term Administration of AZD9291, Gefitinib and Afatinib in the NCI-H3255 NSCLC Xenograft Model (Pharmacology Report 18, Amendment 1)
- In Vivo Anti-tumour Efficacy of AZD9291 in Brain Metastasis Xenograft Models in Mice (Pharmacology Report 19)
- In vitro investigation to determine whether the EGFR T790M mutation would be an acquired resistance mechanism to AZD9291 when used in a first line mutant EGFR NSCLC setting (Pharmacology Report 20, Amendment 1)
- In vitro investigation of resistance to gefitinib, afatinib, (b) (4) or AZD9291 in PC9 cells comparing time to resistance in an EGFR mutant first line setting. (Pharmacology Report 21, Amendment 1)
- To test the activity of AZD9291 and its metabolite AZD5104 and competitor compounds against HER2 and HER3 in ligand driven system and in an amplified setting (Pharmacology Report 22)

SECONDARY PHARMACOLOGY

- AZD9291: Selectivity Screening in Radioligand Binding, Enzyme, Functional and

Electrophysiological Assays in vitro (Report 1112SY)
- ❖ AZ13575104: Selectivity Screening in Radioligand Binding, Enzyme, Functional and Electrophysiological Assays in vitro (Report 1120SY)
- ❖ AZ13597550: Selectivity Screening in Radioligand Binding, Enzyme and Electrophysiological Assays in vitro (Report 1121SY)
- ❖ AZ13597550 : Selectivity Screening in Cardiac Ion Channel Electrophysiological Assays in vitro (Report 3472SV)
- ❖ AZ13575104 : Selectivity Screening in Cardiac Ion Channel Electrophysiological Assays in vitro (Report 3473SV)
- ❖ (b) (4) : Selectivity Screening in Cardiac Ion Channel Electrophysiological Assays in vitro (Report 3535SV)

SAFETY PHARMACOLOGY
- ❖ AZ13540484 and (b) (4) Cardiovascular Effects in Anaesthetised Guinea-Pigs following Intravenous Infusion (0264SG)
- ❖ AZD9291: Cardiovascular Effects in Conscious, Telemetered Beagle Dogs following Single Oral Administration - Report Amendment (1352ZD)
- ❖ AZD9291: Nervous System, Visual, Respiratory and Gastrointestinal Transit Effects in the Han Wistar Rat following Single Oral Administration (3464SR)
- ❖ Cardiovascular Effects of (b) (4) in vivo: Rat Telemetry (ONC.000-574-813)
- ❖ AZD9291: Effects on Human Ether-a-go-go-related Gene (hERG) Encoded Potassium Channel in vitro (VKS0795)

ADME
- ❖ Validation of a High Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS) Method for the Determination of AstraZeneca AZD9291, AZ13597550 and AZ13575104 Concentrations in Rat and Dog Plasma (D9291 KPV005)
- ❖ AZD9291: In Vitro Covalent Binding of [^{3}H]-Labelled AZD9291 to Human Hepatic Proteins in Human Hepatocyte Incubations (111123_CVB_KXZZ856)
- ❖ AZD9291: The Tissue Distribution of Total Radioactivity in the Rat Following Oral Administration of [^{14}C]AZD9291 (Quantitative Whole Body Autoradiography) (Report #8265542)
- ❖ AZD9291: Mass Balance and Radioactive Pharmacokinetics Following Oral and Intravenous Administration of [^{14}C]AZD9291 to the Rat (Report #196187)
- ❖ AZD9291: The disposition of [14C]AZD9291 following intravenous and oral administration in the dog (Report 197725)

TOXICOLOGY
- ❖ AZD9291: Three Month Oral (Gavage) Toxicity Study in the Rat – Report Amendment (Report 52648)
- ❖ AZD9291: Three Month Oral (Gavage) Toxicity Study in the Dog (Report 526253
- ❖ AZD9291: Genetic Toxicity Evaluation Using the Bacterial Reverse Mutation Test in Salmonella typhimurium TA 1535, TA 100, TA 1537 and TA 98 and Escherichia coli WP2uvrA (pKM101) (Report #793061)
- ❖ AZD9291: Genetic Toxicity Evaluation using the Mouse Lymphoma Cell Thymidine Kinase Locus Assay (Report #793056)
- ❖ AZD9291: Genetic Toxicity Evaluation Using the Rat Micronucleus Test After

Two Oral Doses (Report #793538)
- AZD9291: Oral (Gavage) Investigative Dose Range Finding Embryofetal Development and Pre and Post Natal Study in the Rat (Report #496800)
- AZD9291: Evaluation of in vitro phototoxicity on Balb/c 3T3 fibroblasts using the Neutral Red Uptake assay (8273754)

IMPURITY AND METABOLITE QUALIFICATION
- AZD9291: One Month Oral (Gavage) Toxicity Study in the Rat (Report # 528622)
- AZ13575104 and (b) (4): One Month Investigatory Toxicity Study in the Rat (3500KR)
- (b) (4) Genetic Toxicity Evaluation Using the Bacterial Reverse Mutation Test in Salmonella typhimurium TA1535, TA100, TA1537 and TA98 and Escherichia coli WP2 uvrA (pKM101) (Report # 8303534)
- (b) (4): Genetic Toxicity Evaluation Using the Bacterial Reverse Mutation Test in Salmonella typhimurium TA1535, TA100, TA1537 and TA98 and Escherichia coli WP2 uvrA (pKM101) (Report # 8303535)
- (b) (4): Genetic Toxicity Evaluation Using the Bacterial Reverse Mutation Test in Salmonella typhimurium TA1535, TA100, TA1537 and TA98 and Escherichia coli WP2 uvrA (pKM101) (Report # 8303536)
- (b) (4): Genetic Toxicity Evaluation Using the Bacterial Reverse Mutation Test in Salmonella typhimurium TA1535, TA100, TA1537 and TA98 and Escherichia coli WP2 uvrA pKM101(Report # 8306010)

3.2 Studies Not Reviewed

ADME
- Exposure in male beagle dogs following oral administration f a 20 mg dose of AZD9291 mesylate salt as a solution, or solid in a capsule and tablets (Report CPK-13-0099)
- Pharmacokinetics of AZD9291 following intravenous and oral administration in the rat (Report 8308627)
- AZD9291: In vivo pharmacokinetics in plasma, brain and H1975 tumour of AZD9291 in tumour bearing SCID mice following a single oral dose at 5 and 25 mg/kg (Report #BE000343-60)
- AZD9291: Validation of an Analytical Method for the Determination of AZD9291 and Metabolites (AZ13597550 and AZ13575104) in Rat Plasma Using Protein Precipitation Followed by LC-MS/MS (Report #317706)
- AZD9291: Partial Validation of a Method for the Determination of AZD9291 and Metabolites (AZ13597550 and AZ13575104) in Dog Plasma Using Protein Precipitation Followed by LC-MS/MS (Report #318322)
- Investigation of ISR Failure and Further Development of an Analytical Method for the Determination of AZ13575104 in Rat and Dog Plasma by LC-MS/MS (Report # 319719)
- Qualification of a Method for the Determination of AZD9291, AZ13575104 and AZ13597550 in Rat Plasma using Protein Precipitation followed by Liquid Chromatography with Tandem Mass Spectrometric Detection (LC-MS/MS) Study (Report #8309952)

- Administration of AZD9291 in the Rat Following Oral Administration to Produce Plasma to Investigate a Rat Bioanalytical Method (Report #8309953)
- Validation of a High Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS) Method for the Determination of AstraZeneca AZD9291, AZ13597550 and AZ13575104 Concentrations in Rat and Dog Plasma (Report #D9291 KPV005)

TOXICOLOGY

- (b) (4) Maximum Tolerated Dose and Repeated Dose Oral Toxicity Study in the Dog (1324DD)
- AZD9291: One Month Oral Toxicity Study with Assessment of Recovery in the Dog - Report Amendment (1351AD)
- (b) (4) 7 Day Oral Gavage MTD Toxicity Study in the Rat (3278DR)
- (b) (4): 14 Day Oral Toxicity Study in the Rat (3310DR)
- AZD9291: One Month Oral Toxicity Study with Assessment of Recovery in the Rat (3416AR)
- (b) (4) Maximum Tolerated Dose and Repeated Dose Oral Toxicity Study in the Dog (Report # 1324DD)

3.3 Previous Reviews Referenced

- Review of IND 117,879 by Shawna L. Weis, PhD

4 Pharmacology

4.1 Primary Pharmacology

4.1.7 In Vitro Enzyme and Cellular primary pharmacology for AZD9291 and metabolites AZ13575104 and AZ13597550 (Pharmacology Report 12, Amendment 1)

The purpose of this study was to evaluate the activity of AZD9291 and its active metabolites, AZD13575104 and AZD13597550 in kinase assays to determine the extent to which the compounds inhibited mutant EGFR kinase activity. Compounds were tested in a panel of cells exhibiting different EGFR mutational status (NCI H1975 (T790M/L858R); PC9 (Ex19del) and LOVO (wild-type; stimulated with 20 nM EGF for 20 minutes prior to assay)). Cells were incubated with compound for 2 hours in a 12-point dose-response sequence, and EGFR phosphorylation was measured using a modified ELISA system with an anti-phospho-EGFR antibody.

As indicated in Table 2, AZD9291 or its metabolites inhibited cell proliferation with IC_{50}s of between 2-45 nM. IC_{50}s against cells expressing WT EGFR ranged from 33-786 nM.

Table 2: Inhibition of cellular phosphorylation (pEGFR) by AZD9291 and its metabolites (IC50, µM)

Compound	NCI H1975 (T790M/L858R)	PC9 (Ex19del)	LOVO (Wild-Type)
AZD9291	0.015	0.017	0.480
AZ13575104	0.002	0.002	0.033
AZ13597550	0.045	0.026	0.786

The Applicant also evaluated enzyme inhibition using a homogenous time resolved fluorescence (HTRF) assay, the experimental details of which were not provided in the study report. As indicated in Table 3, IC_{50}s for pEGFR production ranged from 0.5-10 nM in this assay.

Table 3: pEGFR suppression by AZD9291 and its metabolites in the HTRF assay (IC50, µM)

Compound	NCI H1975 T790M/L858R	PC9 Ex19del
AZD9291	0.0025	0.0075
AZ13575104	0.0005	0.0024
AZ13597550	0.0036	0.01

4.1.8 To test the ability of AZD9291, AZ13575104 and AZ13597550 to inhibit in vitro proliferation of a panel of NSCLC cell lines expressing either mutant or wild type EGFR (Pharmacology Report 13, Amendment 1)

The purpose of this study was to assess the effects of AZD9291 and two of its active metabolites (AZ13575104 and AZ13597550) on cell proliferation and survival, in a panel EGFR-mutant and -WT cell lines. Cells were cultured in the presence of escalating concentrations of AZD9291, AZ13575104 or AZ13597550 along with the vital dye, Sytox Green. Viable cells (those stained with Sytox Green) were counted, then cells were permeabilized and re-stained, then re-counted to determine the total number of cells per treatment; data were expressed as proportion of live cells relative to the total number of cells/well.

IC_{50}s (nM) for cell proliferation are provided in Table 4. AZD9291 and both pharmacologically active metabolites exhibited greater potency toward mutant forms of EGFR than against WT cell lines (Calu3, Calu6 and NCI-H203).

All three compounds exhibited significantly greater inhibitory activity toward the EGFR mutant cell lines, relative to wild-type EGFR-expressing cell lines.

Table 4: Mean IC_{50}s (nM) for AZD9291 and its metabolites, AZ13575104 and AZ1397550 in cultures of NSCLC cells bearing WT or mutant EGFR

	H1975 (T790M/L858R)	PC9 (Ex19del)	Calu 3 (wild type)	PC9 VanR (Ex19del/T790M)	NCI-H2073 (wild type)	Calu 6 (wild type)
AZD9291	**11**	**8**	**650**	**40**	**461**	**4089**
	(6, 19)	(7, 9)	(457, 924)	(30, 54)	(230, 924)	(3551, 4708)
	n=17	n=17	n=17	n=8	n=12	n=15
AZ13575104	**3**	**3**	**80**	**7**	**28**	**2041**
	(2, 5)	(2, 3)	(28, 231)	(3, 17)	(7, 107)	(1650, 2525)
	n=11	n=11	n=11	n=3	n=8	n=9
AZ13597550	**30**	**16**	**954**		**1361**	**3954**
				ND		
	n=2	n=2	n=2		n=1	n=2

(*Excerpted from the Applicant's NDA)*

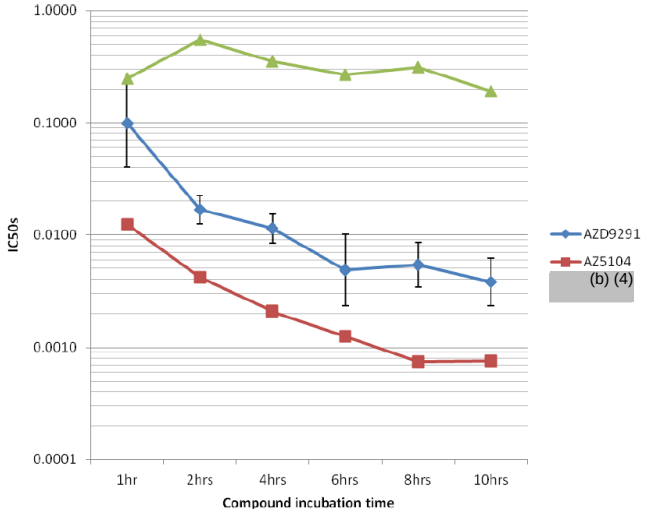
4.1.9 AZD9291 Mechanism of action (Pharmacology Report 14, Amendment 1)

The purpose of this study was to characterize the activity of AZD9291, as measured by pEGFR suppression, in EGFR-WT and -mutant cell lines, and to demonstrate the time-dependent mechanism of pEGFR-inhibition. A secondary objective was to assess the relative potency of AZD9291 against other EGFR inhibitors.

As illustrated in Figure 1 through Figure 3, the IC_{50} for pEGFR formation was time dependent, as illustrated by the left-shifted (i.e. lower) IC_{50} estimate that occurred with increasing periods of incubation. Maximal inhibition was achieved after an incubation period of approximately 8-10 hours.

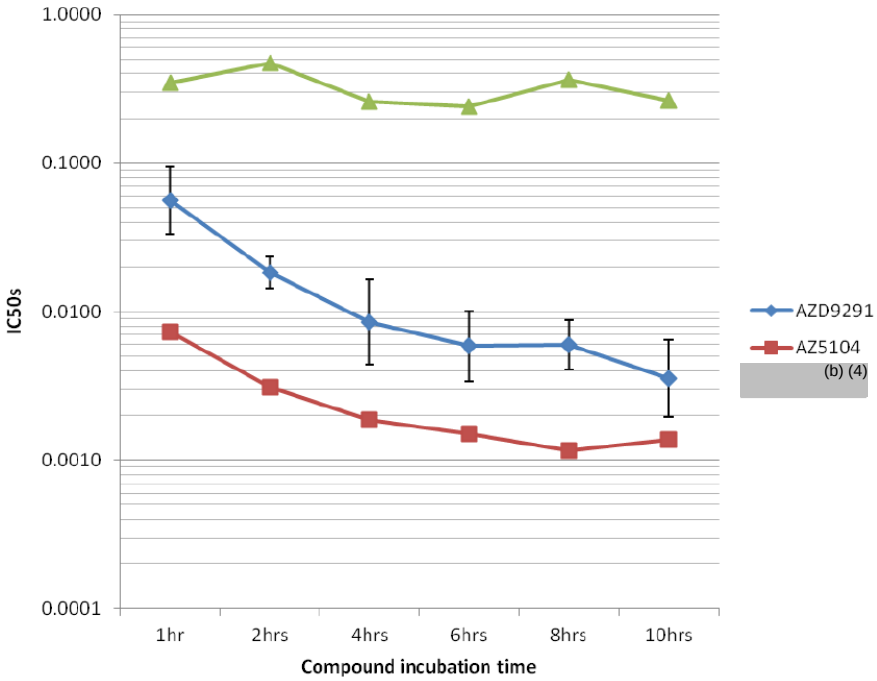
As observed in other studies, the activity of AZD9291 was greater against EGFR-mutant cell lines (e.g. NCI H1975, PC9 VanR, PC9, NCI H3255 and NCI H1650) than against WT cell lines (e.g. LOVO, A431 and NCI H2073) See Figure 1 and Figure 2 compared with Figure 3 (note that the Applicant states that (b) (4) is a reversible EGFR inhibitor, but no further information was provided about this agent).

Figure 1: Time-course for IC_{50} determination (pEGFR formation) in NCI H1975 cells (L858R/T790M)



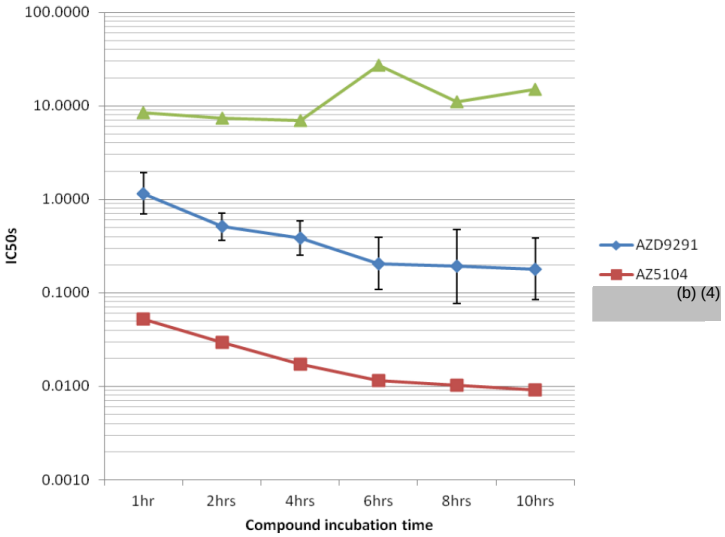
(*Excerpted from the Applicant's NDA)*

Figure 2: Time-course for IC_{50} determination (pEGFR formation) in PC9 cells (Ex19del)



(*Excerpted from the Applicant's NDA)*

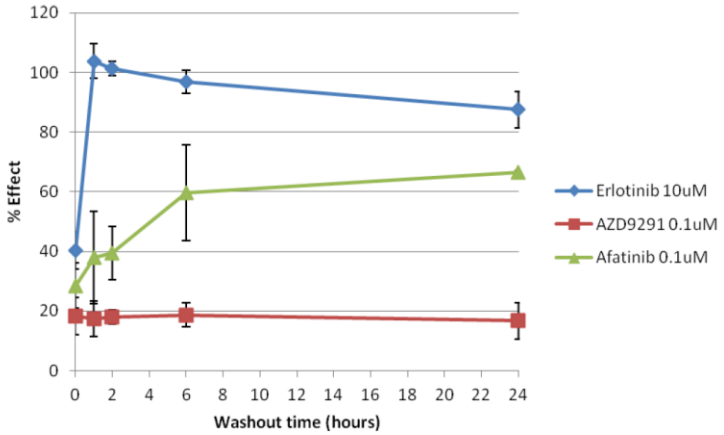
Figure 3: Time-course for IC50 determination (pEGFR formation) in LOVO cells (WT)



(*Excerpted from the Applicant's NDA)*

In addition, suppression was irreversible for the duration evaluated, as suppression of pEGFR was sustained in treated NCI H1975 cells for at least 48 hours following washout (Figure 4). In T790M/L858R mutants, (H1975 cells) this effect was similar to that observed following treatment with afatinib (another irreversible EGFR inhibitor), and is consistent with a mechanism of irreversible binding. T790M cells treated with erlotinib, a reversible inhibitor, exhibited rapid recovery of pEGFR following drug-washout. Data are presented as % pEGFR relative to vehicle (DMSO)-treated controls.

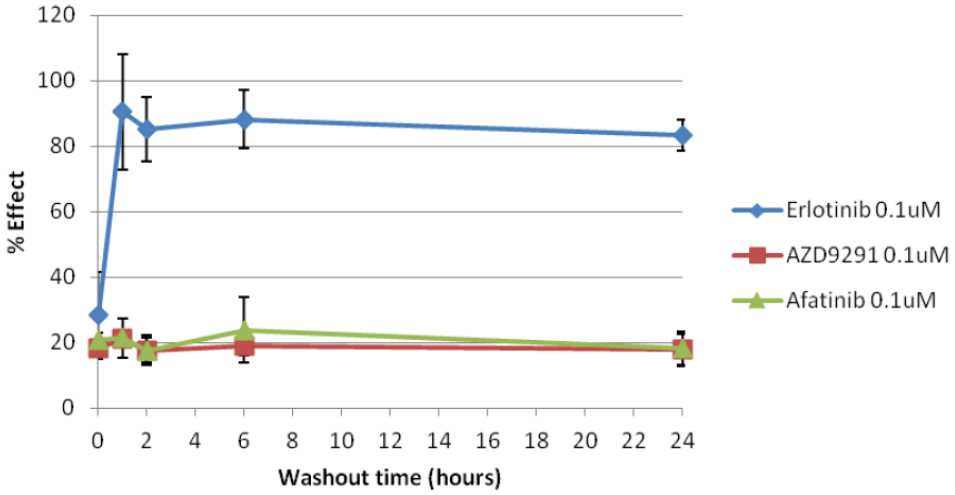
Figure 4: Timecourse of pEGFR suppression in NCI H1975 (T790M/L858R) following drug-washout (%DMSO control vs. time)



(*Excerpted from the Applicant's NDA)*

In Exon 19-deleted cells, AZD9291 and afatinib exhibited comparable potency and similar kinetics of pEGFR suppression following drug-washout (Figure 5)

Figure 5: Timecourse of pEGFR suppression in PC9 (Ex19Del) cells following drug-washout (%DMSO control vs. time)



(*Excerpted from the Applicant's NDA)*

4.1.1 AZD9291, AZ13575104, and A13597550 secondary kinase selectivity (Pharmacology Report 01)

The purpose of this study was to assess the off-target activities of AZD9291 and its active metabolites, AZ5104 and AZ7550. As illustrated in Table 5, AZD9291 and its metabolites significantly inhibit (>80%) numerous kinases when tested at 1 µM, which is twice the clinical Cmax of 500 nM. The Applicant states that targets listed in bold have

(b) (4)

Table 5: Percent kinase inhibition at 1μM and IC50 values for kinases inhibited by AZD9291 or its metabolites

Kinase	AZD9291 % Kinase Inhibition (@ 1μM)	AZD9291 IC50 Kinase Inhibition (nM)	AZ5104 % Kinase Inhibition (@ 1μM)	AZ5104 IC50 Kinase Inhibition (nM)	AZ7550 % Kinase Inhibition (@ 1μM)	AZ7550 IC50 Kinase Inhibition (nM)
ACK1	100	71, 128	100	27, 66	76	156, 344
ALK	66	231, 1622	89	97, 175	58	420, 1804
BLK	**100**	**168, 442**	**100**	**27, 56**	**50**	**977, 1469**
BMX	**19**	**2425, 2381**	**68**	**505, 359**	**14**	**>10000, >10000**
BRK	87	255, 258	99	45, 57	56	843, 420
BTK	**64**	**699, 989**	**91**	**132, 451**	**12**	**5104, 4433**
ErbB2	**97**	**116**	**98**	**61**	**89**	**700**
ErbB4	**94**	**67, 46**	**97**	**7, 11**	**81**	**195, 222**
FAK	67	598, 774	95	136, 320	37	995, 1866
FES	39	389, 1985	83	127, 468	59	449, 1028
FGFR1	77	>10000	31	6018	12	>10000
FLT3	55	562, 2392	75	129, 911	39	302, 2128
FLT4	78	678, 983	82	142, 404	50	1784, 3190
IGF1-R	64	941, 1775	87	78, 395	38	1005, 4119
Ins R	66	432, 880	90	127, 226	39	1256, 1803
IRR	21	281, 2210	95	423, 342	19	840, 3510
ITK	**17**	**6956, 10000**	**81**	**925, 1529**	**23**	**>10000, >10000**
JAK3	**44**	**2640, 3436**	**49**	**1358, 2556**	**19**	**>10000, >10000**
LRRK2	75	375	65	993	35	3933
MLK1	88	85, 409	63	141, 1289	69	88, 448
MNK2	91	95, 155	91	62, 171	75	228, 585
PYK2	59	682, 1476	81	284, 536	28	2288, 4653
TEC	**79**	**420, 497**	**91**	**118, 219**	**43**	**1317, 2191**
TrkB	0	>10000	100	>10000	100	>10000
Txk	**66**	**1590, 2519**	**83**	**426, 621**	**29**	**2443, 5541**
YES	86	8193	22	4803	4	>10000

(Excerpted from Applicant's submission)

The Applicant further evaluated the effect of AZD9291 and its metabolites on IGF1R using a cell-based (pIGF1R) assay in murine embryonic fibroblasts that express high levels of exogenous human IGF1R. As shown in Table 6, IC_{50}s for IGF1R are ~4-20-fold higher than peak clinical exposures, and are therefore likely beyond the range of physiologically-relevant exposures.

Table 6: IC50 for inhibition of pIGF1R by AZD9291, AZ5104 or A7550 (mean±95%CI; nM)

Compound	Mean IC_{50} Inhibition of IGF1R
AZD9291	4614 (1997, 10664) (n=4)
AZ5104	1915 (1350, 2714) (n=4)
AZ7550	>10000, >10000 (n=2)

(b) (4) Reports; 9137, 6649, 6796, 7106

(Excerpted from Applicant's submission)

The off-target activities of AZD9291 and AZ5104 against HER2 activation (suppression of pHER2) were also evaluated in NIH3T3 cells. As shown in Table 7, AZ5104 was more potent in this assay than AZD9291.

Table 7: Apparent IC50 inhibition of pHER2 for AD9291 and AD5104 (mean±95%CI; nM)

Compound	NIH3T3/HER2a
AZD9291	93 (39, 221) (n=3)
AZ5104	18 (9, 35) (n=3)
Afatinib	15 (8, 29) (n= 3)
Lapatinib	7 (5, 11) (n=4)

(b) (4) Reports; 7321, 7107, 7025, 7108

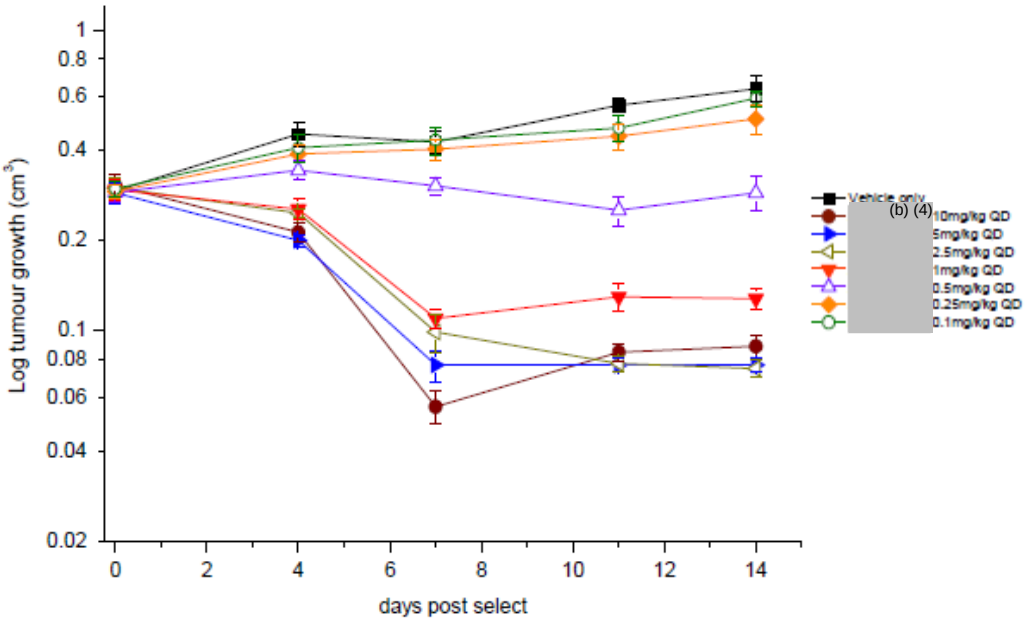
(Excerpted from Applicant's submission)

4.1.2 Effect of AZD9291 on In Vivo Tumour Growth of EGFR Single Mutant, EGFR Double Mutant and EGFR Wild Type Xenograft Models (Pharmacology Report 05, Amendment 1)

The purpose of this study was to correlate the anti-tumor activities of AZD9291 with EGFR mutation status. Female athymic mice (Swiss nu/nu) were implanted with tumors derived from A431 (WT EGFR), LoVo (EGFR WT), or H1975 (EGFRm L858R/T790M) cell lines, and female SCID mice were implanted with tumors derived from PC9 (EGFRm Ex19del), H3255 (EGFRm L858R), and PC9/VanR (EGFRm Ex19del/T790M) cell lines.

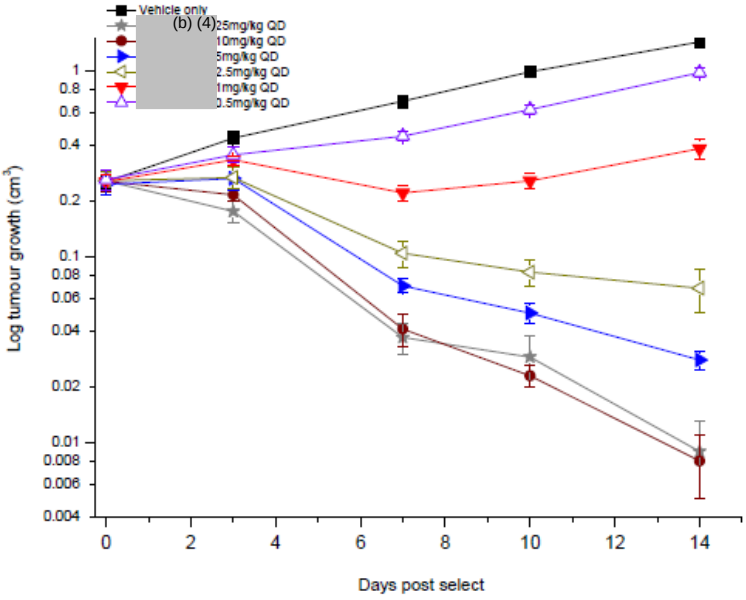
As demonstrated in Figure 6 through Figure 8, AZD9291 induced a dose-related suppression of tumor growth over the range from 0.1 to 10 mg/kg, particularly in EGFR mutant xenografts.

Figure 6: Tumor growth inhibition of AZD9291 in female SCID mice harboring PC9 (EGFR Ex19del) xenografts



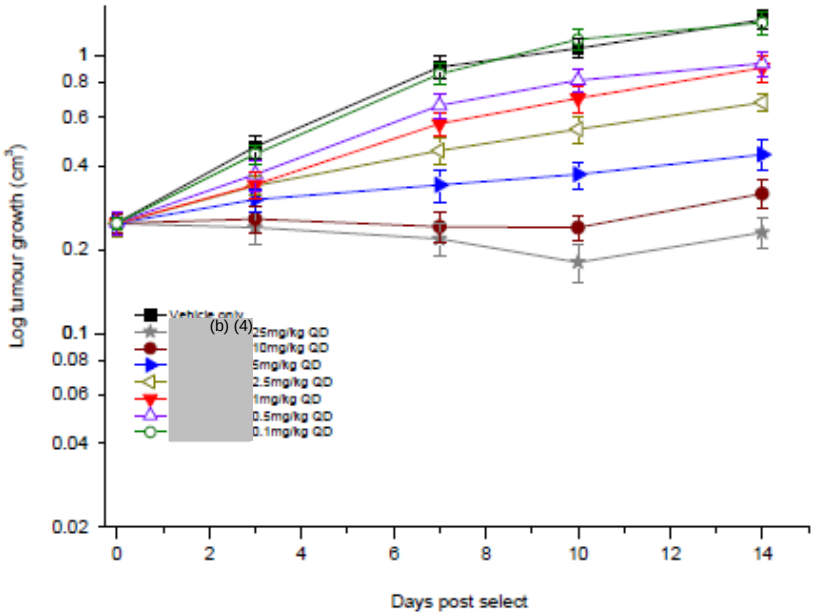
(Excerpted from Applicant's submission)

Figure 7: Tumor growth inhibition of AZD9291 in female athymic (nu/nu) mice harboring H1975 (EGFR T790M/L858R) xenografts



(Excerpted from Applicant's submission)

Figure 8: Tumor growth inhibition of AZD9291 in female athymic (nu/nu) mice harboring A431 (EGFR WT) xenografts



(Excerpted from Applicant's submission)

4.1.3 Effect of AZ13575104, an Active Metabolite of AZD9291, on In Vivo Tumour Growth of EGFR Single Mutant, EGFR Double Mutant and EGFR Wild Type Xenograft Models (Pharmacology Report 06, Amendment 1)

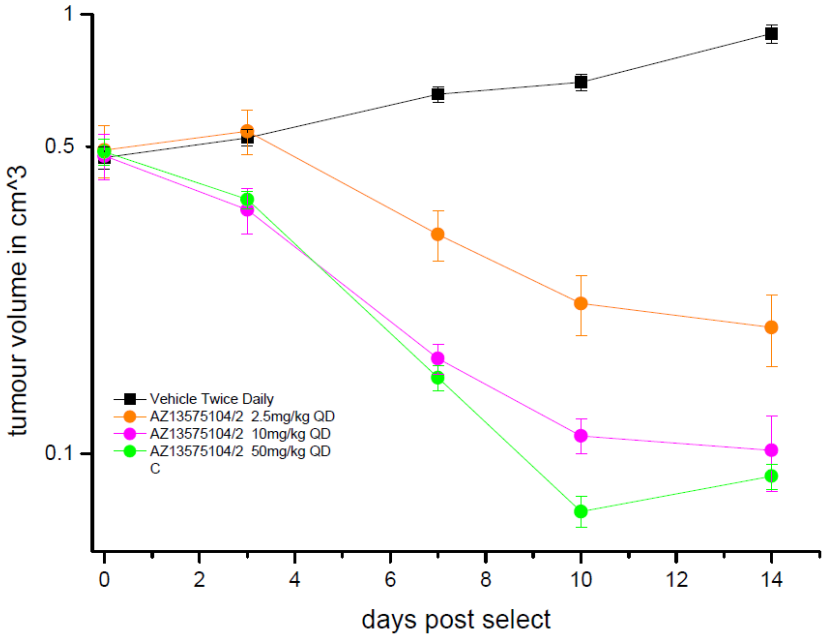
The purpose of this study was to assess the contribution of the metabolite, AZ13575104 to the pharmacological activity of AZD9291 in immunocompromised (nude or SCID) mouse xenograft models of NSCLC. Mutant cells were implanted subcutaneously into the dorsal flank of female mice (at least 6 weeks of age) and allowed go grow until tumor volumes reached 0.2 cm^3 (nude mice) or 0.4 cm^3 (SCID mice). Mice were randomized to receive either vehicle or drug at doses of 2.5, 10, or 50 mg/kg once daily. The EGFR mutant status of the different lines tested and the mouse strains used, are summarized in Table 8

Table 8: Summary of study designs

Model	Cell Line	Phenotype
Swiss nu/nu	H1975	T790M/L858R
Swiss nu/nu	A431	WT
SCID	PC9	Ex19del

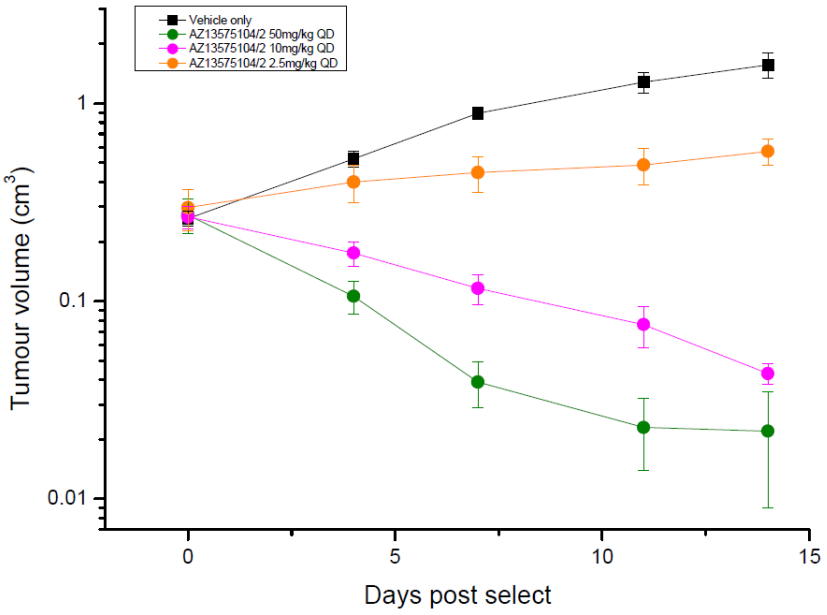
As illustrated in Figure 9 through Figure 11, AZ13575104 exhibited dose-related anti-tumor activity in all models tested; thus, the metabolite appears to contribute to the overall pharmacological activity of AZD9291.

Figure 9: Effect of AZ13575104 on tumor growth in PC9 (EGFRm+;Ex19del) SCID xenografts



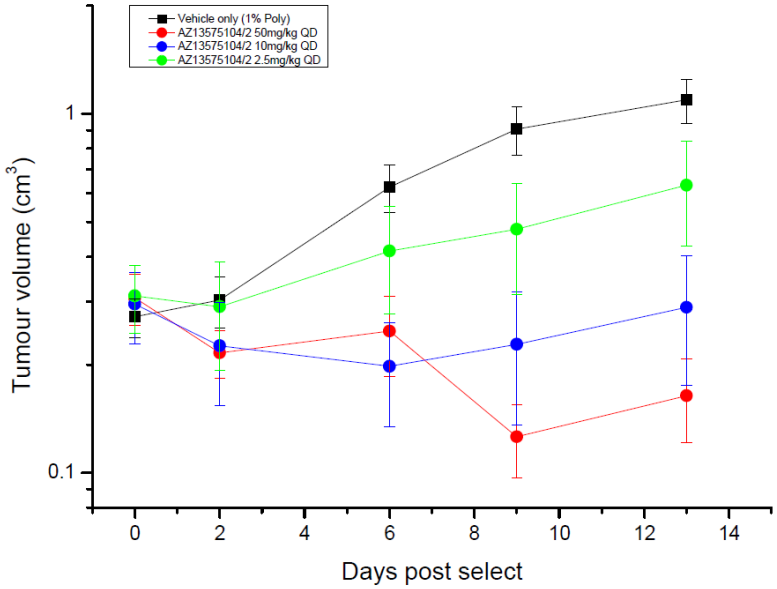
(*Excerpted from the Applicant's NDA)*

Figure 10: Effect of AZ13575104 on tumor growth in H1975 (EGFRm+; L585R/T790M) nu/nu xenografts



(*Excerpted from the Applicant's NDA)*

Figure 11: Effect of AZ13575104 on tumor growth in A431 (EGFRwt) nu/nu xenografts



(Excerpted from Applicant's submission)

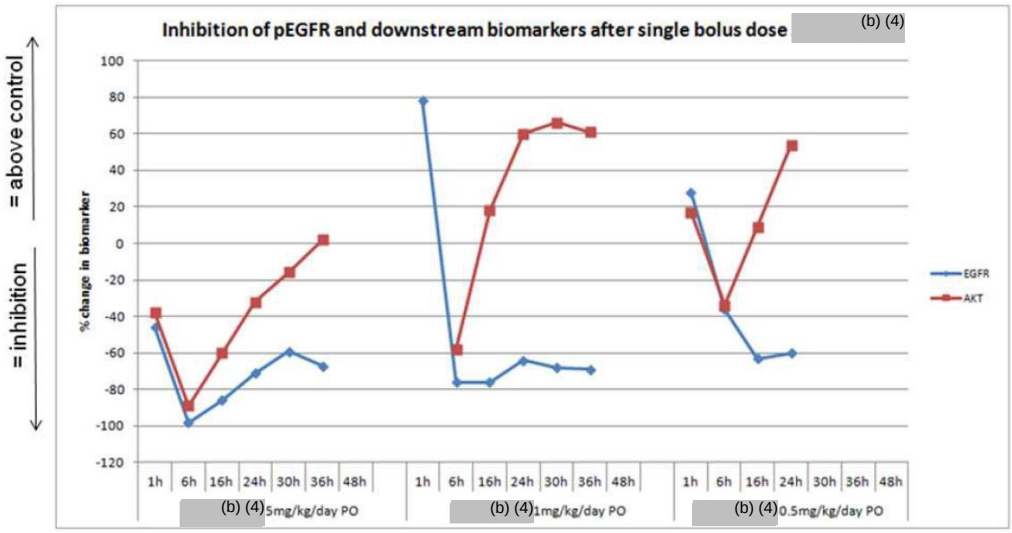
4.1.4 Effect of AZD9291 on phosphorylated biomarkers from in vivo xenografts representing EGFR wild type (A431), activating (PC9) and resistant (H1975) NSCLC disease settings (Pharmacology Report 07, Amendment 1)

The purpose of this study was to assess the mechanism by which AZD9291 exerts anti-tumor activity by exploring activation of the EGFR pathway in tumor-bearing, immunocompromised mice treated with AZD9291.

Animals (SCID for the PC9 model, or nu/nu for the H1975 and A431 models) harboring subcutaneous (SC) tumor implants were randomized to treatment when tumors reached a size of 0.5 cm^3 (nu/nu) or 0.8 cm^3 (SCID). Animals received a single oral dose of either vehicle (control) or AZD9291 (0.5, 1 or 5 mg/kg). Tumors were excised at specific timepoints post-dose, and half of each tumor was snap-frozen prior to analysis by ELISA for EGFR activation markers (EGFR, pEGFR, AKT, pAKT); the other half was processed for immunohistochemistry (IHC; pEGFR and pErk).

As illustrated in Figure 12 and Figure 13, both pEGFR and pAKT were suppressed by AZD9291 administration in EGFR mutant xenografts at all doses tested (note that the green symbol is not defined in the report; however, as no data were plotted, it appears to be an error). Maximal suppression was observed at 6 hours post-dose at the 5 mg/kg dose level in all tumor types (Figure 12 through Figure 14). While doses of 1 and 5 mg/kg were also effective at suppressing pEGFR and pAKT in tumors expressing WT-EGFR, there was no activity observed in the EGFR wild type tumors at a dose of 0.5 mg/kg (Figure 14).

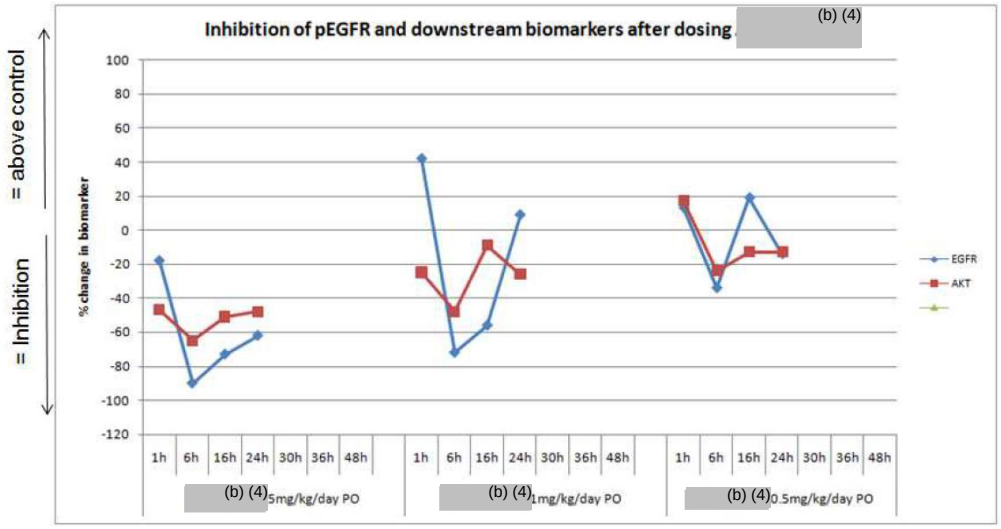
Figure 12: Dose-response and timecourse of pEGFR and pAKT expression following oral administration of AD9291 in H1975 xenografts by ELISA



Data expressed as % inhibition of phosphorylated:total receptor ratio compared to a 6h vehicle dosed control group.

(*Excerpted from the Applicant's NDA)*

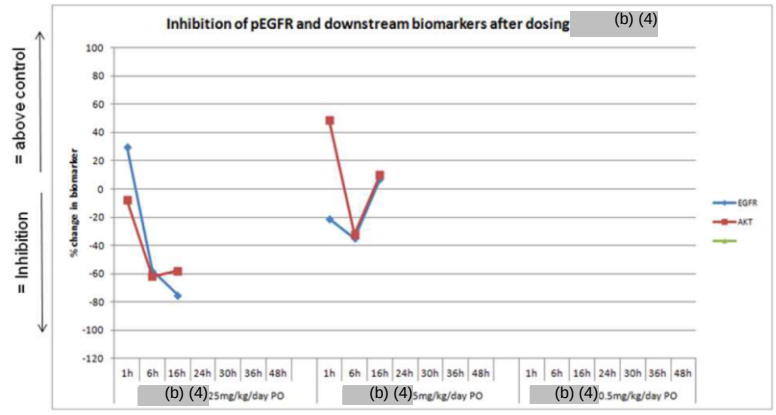
Figure 13: Dose-response and timecourse of pEGFR and pAKT expression following oral administration of AD9291 in PC9 xenografts by ELISA



Data expressed as % inhibition of phosphorylated:total receptor ratio compared to a 6h vehicle dosed control group.

(*Excerpted from the Applicant's NDA)*

Figure 14: Dose-response and timecourse of pEGFR and pERK expression following oral administration of AD9291 in A431 (EGFR WT) xenografts

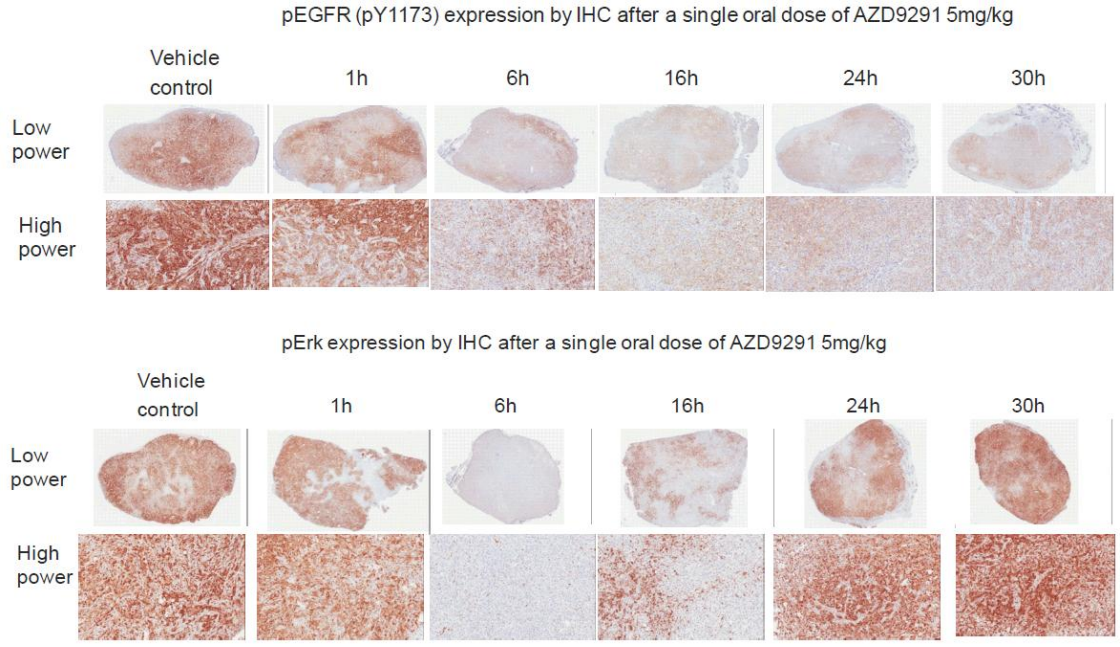


Data expressed as % inhibition of phosphorylated:total receptor ratio compared to a 6h vehicle dosed control group.

(*Excerpted from the Applicant's NDA)*

In H1975 cells, suppression of pEGFR and pERK (a downstream-mediator of EGFR) was evaluated histologically. As illustrated in Figure 15, a single oral dose of AZD9291 induced prolonged suppression of pEGFR, though pErk rebound was observed by approximately 16 hours post-dose, and appeared to be comparable to vehicle controls by 24 hours post-dose.

Figure 15: Immunohistochemical timecourse of pEGFR and pERK suppression in H1975 (EGFRm) tumors treated in vivo with AZ9291



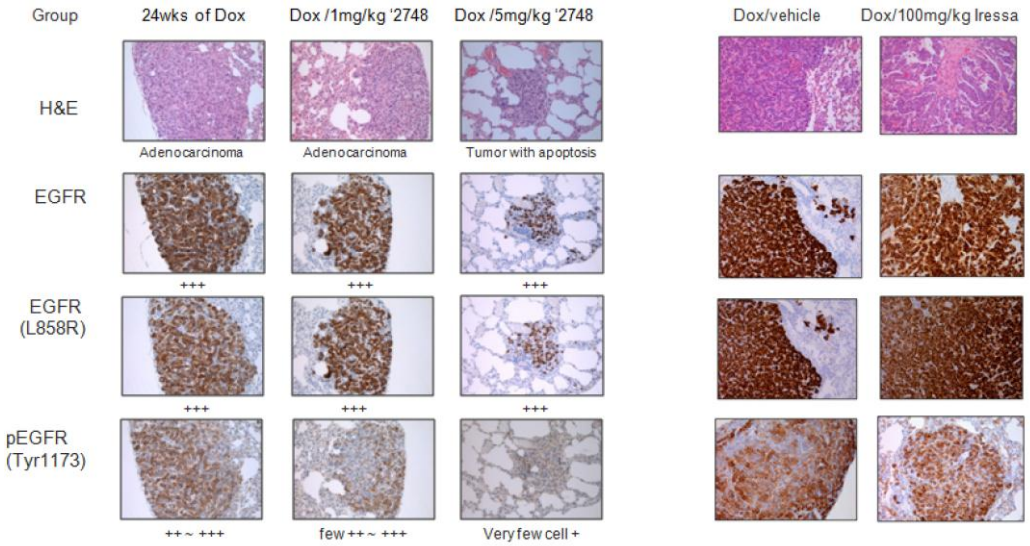
(*Excerpted from the Applicant's NDA)*

4.1.5 Effect of AZD9291 on In Vivo Tumour Growth of EGFR Double Mutant bi-transgenic NSCLC model (Pharmacology Report 10)

The purpose of this study was to assess the effect of AZD9291 in a murine bitransgenic model of T790M/L858R double-mutant NSCLC. Mouse embryos were transduced with two mutant (T790M and L858R) EGFR transgenes under the control of a tetracycline-inducible element. Confirmed-double-mutant animals were reared on a diet containing doxycycline for more than 16 weeks to induce transgene expression and tumor growth, at which time they were randomized to receive AZD9291 (1 or 5 mg/kg/Day) or Iressa (gefitinib; 100 mg/kg/Day). Upon sacrifice, lungs and skin were harvested and stained for microscopic evaluation.

As illustrated in Figure 16, AZD9291 suppressed tumor growth in a dose-dependent fashion over the dose-range evaluated. In comparison, gefitinib failed to suppress tumor growth in this model, consistent with its lack of activity in T790M-mutant tumors.

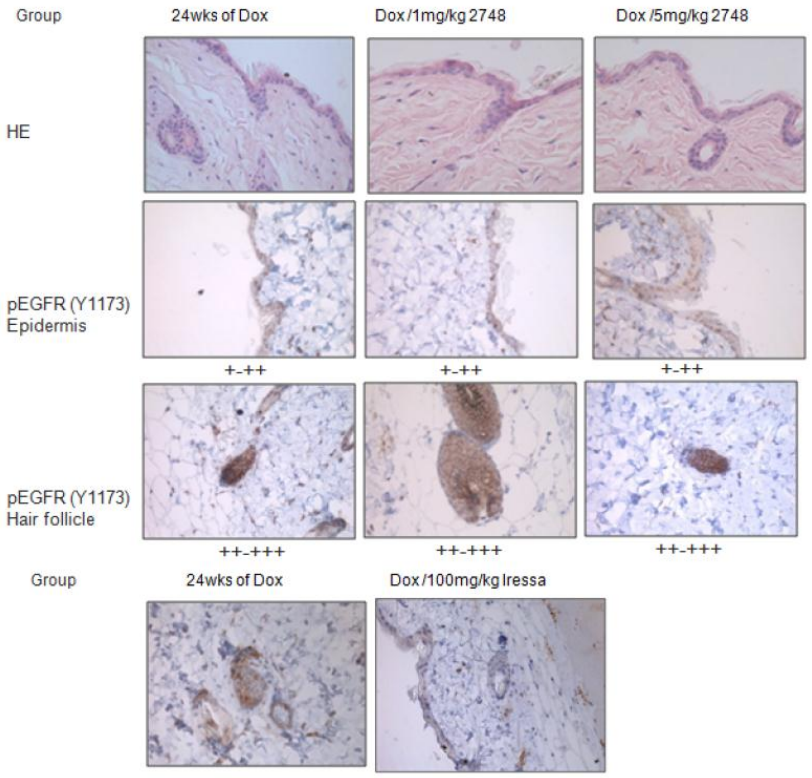
Figure 16: Effect of AZD9291 (b) (4) on bi-transgenic EGFRm+/T790M tumor growth in comparison with Iressa



(*Excerpted from the Applicant's NDA)*

The Applicant also demonstrated that, compared with gefitinib which suppressed WT pEGFR in the skin of treated mice, pEGFR expression was maintained in animals treated with AZD9291 (Figure 17).

Figure 17: IHC for pEGFR (Y1173) in skin epidermis and hair follicles in the bi-transgenic mouse study



(*Excerpted from the Applicant's NDA)*

4.1.6 Mass Spectrometric Analysis of Recombinant EGFRm (T790M) to Determine the Binding Site of (b) (4) (Pharmacology Report 11)

The purpose of this study was to assess the binding site of AZD9291 (b) (4) in isolated recombinant EGFRm (T790M) protein. Purified recombinant T790M mutant EGFR was incubated with test compound, purified by electrophoresis and digested with chymotrypsin before undergoing analysis by mass spectrometry. Analysis of treated- and untreated-samples identified two peptides that were modified by the addition of a 499.26 mass units. (b) (4)

4.1.10 Long Term Administration of AZD9291 in the NCI-H1975 NSCLC Xenograft Model (Pharmacology Report 16, Amendment 1)

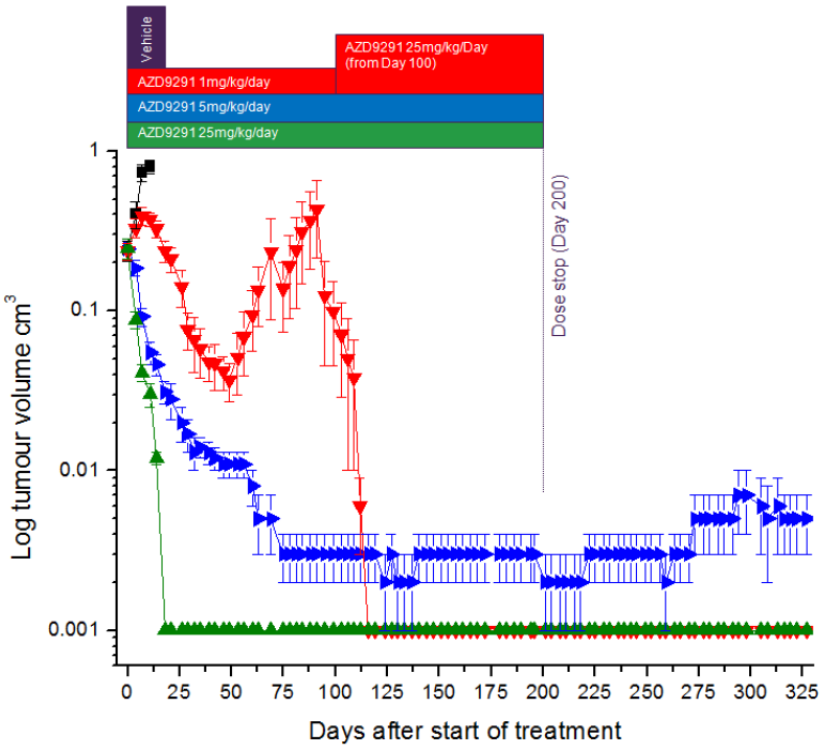
The purpose of this study was to assess the durability of response to AZD9291 in female athymic Swiss nu/nu mice following implantation of an NCI-H1975 (mEGFR L858R/T790M) tumor.

Treatment with vehicle or AZD9291 (0, 1, 5 or 25 mg/kg.) was commenced when tumors reached a size of 0.2 cm^3 and continued through Day 200, after which post-treatment tumor growth was assessed until Day 327. Tumor growth was suppressed in a dose-related fashion between doses of 5 and 25 mg/kg/Day. At a dose of 1 mg/kg/Day, tumor growth was initially suppressed, then began to regrow. On Day 100, animals treated at the 1 mg/kg dose level were switched to a dose of 25 mg/kg, and tumor growth was rapidly suppressed, as illustrated in Figure 18.

Overall, daily oral administration of AZD9291 at doses of ≥ 5 mg/kg/Day robustly suppressed tumor growth in vivo. The Applicant states that 9/12 animals had no visible tumors at the end of the post-treatment regrowth period. In the 5 mg/kg dose group, only 1 of 12 had a measurable tumor but the mass did not appear to be actively growing.

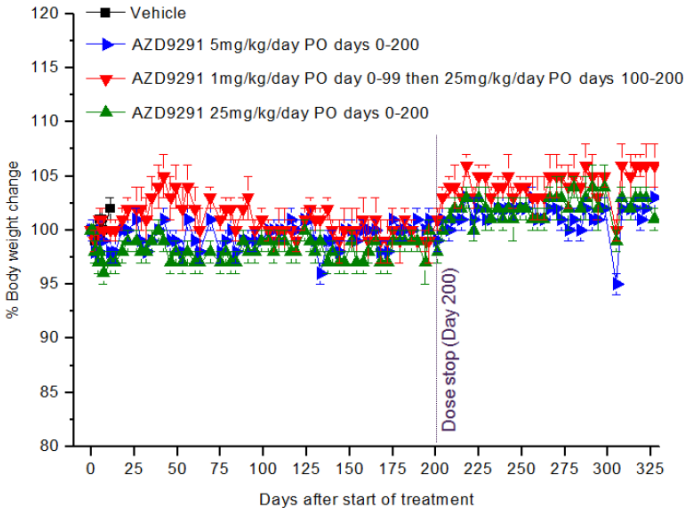
There were no effects of treatment on body weight or mortality over the course of the study (Figure 19).

Figure 18: Tumor growth inhibition in NCI-H1975 nude mouse xenografts during and after treatment with AZD9291



(*Excerpted from the Applicant's NDA)*

Figure 19: Effect of AD9291 treatment on body weight in NCI-H1975 (L858R/T790M) NSCLC xenograft-bearing female nude mice



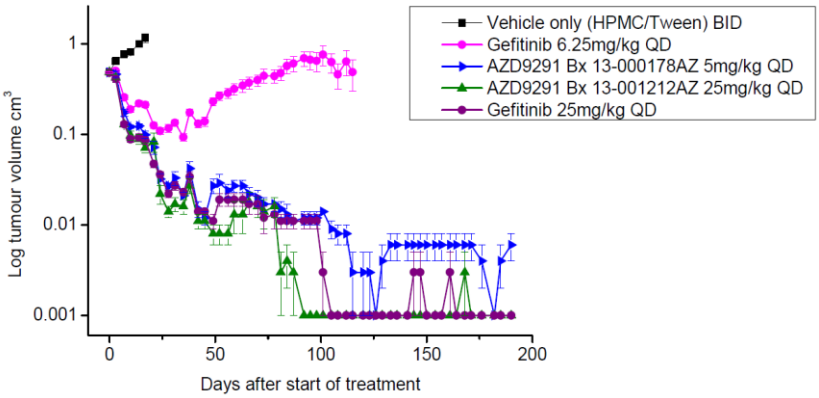
(*Excerpted from the Applicant's NDA)*

4.1.11 Long Term Administration of AZD9291 and Gefitinib in a PC9 NSCLC Xenograft Model (Study 2) (Pharmacology Report 17, Amendment 1)

The purpose of this study was to assess the durability of response to treatment with AZD9291 or gefinitib in female athymic Swiss nu/nu mice following implantation of a PC9 NSCLC xenograft (mEGFR Ex19del).

Treatment with vehicle or AZD9291 was commenced when tumors reached a size of 0.4 cm^3. Daily AZD9291 oral doses of 5 or 25 mg/kg were used and effects were compared with those obtained following administration of daily oral doses of 6.5 mg/kg gefitinib. As indicated in Figure 20, all regimens were highly active in this model except for the low-dose gefitinib arm. Tumor growth was partially suppressed at the low dose of 5 mg/kg AZD9291; however, both gefitinib and high-dose (25 mg/kg) AZD9291 completely suppressed tumor growth in this model. The Applicant states that the 25 mg/kg dose level of gefitinib is not clinically tolerable in humans because of toxicity attributable to inhibition of WT EGFR.

Figure 20: Tumor growth inhibition following administration of AZD9291 or gefitinib in the PC9 NSLC xenograft model



(*Excerpted from the Applicant's NDA)*

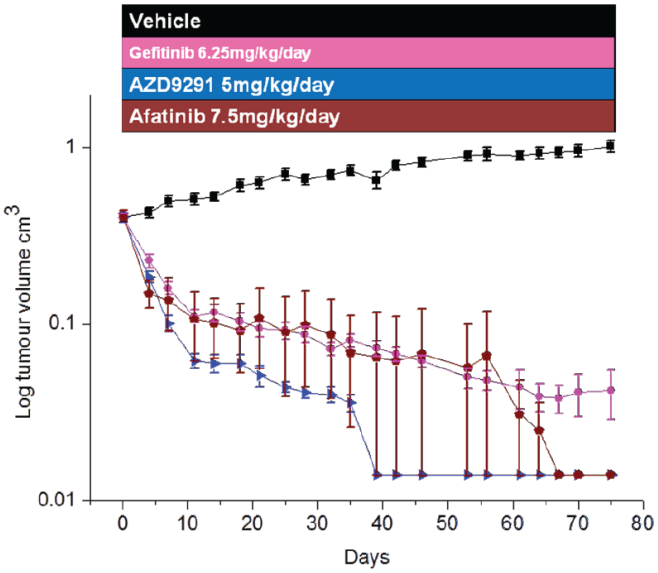
4.1.12 Long Term Administration of AZD9291, Gefitinib and Afatinib in the NCI-H3255 NSCLC Xenograft Model (Pharmacology Report 18, Amendment 1)

The purpose of this study was to compare the activity of AZD9291 (5 mg/kg – a dose that produces comparable exposures to a dose of 20 mg/day dose in humans), with that of afatinib (7.5 mg/kg) or gefitinib (6.25 mg/kg), in an EGFR mutant (L858R) tumor model.

Animals were implanted subcutaneously with NCI-H3255 cells in matrigel and randomized to treatment when tumor volumes reached 0.4 cm^3. Animals received daily oral doses of either vehicle or drug for 75 days, beginning the day after randomization.

As illustrated in Figure 21, there was no significant difference between the three treatments, though AZD9291 appeared to suppress tumor growth more quickly than the others regimens and both AZD9291 and afatinib (both irreversible inhibitors) appeared to suppress tumor growth to a greater extent than gefitinib.

Figure 21: Tumor growth inhibition in NCI-H3255 NSLC xenografts following treatment with gefitinib, afatinib or AZD9291



(Excerpted from the Applicant's NDA)

4.1.13 In Vivo Anti-tumour Efficacy of AZD9291 in Brain Metastasis Xenograft Models in Mice (Pharmacology Report 19)

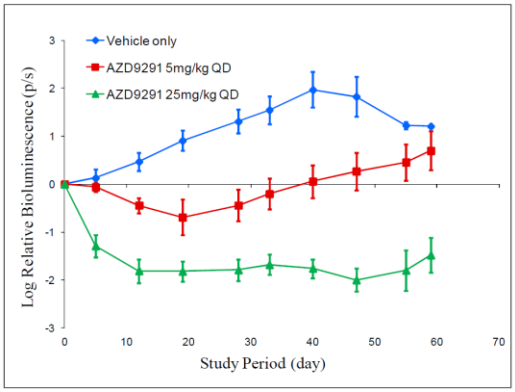
The purpose of this study was to assess the activity of AZD9291 in a murine model of brain metastasis.

Female athymic nu/nu mice received luciferase-transfected PC9 (mEGFR+: Ex19del) tumor cell implants via injection into the intra-internal carotid artery and randomized to treatment when tumor volumes reached 0.4 cm^3. Tumor growth was monitored using the IVIS Xenogen imaging system.

Animals were randomized to treatment groups when tumors reached a mass of approximately 10^7 cells. Animals received daily oral doses of either 0 (vehicle control), 5 or 25 mg/kg AZD9291 (b) (4) and tumor growth was assessed by comparing the bioluminescence intensity on the treatment day compared with the initial bioluminescence intensity.

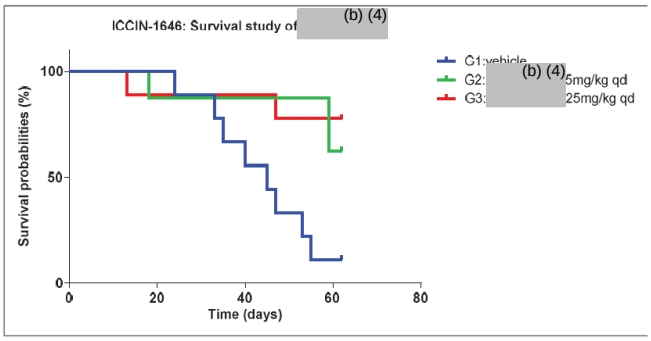
As illustrated in Figure 22, AZD9291 exhibited a dose-dependent inhibition of tumor growth in this model, which correlated with improved survival (Figure 23). As illustrated in Figure 24, CNS exposure to AZD9291 is robust. Peak exposures in the brain were approximately 4-fold higher than in the plasma.

Figure 22: Summary of tumor growth inhibition by AZD9291 in a PC9 brain metastasis model in athymic nu/nu mice



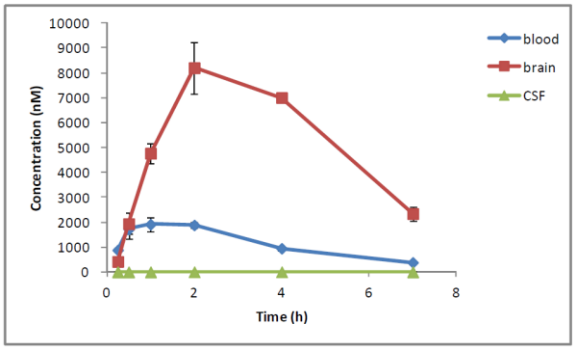
(Excerpted from the Applicant's NDA)

Figure 23: Effect of AZD9291 on survival in the murine NSCLC brain metastasis model in athymic nu/nu mice



(Excerpted from the Applicant's NDA)

Figure 24: Concentration-time curve for AZD9291 in the blood, brain and CSF in athymic nu/nu mice



(Excerpted from the Applicant's NDA)

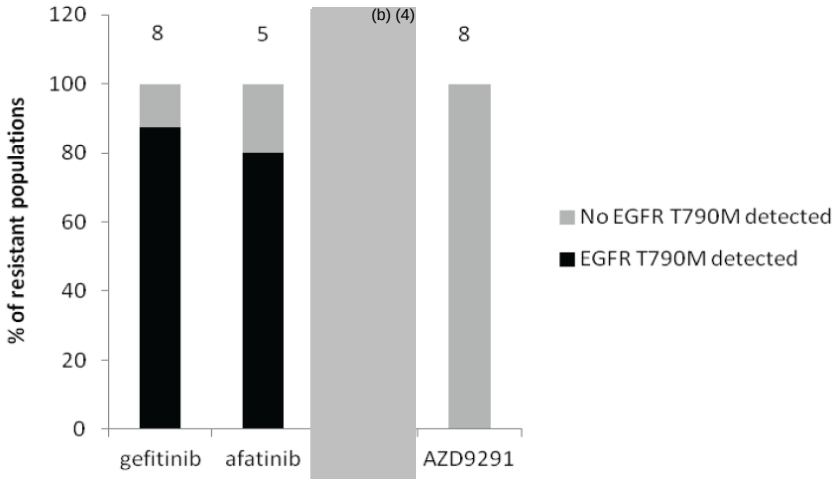
4.1.14 In vitro investigation to determine whether the EGFR T790M mutation would be an acquired resistance mechanism to AZD9291 when used in a first line mutant EGFR NSCLC setting (Pharmacology Report 20, Amendment 1)

The purpose of this study was to characterize the mechanism of resistance when PC9 NSCLC cells (Ex19del) were selected for resistance using gefitinib, afatinib, (b) (4), or AZD9291. T790M is described in the literature as a common secondary mutation acquired following treatment with EGFR kinase inhibitors that contributes to resistance with these agents.

PC9 (mEGFR+; EX19del) cells were treated with either gefitinib (1.5 μM), afatinib (1.5 μM), (b) (4) (1.5 μM) or AZD9291 (160 nM), and DNA from resulting clones was sequenced.

The majority of cells selected for resistance with gefitinib or afatinib had acquired T790M mutations (Figure 25 and Table 9), whereas those selected using (b) (4) and AZD9291 had not, consistent with its known activity as an inhibitor of T790M, indicating that the acquisition of the T790M mutation is unlikely to drive resistance to AZD9291 when used in a first line setting in patients with mutant EGFR NSCLC.

Figure 25: Proportion of resistant cells harboring T790M mutations following treatment with gefitinib, afatinib, (b) (4) or AZD9291



(Excerpted from the Applicant's NDA)

Table 9: Summary of mutation analyses from studies of EX19del PC9 cells selected for resistance with gefitinib, afatinib, (b) (4) or AZD9291

PC9 gefitinib resistant (1.5uM)	GM	EX19del.+T790M
	LR	EX19del
	1	EX19del.+T790M
	2	EX19del.+T790M
	3	EX19del.+T790M
	4	EX19del.+T790M
	5	EX19del.+T790M
	6	EX19del.+T790M
PC9 afatinib resistant (1.5uM)	GM	EX19del
	1	EX19del.+T790M
	3	EX19del.+T790M
	4	EX19del.+T790M
	5	EX19del.+T790M
(b) (4)		
PC9 AZD9291 resistant (160nM)	2	EX19del
	3	EX19del
	5	EX19del
	6	EX19del
	LOB1	EX19del
	LOB2	EX19del
	LOB3	EX19del
	CE	EX19del

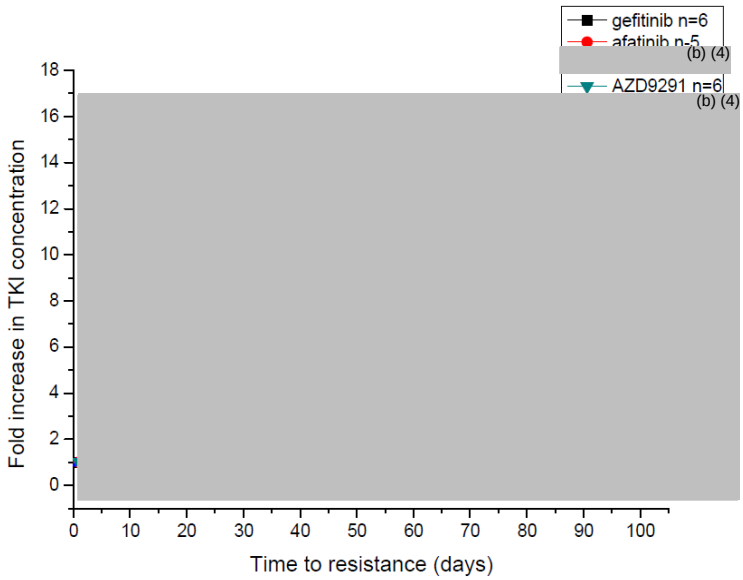
(Excerpted from the Applicant's NDA)

4.1.15 In vitro investigation of resistance to gefitinib, afatinib, (b) (4) or AZD9291 in PC9 cells comparing time to resistance in an EGFR mutant first line setting. (Pharmacology Report 21, Amendment 1)

The purpose of this study was to study the kinetics of resistance in cultured PC9 (Ex19del) cells. Unselected PC9 cells were grown in the presence or absence of gefitinib, afatinib, (b) (4) or AZD9291 at initial concentrations corresponding to each drug's IC_{50}. Treatment medium was replaced every 2-3 days during the growth cycle. When resistant colonies became 80% confluent, the culture was determined to have become to treatment, and cells were re-plated at a density of $1X10^5$/flask at a two-fold higher concentration. This procedure was repeated until the final concentration was 16-fold higher than the initial concentration.

As indicated in Figure 26, all compounds eventually induced resistance to treatment. The rate of resistance acquisition appeared to be slower with AZD9291 under the conditions of this assay.

Figure 26: Kinetics of resistance acquisition in cultured PC9 cells treated with AZD9291, gefitinib, afatinib (b) (4)



(Excerpted from the Applicant's NDA)

4.1.16 To test the activity of AZD9291 and its metabolite AZD5104 and competitor compounds against HER2 and HER3 in ligand driven system and in an amplified setting (Pharmacology Report 22)

The purpose of this study was to assess the ability of AZD9291 and its metabolite, AZ5104 to inhibit HRG1-stimulated HER2 and HER3 phosphorylation in stimulated MCF7 cells, and to inhibit pHER2 production in HER2 amplified BT474 cells.

As illustrated in Table 10, AZD9291 and its metabolite, AZ5104 suppressed pErbB2 production in both HRG1-stimulated and HER2 amplified cells. In addition, AZD9291 and AZ5104 both suppressed pErbB3 production in HRG1-stimulated cells. The potency of AZD9291 was comparable to the other irreversible inhibitors (e.g. AZ5104, and afatinib). Gefitinib and erlotinib (both reversible inhibitors) were less potent in the context of HER2 amplification. The potency of AZD9291 in the context of HER2 amplification was comparable (within ~1.5X) of that observed in the context of HRG1-stimulation. (b) (4)

Table 10: Comparative inhibitory activity of different compounds for pErbB2 and pErbB3 production in HRG1-stimulated MCF-7 cells or HER2-amplified BT474 cells

Compound	Cell Line	pHER2 IC50 (μM)	pHER3 IC50 (μM)
AZD9291	MCF7	0.095	0.128
	BT474	0.1227	--

Compound	Cell Line	pHER2 IC50 (µM)	pHER3 IC50 (µM)
			(b) (4)
AZ5104	MCF7	0.0135	0.016
	BT474	0.0163	--
Gefitinib	MCF7	0.0216	0.077
	BT474	0.2732	--
Afatinib	MCF7	0.0145	0.023
	BT474	0.0201	--
Erlotinib	MCF7	0.0848	0.326
	BT474	2.0487	--
Dacomitinib	MCF7	0.0117	0.029
	BT474	0.0461	--
Clovis	MCF7	--	--
	BT474	0.4877	--

4.2 Secondary Pharmacology

4.2.1 AZD9291: Selectivity Screening in Radioligand Binding, Enzyme, Functional and Electrophysiological Assays in vitro (Report 1112SY)

AZD9291 exhibited off-target activity against 15 of the 97 targets in radioligand displacement assays. In particular, AZD9291 was an antagonist of the alpha2A adrenergic receptor, and the serotonin 5-HT2A and 3B receptors. Some activity against potassium and sodium channels was observed at high concentrations. A summary of the mean IC_{50}s for the targets identified is provided in Table 11.

Table 11: Summary of AZD9291 off-target pharmacological activity (mean IC50s)

Target [a]	IC_{50} (µM)	K_i (µM)	% inhibition
5-HT_{2A} receptor	0.40[b]	0.11[b]	
5-HT_{2B} receptor	0.52	0.33	
Adrenergic α_{2A} receptor	1.70[b]	0.64[b]	
Insulin receptor, Protein Tyrosine Kinase	0.75[b]	nd	
Somatostatin sst_4 receptor	1.16[b]	1.05[b]	
5-HT_{1B} receptor (rat)	1.52	1.16	
Dopamine D_1 receptor	2.49	1.25	
Adrenergic α_{1D} receptor	3.10	1.52	
Dopamine D_{2L} receptor	4.72	1.57	
Adenosine A_3 receptor	1.94	1.79	
κ Opiate receptor	6.07	2.43	
Dopamine transporter	3.66	2.91	
Norepinephrine transporter	5.55	5.51	
YES1, protein Tyrosine Kinase	25.8	nd	
$hK_v11.1$	16.22	nd	
$hNa_v1.5$	>33	nd	29% at 33 µM
HKvLQT1/hmink	>33	nd	34% at 33 µM

[a]All human except where noted; [b]Geometric mean; nd not determined

(Excerpted from the Applicant's NDA)

4.2.2 AZ13575104: Selectivity Screening in Radioligand Binding, Enzyme, Functional and Electrophysiological Assays in vitro (Report 1120SY)

AZ13575104 exhibited off-target activity for 17 of the 97 targets evaluated in radioligand binding displacement assays (Table 12). It was found to be an agonist of the adrenal α2A receptor and an antagonist of the serotonin 5-HT2A and 2B receptors. Partial inhibition of the adenosine A3 receptor and L-type calcium channels was observed at high exposures (10 μM).

Table 12: Summary of in vitro binding and enzyme inhibition assays with AZ13575104

Target [a]	IC_{50} (μM)	K_i (μM)	% inhibition at 10 μM
EGF Receptor, Protein Tyrosine Kinase	0.0006	nd	
5-HT_{2A} receptor	0.17	0.05	
Insulin receptor, Protein Tyrosine Kinase	0.11	nd	
5-HT_{2B} receptor	0.30	0.19	
Adrenergic α_{1A} receptor (rat)	1.19	0.48	
Adrenergic α_{1D} receptor	0.81	0.40	
Adrenergic α_{2A} receptor	4.44	1.67	
5-HT_{1B} receptor (rat)	nd	nd	80
Adenosine Transporter (guinea pig)	nd	nd	83
Peripheral benzodiazepine receptor (rat)	nd	nd	79
Dopamine D_1 receptor	nd	nd	78
Somatostatin sst_4 receptor	nd	nd	72
FGFR1, Protein Tyrosine Kinase	nd	nd	70
Growth hormone secretagogue (Ghrelin)	nd	nd	67
Calcium Channel L-Type, Benzothiazepine (rat)	nd	nd	56
Calcium Channel L-Type, Dihydropyridine (rat)	nd	nd	55
Adenosine A_3 receptor	nd	nd	54

[a]All human except where noted; nd not determined.

(Excerpted from the Applicant's NDA)

4.2.3 AZ13597550: Selectivity Screening in Radioligand Binding, Enzyme and Electrophysiological Assays in vitro (Report 1121SY)

At 10 μM the AZD9291 metabolite, AZ13597550 exhibited off-target activity against 22 of the 97 targets screened in radioligand binding and enzyme assays (Table 13). At high concentrations, AZ13597550 also had activity against several of the human recombinant voltage-gated cardiac ion channels (Table 14).

Table 13: Effect of AZ13597550 in in vitro radioligand binding, enzyme and electrophysiology assays

Target [a]	IC_{50} (μM)	K_i (μM)	% inhibition at 10 μM
EGF Receptor, Protein Tyrosine Kinase	0.0027	nd	
Insulin receptor, Protein Tyrosine Kinase	0.62	nd	
Dopamine D_{2L} receptor	1.41	0.47	
5-HT_{2B} receptor	0.99	0.63	
5-HT_{2A} receptor	2.57	0.74	
Growth hormone secretagogue (Ghrelin)	2.65	1.48	
Somatostatin sst_4 receptor	nd	nd	82
Tachykinin NK_1 receptor	nd	nd	82
Adenosine A_3 receptor	nd	nd	81
Adrenergic α_{1D} receptor	nd	nd	81
Dopamine transporter	nd	nd	77
μ Opiate receptor	nd	nd	77
Adrenergic α_{1A} receptor (rat)	nd	nd	72
5-HT_{1A} receptor	nd	nd	68
Adrenergic α_{2A} receptor	nd	nd	67
κ Opiate receptor	nd	nd	65
Norepinephrine transporter	nd	nd	65
Dopamine D_1 receptor	nd	nd	64
Calcium Channel L-Type, Dihydropyridine (rat)	nd	nd	60
Melanocortin MC_4 receptor	nd	nd	59
Hisatmine H_2 receptor	nd	nd	58
5-HT_{1B} receptor (rat)	nd	nd	51

[a]All human except where noted; nd not determined.

(Excerpted from the Applicant's NDA)

Table 14: Effect of AZ13597550 in in vitro electrophysiology assays

Channel	Active / Inactive	IC_{50} (μM)	Maximum test concentration (μM)	% Inhibition
hCa$_v$1.2/β2/α2δ	Inactive			
hNa$_v$1.5	Active	>33.3	33	31%
hK$_v$4.3 / hKChIP2.2	Active	>33.3	33	21%
hK$_v$LQT1/hmink	Active	>33.3	33	38%
hK$_v$11.1	Active	>33.3	33	28%

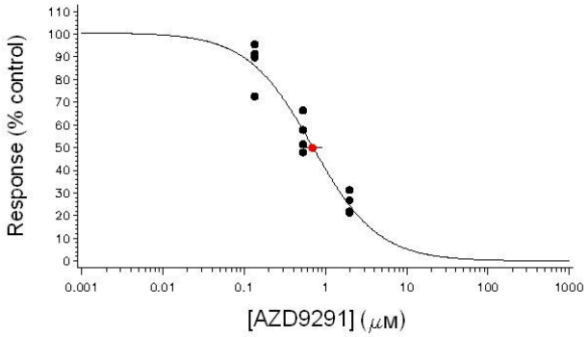
(Excerpted from the Applicant's NDA)

4.3 Safety Pharmacology

4.3.1 AZD9291: Effects on Human Ether-a-go-go-related Gene (hERG) Encoded Potassium Channel in vitro (Report# VSK0795)

In the GLP hERG patch-clamp electrophysiology assay, hERG-expressing CHO cells were exposed to ascending concentrations of AZD9291 to assess the potential for the drug to inhibit the hERG potassium channel. As indicated in Figure 27, the IC_{50} for hERG inhibition by AZD9291 was 0.69 µM (690 nM). Note that this estimate of the hERG IC_{50} has been adjusted using actual perfusate concentration measured in test solutions, which demonstrated that the cells were exposed to concentrations of 44.9% to 66.0% of nominal over the range evaluated (Table 15). The Applicant considers this reduction in test concentration to be the result of drug-adsorption to the cell and/or perfusion culture apparatus. Based on this IC_{50} there is some potential for hERG inhibition at the clinical Cmax of 0.5µM; however, as the plasma protein binding properties of osimertinib were not characterized, the extent to which this represents a clinically significant risk is unclear.

Figure 27: AZD9291 hERG Concentration-Effect Curve



(Excerpted from the Applicant's NDA)

Table 15: Analytical chemistry results from the in vitro hERG assay

Nominal concentration (μM)	Stock/Perfusate	Measured concentration (μM)	Mean measured concentration (μM)	% Nominal concentration
0	Stock 1	ND	-	-
0	Stock 2	ND		
0	Perfusate 1	ND	-	-
0	Perfusate 2	ND		
0.3	Stock 1	0.321	0.243	81.0
0.3	Stock 2	0.165		
0.3	Perfusate 1	0.117	0.135	44.9
0.3	Perfusate 2	0.152		
1	Stock 1	1.01	0.892	89.2
1	Stock 2	0.773		
1	Perfusate 1	0.493	0.531	53.1
1	Perfusate 2	0.570		
3	Stock 1	3.10	2.86	95.2
3	Stock 2	2.61		
3	Perfusate 1	1.84	1.98	66.0
3	Perfusate 2	2.12		

ND None detected.

(Excerpted from the Applicant's NDA)

4.3.2 AZ13597550: Selectivity Screening in Cardiac Ion Channel Electrophysiological Assays in vitro (Report #3472SV)

The purpose of this study was to assess the inhibitory potential of the AZD9291 metabolite, AZ13597550, in a panel of cell-based electrophysiological assays using cells that express one of three cardiac-voltage gated ion channels (the human potassium channel, hK_v15; the human calcium channel, $hCa_v3.2$; and hHCN4, the hyperpolarization activated cyclic nucleotide gated potassium channel).

As indicated in Table 16, AZ13597550 inhibited the $hCa_v3.2$ cell line at a concentration of ~32 μM in this assay, which is beyond the range of expected plasma concentrations. Data from positive and negative controls were not provided.

Table 16: Effect of AZ13597550 in electrophysiology assays using CHO or HEK cells expressing human cardiac voltage-gated ion channels

Channel (cell line)	IC_{50} (μM)	Maximum test concentration (μM)	Number of replicates
$hK_v1.5$ (CHO)	>100*	100	1
$hCa_v3.2$ (HEK)	31.91	100	1
hHCN4 (CHO)	>100	100	2

*activity detected above baseline assay signal, but no IC_{50} could be calculated

4.3.3 AZ13575104: Selectivity Screening in Cardiac Ion Channel Electrophysiological Assays in vitro

The purpose of this study was to assess the inhibitory potential of the AZD9291 metabolite, AZ13575104, in a panel of cell-based electrophysiological assays using cells that express one of three cardiac-voltage gated ion channels (the human potassium channel, hK_v15; the human calcium channel, $hCa_v3.2$; and hHCN4, the hyperpolarization activated cyclic nucleotide gated potassium channel).

As indicated in Table 17, AZ13575104 inhibited the $hK_v1.5$ and $hCa_v3.2$ channels in this study. Both effects occurred at concentrations that are beyond the range of expected plasma concentrations. Data from positive and negative controls were not provided.

Table 17: Effect of AZ13575104 in electrophysiology assays using CHO or HEK cells expressing human cardiac voltage-gated ion channels

Channel (cell line)	IC50 (µM)	Maximum test concentration (µM)	Number of replicates
$hK_v1.5$ (CHO)	>89.64*	100	2
$hCa_v3.2$ (HEK)	37.54	100	1
hHCN4 (CHO)	>100	100	2

* Mean of one "defined" and one "out of range" (">") IC_{50} value

4.3.4 (b) (4): Selectivity Screening in Cardiac Ion Channel Electrophysiological Assays in vitro (Report #3535SV)

The purpose of this study was to assess the inhibitory potential of the AZD9291 (b) (4), in a panel of cell-based electrophysiological assays using cells that express one of three cardiac-voltage gated ion channels (the human potassium channel, hK_v15; the human calcium channel, $hCa_v3.2$; and hHCN4, the hyperpolarization activated cyclic nucleotide gated potassium channel).

Under the conditions of this assay, AZD9291 did not inhibit the ion channels tested (Table 18). Data from positive and negative controls were not provided.

Table 18: Effect of (b) (4) in electrophysiology assays using CHO or HEK cells expressing human cardiac voltage-gated ion channels

Channel (cell line)	IC50 (μM)	Maximum test concentration (μM)	Number of replicates
$hK_v1.5$ (CHO)	>100	100	1
$hCa_v3.2$ (HEK)	>100*	100	1
hHCN4 (CHO)	>100	100	1

* Activity detected above the baseline assay signal, but IC_{50} was not determined

4.3.5 AZ13540484 and (b) (4) Cardiovascular Effects in Anaesthetised Guinea-Pigs following Intravenous Infusion (Report: 0264SG – Report Amendments 1-2)

The purpose of this study was to evaluate the cardiovascular effect of AZD9291 (b) (4) in the anesthetized guinea pig. Male guinea pigs were surgically telemetered to assess arterial blood pressure, left ventricular pressure and ECG parameters. Each animal received two consecutive IV doses (15-minute infusions) of vehicle, 5, or 40 mg/kg AZD9291, and was monitored for cardiovascular function during each infusion and for a 30-minute post-dose washout period.

AZD9291 caused an increase in diastolic arterial pressure, PR, QRS, QTcB durations, and left ventricular systolic pressure. AZD9291 was also associated with a reduction in heart rate and dP/dtmax (vehicle-adjusted).

Parameter	% Change from Baseline	Vehicle Adjusted % Change from Baseline	Timepoint
DBP	↑6-14%	↑14-21%	Washout (35-60 mins)
MAP	↑12-18%	↑11-19%	End of 2nd infusion through end of washout (25-60 minutes)
HR	↓4-7%	↓6-8%	End of 2nd infusion through end of washout (25-60 minutes)
QRS	↑15-29%	↑11-26%	End of 2nd infusion through end of washout (20-50 minutes)
PR	↑12%	↑7%	End of 2nd infusion (30 minutes)
QTcB	↑4-9%	↑4-9%	End of 2nd infusion through end of washout (20-60 minutes)
LVSP	↑11-15%	↑10-16%	End of 2nd infusion through end of washout (30-50 minutes)
dP/dtmax	↑4%	↓18%	End of 2nd infusion (30 min)

4.3.6 AZD9291: Cardiovascular Effects in Conscious, Telemetered Beagle Dogs following Single Oral Administration (Report # 1352ZD – Amendments 1-3)

The purpose of this study was to evaluate the effect of AZD9291 and its two pharmacologically-active metabolites, AZ13597550 and AZ13575104 on cardiovascular function in the beagle dog.

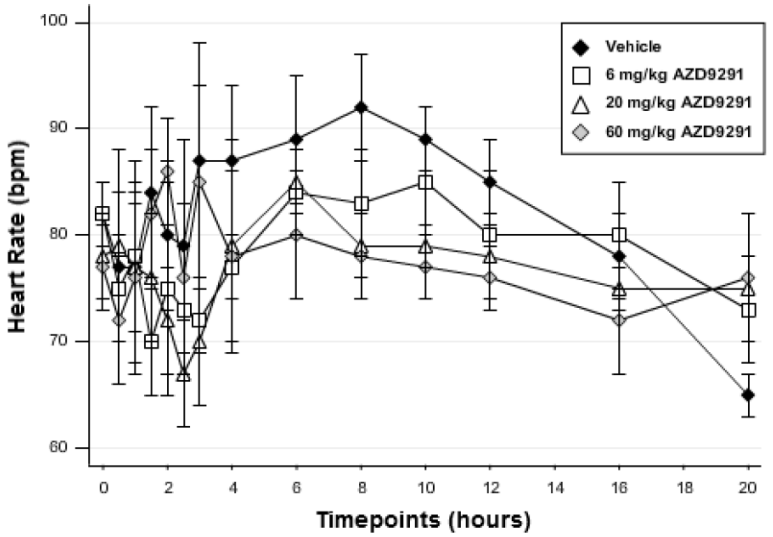
The study employed an ascending dose design in experimentally non-naïve dogs. Four conscious, surgically telemetered (DSI® PhysioTel), male dogs received doses of vehicle on Day 1, and then single ascending doses of AZD9291 (6, 20, and 60 mg/kg/dose) on Days 3, 7 and 10, respectively. Telemetry recordings were performed at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16 and 20 hours after dosing. The Time 0 measurement was the mean of pre-dose measurements performed at -1, -0.75, -0.5 and -0.25 hours for each dose level.

At 2 to 2.5 hours post-dose, there was a small, statistically-significantly and apparently-dose-related decrease in left ventricular dP/dt+ (the maximum rate of ventricular pressure increase), a parameter that is associated with the force of left ventricular contractility, in treated dogs compared with concurrent controls (Figure 29).

AZD9291 also produced a small non-dose-related decrease in heart rate (HR) in treated- versus concurrent control-dogs (Figure 30) and an increase in the heart rate-corrected QT interval (QTcR; Figure 30).

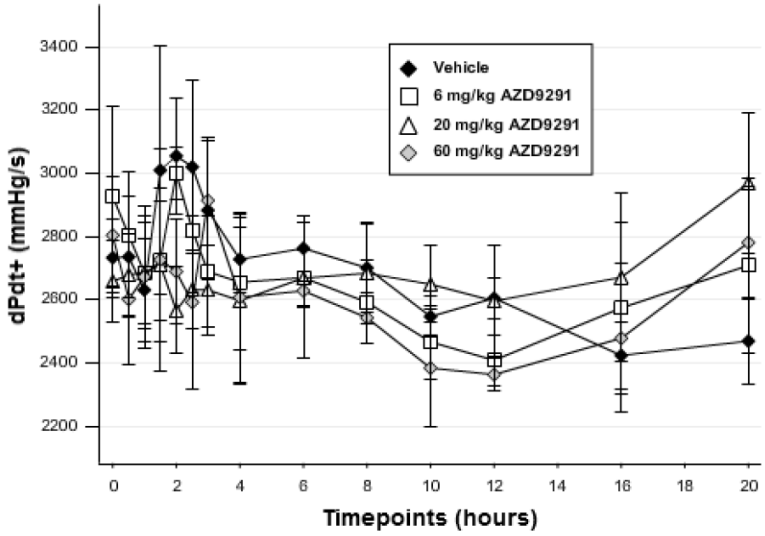
Due to the small magnitude of these changes, particularly in the context of the small sample size and non-cross-over study design, it is unclear whether these findings represent clinically-significant effects on cardiovascular function. There were no effects of AZD9291 at any dose level on the duration of the PR or QRS intervals. No effects were observed on left ventricular systolic or diastolic blood pressure, or on body temperature. Plasma concentrations measured in this study are reported in Table 19; however later bioanalytical stability analyses suggest that the measurements for AZD9291 and its metabolites may be under-estimated by as much as 31%.

Figure 28: Effect of AZD9291 on Heart Rate in the Conscious Telemetered Beagle Dog



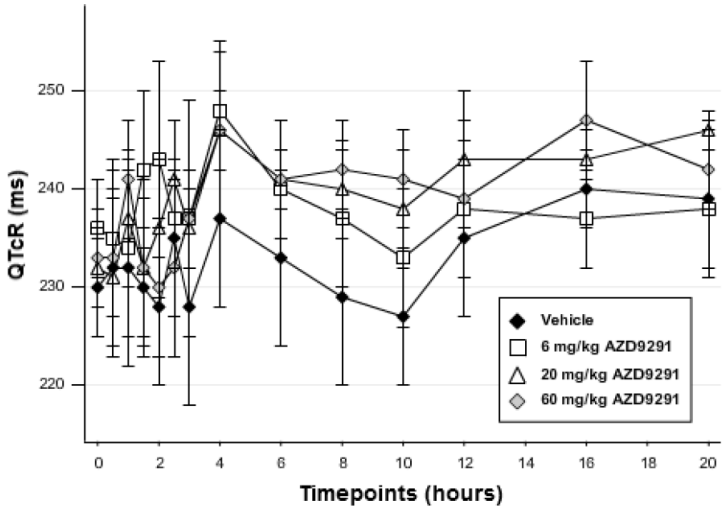
(Excerpted from the Applicant's NDA)

Figure 29: Effect of AZD9291 on dP/dt+ in the Conscious Telemetered Beagle Dog



(Excerpted from the Applicant's NDA)

Figure 30: Effect of AZD9291 on Heart Rate-Corrected QT (QTcR) Intervals in the Conscious Telemetered Beagle Dog



(Excerpted from the Applicant's NDA)

Table 19: Mean Plasma Concentrations Measured in the Conscious Telemetered Beagle Dog at 4-Hours Post-Dose

Dose	0 mg/kg	6 mg/kg	20 mg/kg	60 mg/kg
AZD9291				
C_{max} (µmol/L)	0	0.516 ± 0.390	1.71 ± 0.996	2.51 ± 1.78
T_{max} (hours)	0	4	4	4
AZ13597550				
C_{max} (µmol/L)	0	0.0713 ± 0.0415	0.216 ± 0.114	0.266 ± 1.175
T_{max} (hours)	0	4	4	4
AZ13575104				
C_{max} (µmol/L)	0	0.0150 ± 0.00621	0.0429 ± 0.0205	0.0442 ± 0.0240
T_{max} (hours)	0	4	4	4

(Excerpted from the Applicant's NDA)

4.3.7 AZD9291: Nervous System, Visual, Respiratory and Gastrointestinal Transit Effects in the Han Wistar Rat following Single Oral Administration (Report #3464SR, Report Amendments 1-2)

The purpose of this study was to assess effects on the CNS, ocular parameters, respiration and GI transit following a single oral dose of 10, 40 or 100 mg/kg in male Han Wistar rats. Study designs and animal allocations for these studies are described in Table 20.

Table 20: Groups and Dose Levels

Group	Test item	Dose level mg/kg	Dose level µmol/kg	Dose volume (mL/kg)	Formulation concentration (mg/mL)	Animal numbers	Cage numbers
CNS (Irwin) and OptoMotry phase groups							
1	Control	0	0	10	0	1 to 6	1 and 2
2	AZD9291	10	20	10	1	7 to 12	3 and 4
3	AZD9291	40	80.1	10	4	13 to 18	5 and 6
4	AZD9291	100	200.2	10	10	19 to 24	7 and 8
Respiratory (WBP) phase groups							
5	Control	0	0	10	0	25 to 32	9 and 10
6	AZD9291	10	20	10	1	33 to 40	11 and 12
7	AZD9291	40	80.1	10	4	41 to 48	13 and 14
8	AZD9291	100	200.2	10	10	49 to 56	15 and 16
9	Theophylline	10	55.5	10	1	57 to 64	17 and 18
GI (charcoal meal) phase groups							
10	Control	0	0	10	0	65 to 72	19 and 20
11	AZD9291	10	20	10	1	73 to 80	21 and 22
12	AZD9291	40	80.1	10	4	81 to 88	23 and 24
13	AZD9291	100	200.2	10	10	89 to 96	25 and 26

Formulation concentrations were nominal and are expressed in terms of the parent form of AZD9291.

(Excerpted from the Applicant's NDA)

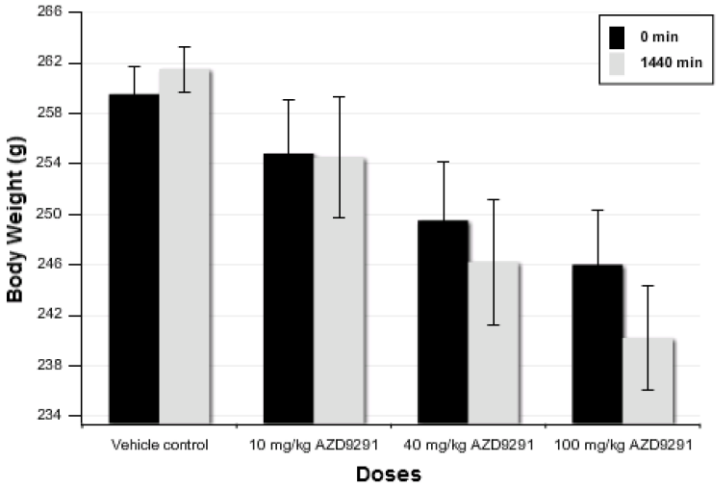
Functional Observational Battery:

Method: CNS evaluations were performed at approximately 15, 30, 60, 120, 240 minutes, and at 24 hours post-dose. Observations included occurrence of vocalization, stereotypies, aggressiveness, abnormal gait, straub tail, twitches, convulsions, body posture, sedation, catalepsy, ptosis, exophthalmos, salivation, lacrimation, piloerection and death. Increases or decreases in the following parameters were noted: pupil response, body tone, spontaneous activity, and response to touch. In addition, decreases in pinna reflex, traction response and grip strength were noted. Abnormal respiration, urination, defecation, sniffling, grooming, scratching, were recorded if observed.

Results:

A decrease in mean body weight as observed. This decrease was only statistically different from vehicle control in high dose group.

Figure 31: Effect of AZD9291 on overnight body weight



(Excerpted from the Applicant's NDA)

A statistically-significant but non-dose-related decrease in pupil size was observed at the 1440 minute timepoint in the 40 mg/kg dose group. The value was within the range observed in vehicle controls and is of uncertain relationship to the test article.

Table 21: Pupil Size Following Single Dose AZD9291

Time(mins)	Vehicle control	10 mg/kg AZD9291	40 mg/kg AZD9291	100 mg/kg AZD9291
15 min	1.17 ±0.17	1.33 ±0.11	1.42 ±0.08	1.33 ±0.11
30 min	1.33 ±0.11	1.42 ±0.08	1.42 ±0.08	1.50 ±0.13
60 min	1.08 ±0.08	1.17 ±0.11	1.33 ±0.11	1.17 ±0.11
120 min	1.33 ±0.11	1.08 ±0.08	1.42 ±0.08	1.17 ±0.11
240 min	1.42 ±0.08	1.17 ±0.11	1.42 ±0.08	1.42 ±0.08
1440 min	1.50 ±0.00	1.33 ±0.11	1.17 ±0.11 *	1.42 ±0.08

Significance Level * P<0.05; ** P<0.01; *** P<0.001 compared to vehicle control (ANOVA on rank scores; 2-sided)

(Excerpted from the Applicant's NDA)

Visual Acuity:

Method: Treatment-related effects on visual acuity were observed in treated animals. Data were collected at a single time point following the 4h modified Irwin assessment. The purpose of this assessment was to determine whether ocular effects observed in toxicology studies could be measured behaviorally following a single oral dose of AZD9291. To evaluate potential visual effects, the OptoMotry system was used, in which the maximum movement frequency that the animal performs while tracking a moving grid is monitored, as well as by counting the number of times focal point was repositioned in a defined period of time (a measure of the animal's activity).

Results:

A decrease in visual acuity was observed in mid- and high-dose groups compared with vehicle controls.

Table 22: Visual Acuity Following Administration of a Single Oral Dose of AZD9291 to male Han Wistar rats

Visual acuity mean (±S.E.M)	**Vehicle control**	**10 mg/kg AZD9291**	**40 mg/kg AZD9291**	**100 mg/kg AZD9291**
Pre-dose	0.35 ± 0.01	0.37 ± 0.02	0.4 ± 0.02	0.35 ± 0.03
Post-dose	0.43 ± 0.02	0.36 ± 0.01	0.32 ± 0.04*	0.32 ± 0.03*

Significance Level * P<0.05; ** P<0.01; *** P<0.001 vs vehicle control group

(Excerpted from the Applicant's NDA)

There was also a decrease in spatial repositioning following treatment with AZD9291. The Applicant states that this is potentially secondary to either behavioral adaptation or to a non-dose-dependent effect of the drug on overall activity level.

Table 23: Spatial Repositioning [mean (±SEM)] data Obtained Following Administration of a Single Oral Dose of AZD9291 to male Han Wistar rats

Visual activity mean (±S.E.M)	**Vehicle control**	**10 mg/kg AZD9291**	**40 mg/kg AZD9291**	**100 mg/kg AZD9291**
Pre-dose	10.83 ± 2.44	7.67 ± 2.20	8.83 ± 2.40	8.50 ± 1.18
Post-dose	18.0 ± 2.62	11.17 ± 1.82*	5.83 ± 1.01***	6.17 ± 1.58***

Significance Level * P<0.05; ** P<0.01; *** P<0.001 vs vehicle control group

(Excerpted from the Applicant's NDA)

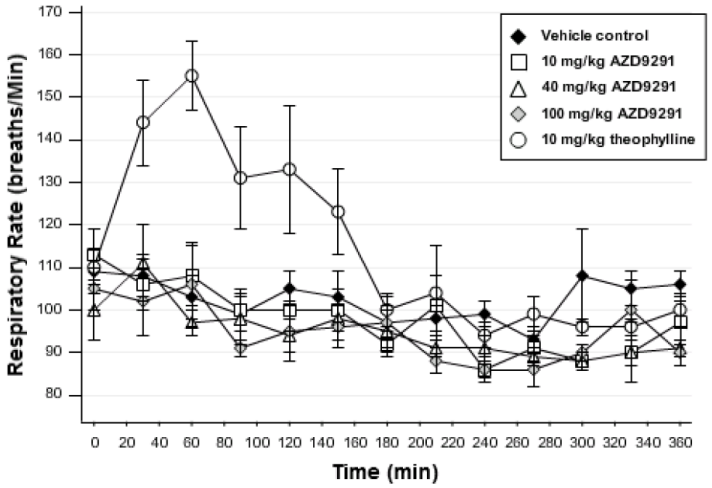
Respiratory Function:

Method: Respiratory evaluations were performed using whole body plethysmography. Following a 90 minute pre-test acclimation period, animals received vehicle or test article and were returned the plethysmograph for approximately 6 hours. Respiratory signals, which included respiratory rate, tidal volume, peak inspiratory and peak expiratory flows and inspiration and expiration times, were collected at 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360 minutes post-dose, where the time 0 timepoint is the mean of the last 30 minutes of the acclimation period.

Results:

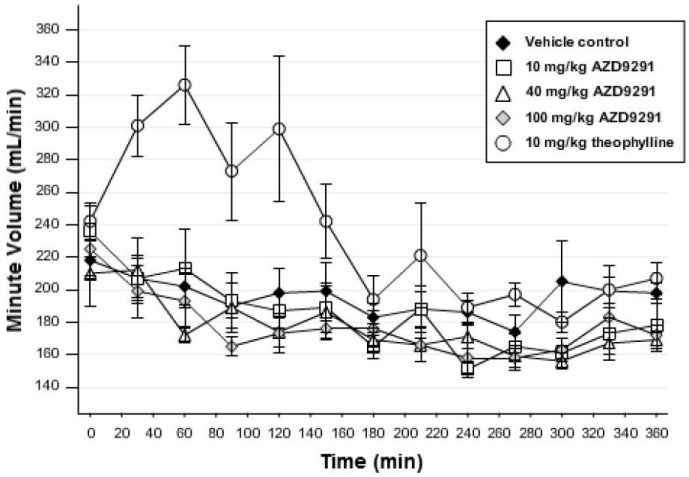
A dose-related effect on expiratory rate was observed (Figure 32) in treated groups relative to controls at 300-360 minutes post-dose. Minute volume was also statistically decreased between 240-300 minutes post-dose at the 10 mg/kg dose level, and at 300 minutes post-dose at the 40 and 100 mg/kg dose levels (Figure 33). These changes were considered within the normal range and were transient, and are thus of uncertain relationship to treatment. An increase expiratory time was also observed in all treated dose groups. While the increase was observed relative to both baseline measurements and concurrent vehicle controls, the statistical evaluation only accounted for changes relative to vehicle controls (Table 24).

Figure 32: Effect of AD9291 on Respiratory Rate in the Rat



(Excerpted from the Applicant's NDA)

Figure 33: Effect of AZD9291 on Minute Volume in the Rat



(Excerpted from the Applicant's NDA)

Table 24: Effect of AZD9291 on expiratory time

Time(mins)	Vehicle control	10 mg/kg AZD9291	40 mg/kg AZD9291	100 mg/kg AZD9291	10 mg/kg theophylline
0	109 ±5	113 ±6	100 ±7	105 ±5	110 ±4
30	108 ±5	106 ±6	111 ±9	102 ±8	144 ±10 ***
60	103 ±5	108 ±7	97 ±3	106 ±10	155 ±8 ***
90	99 ±4	100 ±5	98 ±6	91 ±2	131 ±12 **
120	105 ±4	100 ±5	94 ±4	95 ±7	133 ±15 **
150	103 ±6	100 ±5	98 ±5	96 ±5	123 ±10 *
180	97 ±4	92 ±3	95 ±5	97 ±6	100 ±4
210	98 ±3	101 ±7	91 ±2	88 ±3	104 ±11
240	99 ±3	86 ±2	91 ±3	86 ±3	94 ±3
270	93 ±3	91 ±3	89 ±2	86 ±4	99 ±4
300	108 ±11	88 ±2 **	88 ±2 **	90 ±2 *	96 ±2
330	105 ±4	90 ±7 *	90 ±3 *	100 ±7	96 ±2
360	106 ±3	97 ±5	91 ±2 *	90 ±3 *	100 ±4

Significance Level * P<0.05; ** P<0.01; *** P<0.001 compared to vehicle control

(Excerpted from the Applicant's NDA)

GI Function:

Method: GI evaluations were performed using the charcoal meal method, in which animals received a bolus of charcoal (2 mL/kg) by oral gavage at 240 minutes following oral administration of AZD9291. Animals were killed 15-20 minutes post-administration and the distance traveled by the charcoal bolus was measured in treated and control animals.

Results:

A dose-related decrease was observed in gastric emptying (stomach weight at 15-20 minutes post-charcoal dose; Table 25) and intestinal transport (measured as percent of small intestine length traversed by charcoal front in 15-20 minutes; Table 26).

Table 25: Weight of stomach contents (grams)

AZD9291	Vehicle control	10 mg/kg AZD9291	40 mg/kg AZD9291	100 mg/kg AZD9291
Median	0.82	1.65 *	2.94 **	4.27 ***
Mean	0.92	1.49	2.69	4.72
SEM	0.14	0.19	0.35	0.54
N	8	8	7	8

NS Not Significant *P<0.05; **P<0.01; ***P<0.001

(Excerpted from the Applicant's NDA)

Table 26: Intestinal transport distance (%)

AZD9291	Vehicle control	10 mg/kg AZD9291	40 mg/kg AZD9291	100 mg/kg AZD9291
Median	59.05	57.05 NS	44.28 *	49.27 **
Mean	60.61	53.92	48.46	49.74
SEM	2.61	3.20	3.73	2.06
N	8	8	8	8

NS Not Significant *P<0.05; **P<0.01; ***P<0.001

(Excerpted from the Applicant's NDA)

Plasma concentrations measured in this study are reported in Table 27; however, later bioanalytical stability analyses suggest that the measurements for AZD9291 and its metabolites may be under-estimated by as much as 31%. Data for metabolite AZ13597550 is presented in Table 28. For metabolite AZ13575104, the presence of a co-eluting peak may have affected the exposure assessment; thus, the levels were not reported for this metabolite.

Table 27: Summary of mean plasma concentrations for AZD9291 in male Han Wistar rats following oral administration of AZD9291

Group	Dose (mg/kg)	Nominal sampling time					
		4h 20m			6h 20m		
		Mean	S.D.	n	Mean	S.D.	n
CNS (Irwin) and OptoMotry phase groups							
2	10	0.266	0.0325	6	NS	NA	NA
3	40	0.557	0.0663	6	NS	NA	NA
4	100	0.876	0.218	6	NS	NA	NA
Respiratory (WBP) phase groups							
6	10	NS	NA	NA	0.203	0.0530	8
7	40	NS	NA	NA	0.579	0.136	8
8	100	NS	NA	NA	0.995	0.281	8
GI (charcoal meal) phase groups							
11	10	0.226	0.0464	8	NS	NA	NA
12	40	0.912	0.358	8	NS	NA	NA
13	100	1.47	0.200	8	NS	NA	NA

NS No sample(s)
NA Not applicable

(Excerpted from the Applicant's NDA)

Table 28: Summary of mean plasma concentrations for AZ13597550 in male Han Wistar rats following oral administration of AZD9291

Group	Dose (mg/kg)	Nominal sampling time					
		4h 20m			6h 20m		
		Mean	S.D.	n	Mean	S.D.	n
CNS (Irwin) and OptoMotry phase groups							
2	10	0.0329	0.00929	6	NS	NA	NA
3	40	0.0789	0.00376	6	NS	NA	NA
4	100	0.111	0.0217	6	NS	NA	NA
Respiratory (WBP) phase groups							
6	10	NS	NA	NA	0.0243	0.00610	8
7	40	NS	NA	NA	0.0818	0.0147	8
8	100	NS	NA	NA	0.127	0.0383	8
GI (charcoal meal) phase groups							
11	10	0.0283	0.00610	8	NS	NA	NA
12	40	0.117	0.0376	8	NS	NA	NA
13	100	0.161	0.0221	8	NS	NA	NA

NS No sample(s)
NA Not applicable

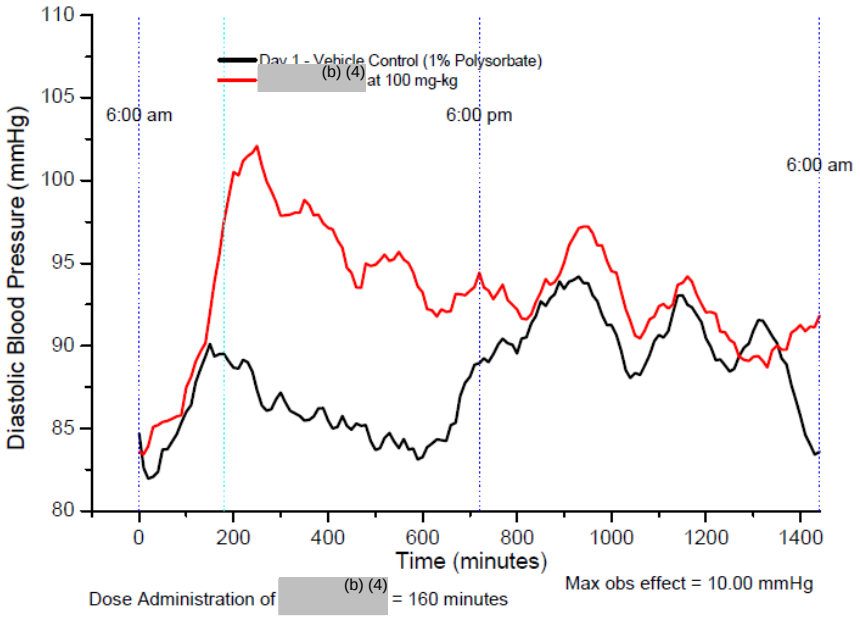
(Excerpted from the Applicant's NDA)

4.3.8 Cardiovascular Effects of (b) (4) in vivo: Rat Telemetry (Report # PH/E/14191)

The purpose of this study was to evaluate potential effects of AZD9291 (a.k.a. (b) (4)) on cardiovascular function in telemetered rats (N = 4 males per group) following administration of a single oral dose of 20, 50, or 100 mg/kg. Animals were telemetrically monitored for electrophysiological effects for up to 24 hours post-dose.

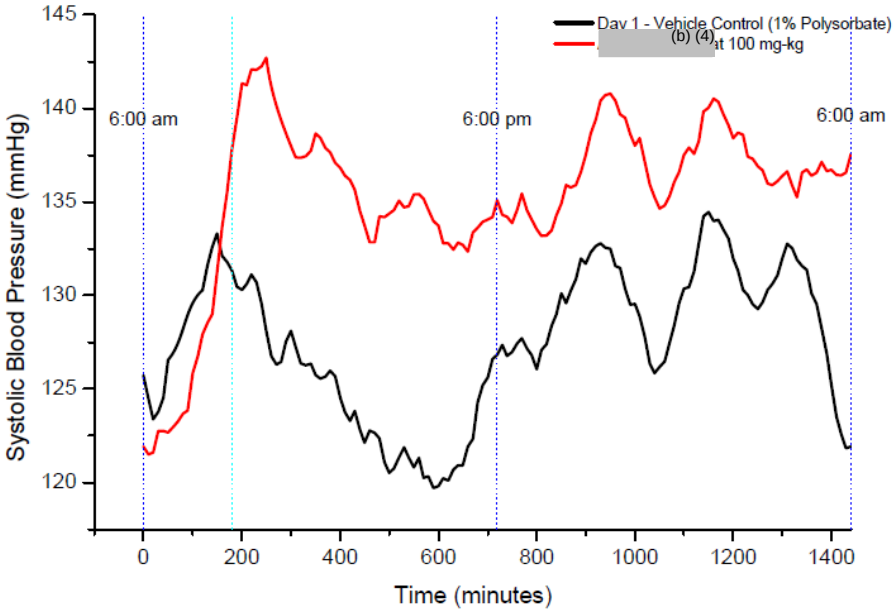
Following a single oral dose of 100 mg/kg, AZD9291 increased systolic and diastolic blood pressure (Figure 34 and Figure 35) in the rat, primarily during the hours of rest (Figure 36), and the effect persisted for more than 24 hours post-dose. There was no effect on heart rate. Similar effects were observed at doses of 50 mg/kg; however, pressures returned to baseline by the end of the observation period at that dose level (data not shown). There was no effect on overall activity (Figure 36).

Figure 34: Effect of AZD9291 on the Diastolic Blood Pressure of Rats



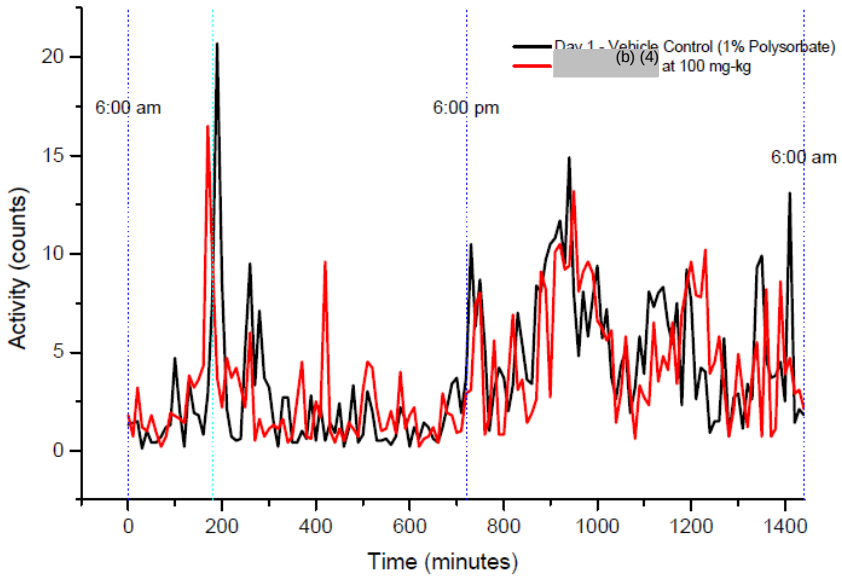
(Excerpted from the Applicant's NDA)

Figure 35: Effect of AZD9291 on the Systolic Blood Pressure of Rats



(Excerpted from the Applicant's NDA)

Figure 36: Activity Response following a 100 mg/kg oral dose of AZD9291 in the Rat



(Excerpted from the Applicant's NDA)

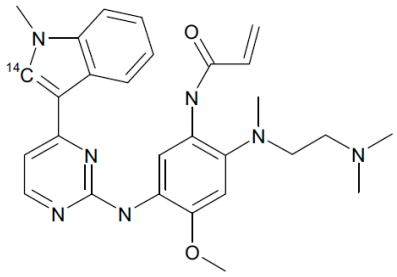
5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

5.1.1 AZD9291: The disposition of [14C]AZD9291 following intravenous and oral administration in the dog (Report 197725)

The purpose of this study was to assess the modes and rate of elimination of [^{14}C]AZD9291 and its metabolites, AZ13575104 and AZ13597550, following administration of either a single IV dose of 1 mg/kg or a single oral dose of 2 mg/kg in male beagle dogs (source: (b) (4) ages 19-20 months, and 9.7-10.8 kg upon dosing; the report does not state whether the dogs were experimentally-naïve).

Figure 37: Position of the ^{14}C label on AZD9291 for the dog disposition study

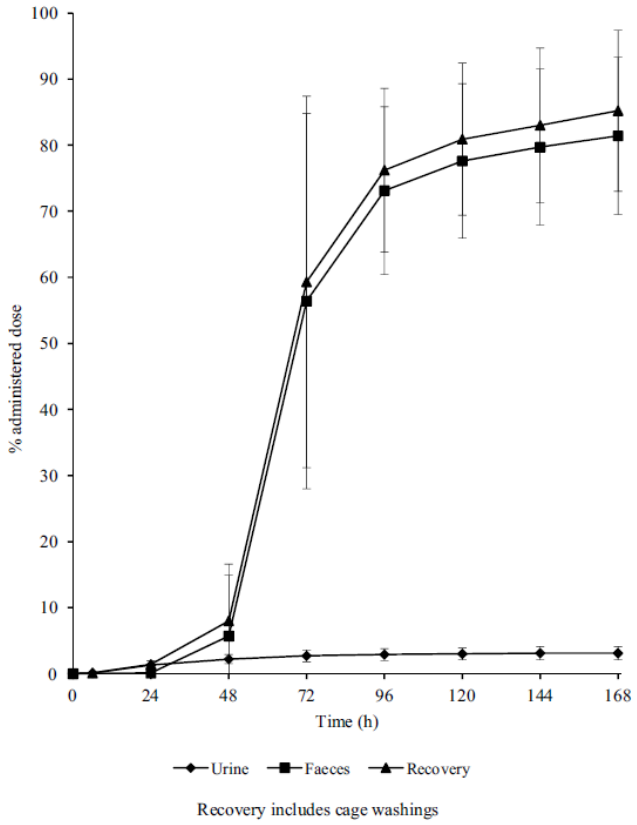


(Excerpted from the Applicant's NDA)

The specific activity of the IV and oral formulations were 2.02 MBq/mg and 0.99 MBq/mg, respectively. Whole blood samples were collected at the following time points: 0.083 (IV), 0.167 (IV), 0.25 (oral), 1, 2, 4, 6, 12, 24, 48, 72, 96, 120, 144, and 168 hours post-dose.

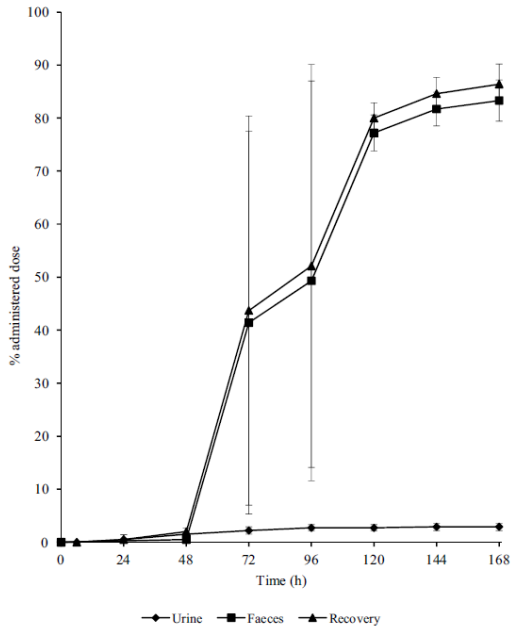
Urine was collected at 00-6 and 6-24 hours, and daily for 168 hours thereafter. Feces were collected for up to 168 hours. After completion of sample collection, cages were washed and wash volumes were retained for analysis.

Figure 38: Mean cumulative recovery of radioactivity in excreta following administration of a 1 mg/kg IV dose of [^{14}C]AD9291 to male beagle dogs



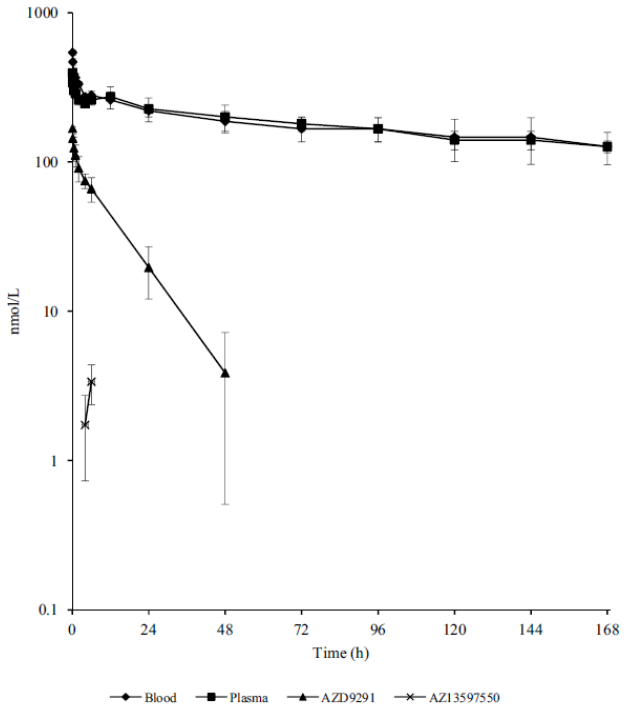
(Excerpted from the Applicant's NDA)

Figure 39: Mean cumulative recovery of radioactivity in excreta following administration of a 2 mg/kg oral dose of [^{14}C]AD9291 in male beagle dogs



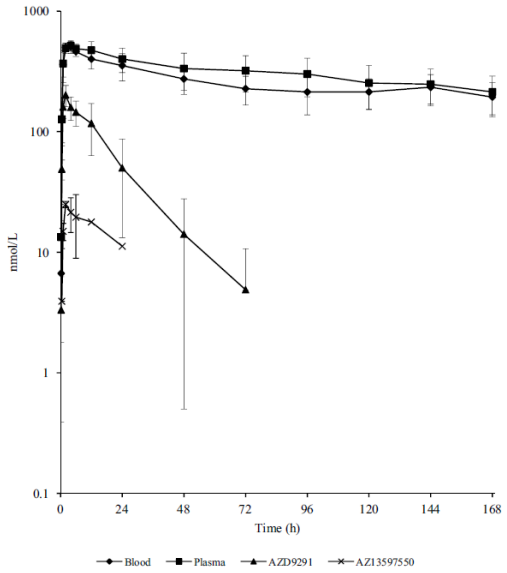
(Excerpted from the Applicant's NDA)

Figure 40: Mean concentration of radioactivity in blood and plasma and concentrations of AD9291 and AZ13597550 following administration of a single IV dose of 1 mg/kg [^{14}C]AD9291 in male beagle dogs



(Excerpted from the Applicant's NDA)

Figure 41: Mean concentration of radioactivity in blood and plasma and concentrations of AD9291 and AZ13597550 following administration of a single oral dose of 2 mg/kg [^{14}C]AD9291 in male beagle dogs



(Excerpted from the Applicant's NDA)

Parameter	Route	Total Radioactivity[+]	AZD9291	AZ13597550
Tmax (hours)	IV	--	--	6
	PO	4	2	4
C_{max} (nmol/L)*	IV	457	195	3.37
	PO	540	201	26.5
$AUC_{(0-\tau)}$ (nmol*hr/L)	IV	30300	1550	ND
	PO	53000	3740	620
AUC_{met}[#]	IV	1950	NA	NC
	PO	1420	NA	16.6
CL (L/h/kg)	IV	NA	1.28	NA
V_d (L/kg)	IV PO	NA	18.2	NA
$T_{1/2}$ (h)	IV	197[§]	10.3	ND
	PO	179[§]	13.1	15.9
%F	PO	NA	115	NA

[+]C_0 nmol equiv/L and AUC nmol equiv*hr/L for total radioactivity
*Cmax for AA13597550 occurred at 6h
[#]AUC_{met} = $AUC_{(0-\tau)}$ of AZ13597550 or total radioactivity expressed as a percentage of AZD9291
[§]value derived where criteria for determination of the terminal phase were not met
NA = not applicable; ND = not determined; NC = not calculated

Total recovery was between 85.2 and 86.4% of the administered dose for the IV and PO routes, respectively. As indicated in Figure 38 and Figure 39, the major route of elimination is via the GI tract; urinary recovery accounted for less than 5% of the total recovered dose. As illustrated in Figure 40 and Figure 41, there was no evidence of partitioning to the cellular components of whole blood.

Exposure to AZ13597550 was approximately 17% of the total dose administered. ISR, indicative of methodological reproducibility, for the AZ13575104 metabolite failed; thus data for that metabolite were not reported.

5.1.2 AZD9291: In Vitro Covalent Binding of [^{3}H]-Labelled AZD9291 to Rat Hepatic Proteins in Rat Hepatocyte Incubations (Report: 120118_CVB_KXZZ856)

The purpose of this study was to assess the extent of covalent binding of [^{3}H]AZD9291 or its metabolites to rat hepatic proteins using cultured rat hepatocytes.

Cells were cultured for 4 hours in the presence of either 10 µM [^{3}H]AZD9291 or [^{3}H]Zomepriac (positive control). Cells were cultured for 4 hours at 37°C/5% CO_2. Samples were analyzed by LC-MS/MS.

As illustrated in Table 29, AZD9291 binds to hepatic proteins. The net covalent binding (CVB_{net}) was 217 pmol eq./mg protein, which exceeded that of the positive control. The metabolic turnover (calculated as 1-[(concentration parent at end) / concentration of parent (initial)] * 100) was estimated to be 71%, suggesting that the fraction of [^{3}H]AZD9291 that was covalently bound (f_{cvb}) was 0.020, which is similar to the positive control.

Table 29: Covalent binding and fraction of covalent binding of [^{3}H]AZD9291 and [^{3}H]zomepirac in cultured hepatocyte incubations

	[^{3}H]- AZD9291	[^{3}H]-Zomepirac
$CVB_{T=End}$[a] (pmol eq./mg protein)	228	58.5
$CVB_{T=0}$[a] (pmol eq./mg protein)	10.9	14.4
CVB_{net}[a] (pmol eq./mg protein)	217 ±20.9	44.0 ±3.8
LC Retention time (min)	0.81	1.17
Turnover of parent[a] (%)	71.0 ±2.5	12.2 ±3.0
f_{CVB}[a]	0.0200 ±0.0025	0.0241 ±0.0042

a Calculations are made from values not rounded

(Excerpted from the Applicant's NDA)

5.1.3 AZD9291: The Tissue Distribution of Total Radioactivity in the Rat Following Oral Administration of [^{14}C]AZD9291 (Quantitative Whole Body Autoradiography) (Report #8265562)

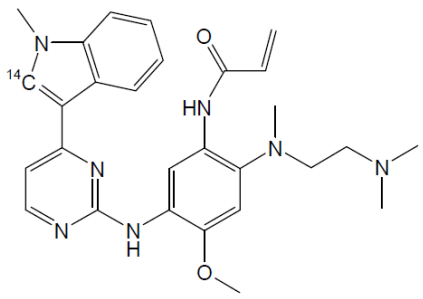
The purpose of this study was to evaluate the tissue distribution of [^{14}C]AZD9291 by quantitative whole body autoradiography in the albino and partially-pigmented rat, following a single oral dose of 4 mg/kg (8 µmol/kg; 7.4 MBq/kg). The study design and timepoints evaluated are provided in Table 30.

Table 30: Design of the rat distribution study

Dose group	Animal strain/sex	Sampling times
A	Partially Pigmented (Lister-Hooded)/Male	0.5, 1, 6 24 hours and 2, 7, 21 and 60 days post-dose
B1	Albino (Han Wistar)/Male	1, 6 and 24 hours post-dose
B2	Albino (Han Wistar)/Female	1, 6 and 24 hours post-dose

(Excerpted from the Applicant's NDA)

Figure 42: Position of the ^{14}C label in the rat mass balance study



(Excerpted from the Applicant's NDA)

As illustrated in Figure 45, the drug was broadly distributed, with low-level binding present in a large number of tissues. Binding was highest in the uveal tract of the pigmented rat at all time-points evaluated (through 60 days), suggesting a potential for accumulation in the eye. Uveal binding was lower in the albino rat than in the pigmented rat, suggesting that retention in the eye was mediated by interactions with melanin.

A low level of radiolabeled material was observed in the brain and spinal cord at all time-points tested (through Day 21).

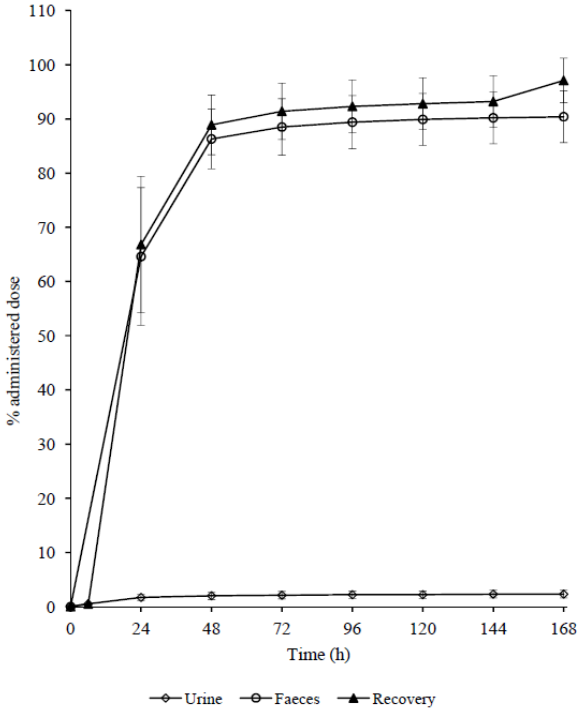
As illustrated in Table 31, the study achieved mass balance, as recovery of the radiolabel was ≥90%. AZD9291 was predominantly eliminated in feces. Urinary elimination accounted for a relatively small proportion of the administered dose (<4% by both routes of administration).

Table 31: Mean recovery of radioactivity (0-168 hours) after single I or PO dose of [^{14}C]AZD9291 in male and female rats (% of administered dose)

Type of	Intravenous dose		Oral dose	
Sample	Male	Female	Male	Female
Urine	3.8 ± 0.8	2.8 ± 0.5	2.3 ± 0.9	2.3 ± 0.5
Faeces	84.4 ± 4.2	80.9 ± 1.0	93.4 ± 2.1	87.4 ± 5.0
Expired Air	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cage Wash	1.0 ± 0.4	1.5 ± 1.1	0.7 ± 0.1	0.9 ± 0.7
Carcass	4.6 ± 0.4	4.8 ± 0.5	3.0 ± 0.3	4.2 ± 0.6
Total	93.7 ± 5.0	90.0 ± 2.1	99.3 ± 2.5	94.8 ± 4.5

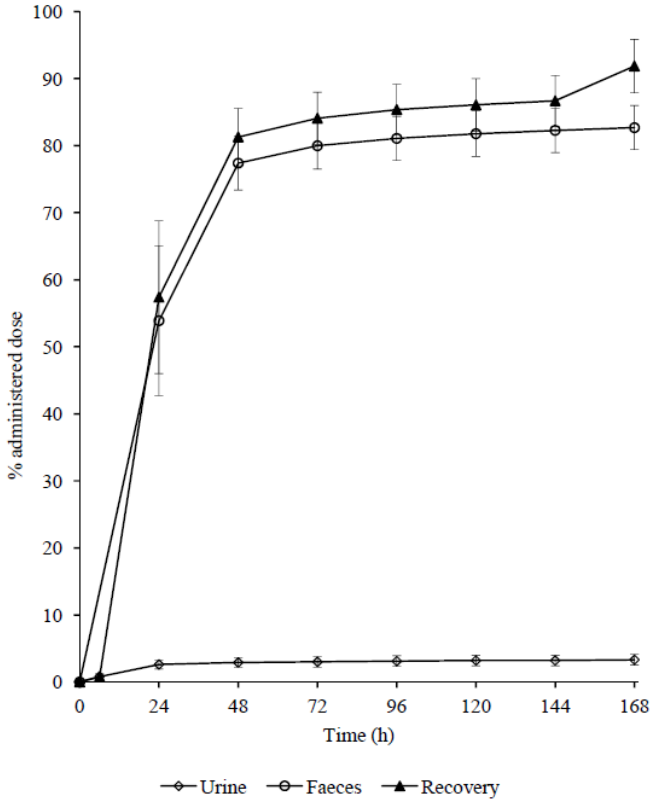
(Excerpted from the Applicant's NDA)

Figure 43: Mean cumulative recovery of radioactivity in excreta following administration of a single oral dose of [14C]AZD9291 (10 mg/kg) to male and female rats



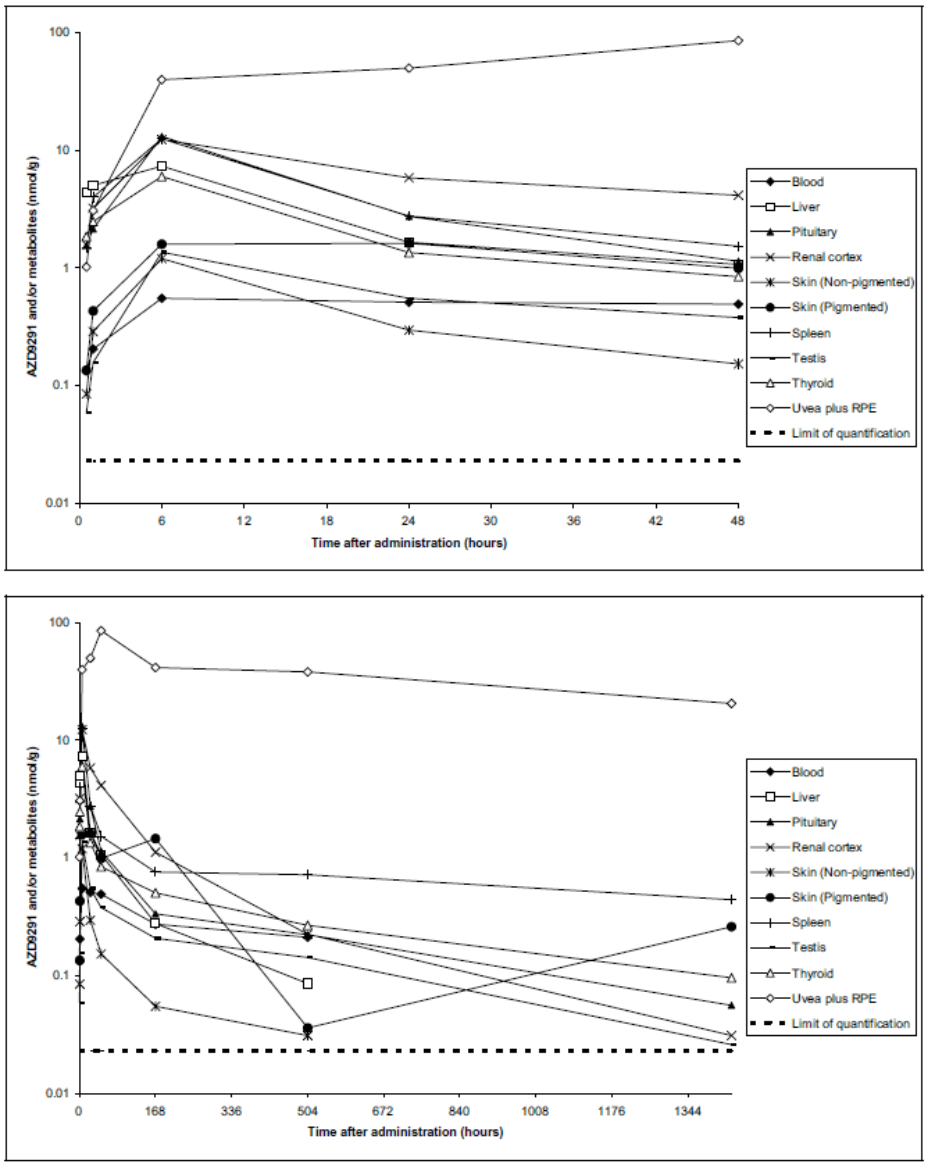
(Excerpted from the Applicant's NDA)

Figure 44: Mean cumulative recovery of radioactivity in excreta following administration of a single oral dose of [14C]AZD9291 (2 mg/kg) to male and female rats



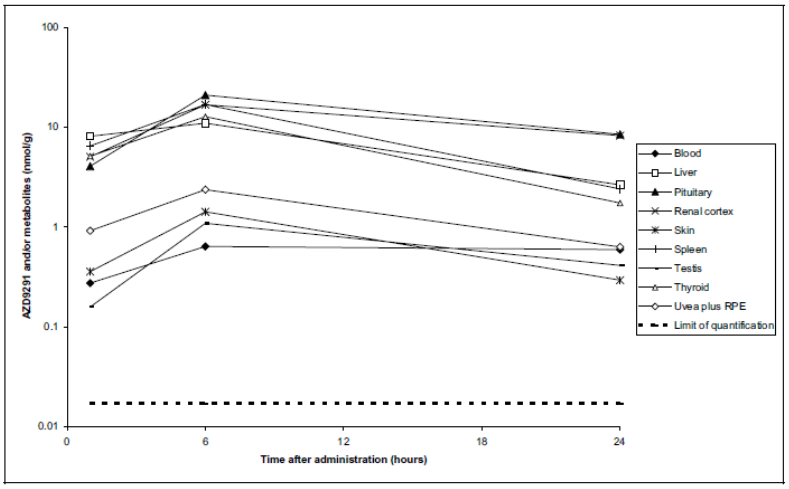
(Excerpted from the Applicant's NDA)

Figure 45: Concentrations of [^{14}C]AZD9291 and/or its Metabolites in Selected Tissues at Different Times After Oral Administration of 4 mg/kg to Male Partially Pigment Rats



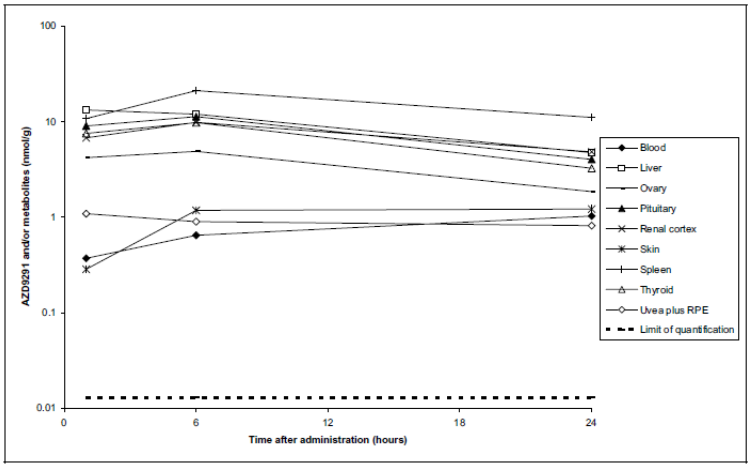
(Excerpted from the Applicant's NDA)

Figure 46: Concentrations of [^{14}C]AZD9291 and/or its Metabolites in Selected Tissues at Different Times after Oral Administration of 4 mg/kg to Male Albino Rats



(Excerpted from the Applicant's NDA)

Figure 47: Concentrations of [^{14}C]AZD9291 and/or its Metabolites in Selected Tissues at Different Times after Oral Administration of 4 mg/kg to Female Albino Rats



(Excerpted from the Applicant's NDA)

5.1.4 AZD9291: Mass balance and radioactive pharmacokinetics following oral and intravenous administration of [^{14}C]AZD9291 to the rat (Report #196187)

The purpose of this study was to assess the mode of elimination for AZD9291 in the rat following administration of a single IV (2 mg/kg; 4µmol/kg) or oral dose (10 mg/kg; 20µmol/kg) of [^{14}C]AZD9291 in the male and female Han Wistar rat. The specific activity was 2.16 GBq/mmol at 501.6 g/mol. Following dose-administration, animals were monitored for up to 168 hours. Expired air was collected from each animal for 0-24

hours post-dose, and 2 animals/sex were sacrificed at 1, 3, and 12 hours after dosing and blood/plasma collection.

Table 32: Design of the rat mass balance study

No. and sex of rats	Dose route	Dose level mg/kg (µmol/kg)	Radioactive dose level MBq/kg	Study type
3 male/3 female	Intravenous	2 (4)	8.52	Excretion balance
3 male/3 female	Oral	10 (20)	10	Excretion balance
6 male/6 female	Oral	10 (20)	10	Blood and plasma radioactivity

(Excerpted from the Applicant's NDA)

As indicated in Table 32, the study achieved mass balance, as > 90% of the radiolabel was recovered.

The major route of elimination was via the feces (Table 33). Urinary elimination accounted for less than 5% of the excreted material, and there were no sex differences in the routes or rates of elimination. The blood to plasma ratio was > 1, suggesting that the drug partitions to red blood cells (Table 34).

Table 33: Summary of mean (±SD) total recovery of radiation after a single IV or oral dose of [^{14}C]AZD9291 to male and female rats (% administered dose)

Type of	Intravenous dose		Oral dose	
Sample	Male	Female	Male	Female
Urine	3.8 ± 0.8	2.8 ± 0.5	2.3 ± 0.9	2.3 ± 0.5
Faeces	84.4 ± 4.2	80.9 ± 1.0	93.4 ± 2.1	87.4 ± 5.0
Expired Air	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cage Wash	1.0 ± 0.4	1.5 ± 1.1	0.7 ± 0.1	0.9 ± 0.7
Carcass	4.6 ± 0.4	4.8 ± 0.5	3.0 ± 0.3	4.2 ± 0.6
Total	93.7 ± 5.0	90.0 ± 2.1	99.3 ± 2.5	94.8 ± 4.5

Table 34: Summary of mean plasma and blood concentrations of total radioactivity following oral administration of 10 mg/kg [^{14}C]AZD9291 to male and female rats

Time after dose (h)	Mean concentration of radioactivity (n=2 per sex/timepoint) (µmol equiv/L)				Blood/plasma ratio	
	Male		Female		Male	Female
	Blood	Plasma	Blood	Plasma		
1	1.32	0.84	1.56	1.03	1.57	1.53
3	2.28	1.38	2.30	1.48	1.65	1.54
12	2.45	0.90	2.45	1.20	2.72	2.04

6 General Toxicology

6.2 Repeat-Dose Toxicity

6.2.1 AZD9291: Three Month Oral (Gavage) Toxicity Study in the Rat - Report

Amendment

Study no.:	(b) (4) 526248
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	06 November 2013 (1st dose)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Drug: AZD9291 mesylate salt Lot: 02-kcwg806-87 Purity: 95.6% (HPLC % area)

Key Study Findings:

- Target organs included the eyes (atrophy of the corneal epithelium and retinal dysplasia), skin (inflammatory cell infiltration), lung (inflammatory cell infiltration), kidneys (tubular basophilia and inflammatory cell infiltration) and GI tract.
- Hematology changes included decreased red blood cell indices, which correlated with increased incidences of erythrophagocytosis in high dose animals; increased WBC indices; increased fibrinogen and decreased APTT.
- Clinical chemistry changes included increased ALT and AST; decreased triglycerides, albumin, and total protein. Decreased calcium was also observed; however, this is likely secondary to the observed decreases in albumin.

Methods

Doses:	0, 1, 10, or 40/20 mg/kg/Day (males/females)**
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	MilliQ water pH adjusted with methane sulfonic acid to a final pH of 3.0-3.5
Species/Strain:	*Rattus norvegicus* (Han Wistar)
Number/Sex/Group:	10/sex/group
Age:	Approximately 8-10 weeks at initiation of dosing
Weight:	Males: 253-337g (Day 1 dosing) Females: 163-211 g (Day 1 dosing)
Satellite groups:	Toxicokinetics (3/sex)
Unique study design:	❖ **Dose holiday between Days 56-62 and

dose reduction from 40 to 20 mg/kg/Day in high dose males due to poor tolerability.
- ❖ Assessment of male fertility during Week 10 by pairing with undosed females.
- ❖ No recovery cohorts

Deviation from study protocol: Reported deviations were unlikely to have affected overall study interpretation; however, the protocol was not appended to the study report; thus, it is not possible to determine whether other protocol deviations may have occurred beyond those that were reported.

Observations and Results

Mortality

There were no unscheduled deaths.

Clinical Signs

Treatment-related clinical signs observed in mid- and high-dose males and females included: dehydration, liquid/soft feces, decreased activity, skin changes (flaking, scabs, dryness, alopecia, ungroomed/stained fur), swollen muzzle, salivation, ploughing (a behavior characterized by using the head to push cage bedding; may be associated with abdominal discomfort), and hunching.

Body Weights

High dose (40 mg/kg) male mean body weights were significantly reduced ($p < 0.05$) compared with controls between Days 36-78. On Day 56, high dose males were given a dosing holiday and on Day 62, dosing was resumed at a dose of 20 mg/kg. There were no statistically significant changes in female body weights versus concurrent controls during the study.

Figure 48: Mean Male Body Weights

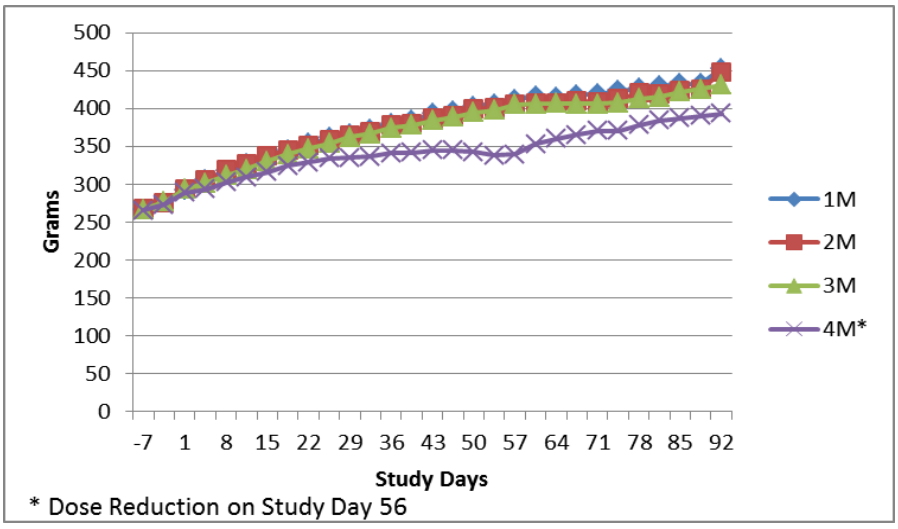
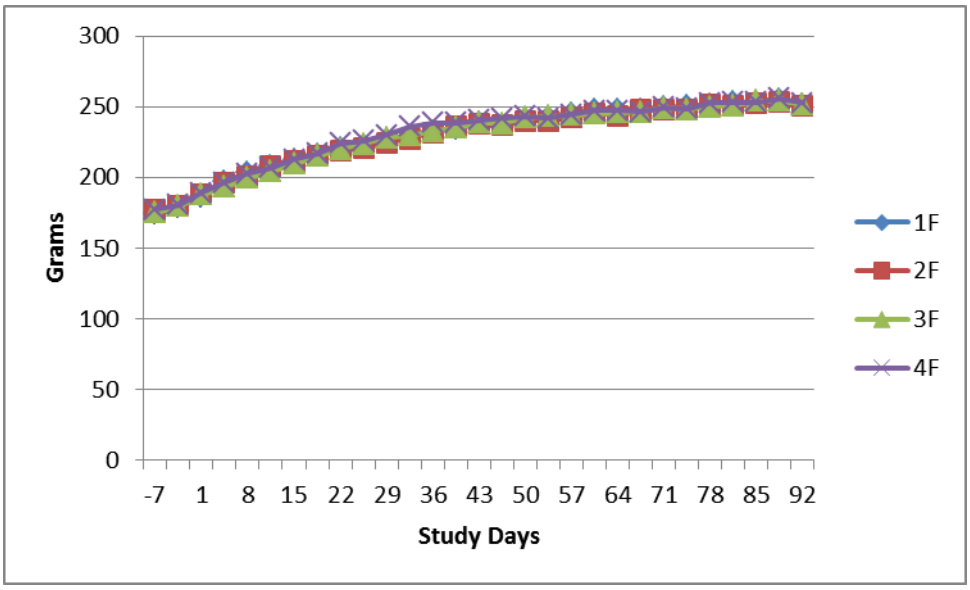


Figure 49: Mean Female Body Weights



Feed Consumption

Reduced feed consumption ($p < 0.05$) was observed in high-dose males between Study Days 36 to 57, which correlated with reduced body weights during that period.

There were no treatment-related effects of treatment on female feed consumption.

Ophthalmoscopy

The ophthalomologist's narrative indicates that there were no treatment-related effects; however, in treated animals there appeared to be an increase in the incidence of "cornea opacity" relative to the incidence noted during baseline exams. This finding correlated with an increase in the histological finding of corneal atrophy in treated animals.

Hematology

Treatment-related increases in many WBC subsets were observed in high dose males and females (Table 35). Treatment-related decreases in some or all RBC subsets were also observed, some statistically significant versus concurrent controls. Treatment-related decreases in APTT were observed in both sexes, without demonstrable effects on PT in either sex. An increase in fibrinogen was also observed in high dose males and females compared with concurrent controls.

Table 35: Changes in mean hematology parameters in the rat (%Δ vs. concurrent control)

Parameter	2M	3M	4M*	2F	3F	4F
WBC	15.6	25.9	115.0	4.9	2.2	102.0
NEUT	13.3	41.8	360.2	32.7	72.7	587.3
LYMPH	15.5	21.5	47.5	-0.3	-9.7	19.9

Parameter	2M	3M	4M*	2F	3F	4F
MONO	0.0	20.0	**190.0**	42.9	14.3	**242.9**
EOS	50.0	8.3	58.3	0.0	0.0	70.0
BASO	50.0	50.0	**300.0**	0.0	0.0	**150.0**
LUC	0.0	0.0	66.7	0.0	0.0	50.0
RBC	2.5	-2.3	**-4.6**	0.5	-1.6	-3.7
HGB	0.0	-3.1	**-5.0**	0.7	-2.6	**-5.3**
HCT	0.8	-3.5	-5.3	0.2	-2.4	-5.3
MCV	-1.7	-1.3	-0.9	-0.4	-1.1	-1.6
MCH	-1.7	0.0	0.0	0.0	-0.5	-1.6
MCHC	-0.6	0.9	0.9	0.0	0.3	-0.3
RDW	-15.9	1.8	2.4	-3.4	-4.7	19.6
PLT	18.3	20.0	**30.4**	1.7	-1.1	6.6
RETIC	4.9	4.6	4.5	2.7	-0.6	22.3
PT	1.4	3.2	1.4	-1.8	1.4	5.0
APTT	-2.7	-5.9	**-9.7**	0.6	2.8	-6.8
FIB	5.0	0.4	**27.8**	5.6	5.0	**53.8**

Red = 0.05 < *p* < 0.001

Clinical Chemistry

Treatment-related increases were observed in AST and ALT in high dose males and/or females. There were no correlating histopathological findings in the liver that would have explained the increased liver enzyme levels.

Decreases in triglycerides, cholesterol, and albumin particularly in high dose males and/or females are potentially related to treatment; however, some of these effects in males may be attributable to inappetence. The decrease in serum calcium is likely attributed to the decrease in serum albumin.

Table 36: Changes in mean chemistry parameters in the rat (%Δ vs. concurrent control)

	2M	3M	4M*	2F	3F	4F
AST	4.9	19.7	**37.7**	-7.2	0.0	18.8
ALT	0.0	17.1	**41.5**	2.7	18.9	**43.2**
ALP	-1.3	13.8	11.3	9.1	6.8	-2.3
TBIL	0.0	0.0	0.0	0.0	0.0	0.0
BILEAC-P	120.0	20.0	80.0	-20.0	80.0	50.0
UREA	9.4	3.8	**15.1**	-8.1	**-16.1**	-11.3
CREAT	15.4	5.1	2.6	-4.7	0.0	**-11.6**
GLUC	4.4	1.3	-2.6	14.9	12.3	19.6
CHOL	0.0	-6.3	-18.8	-6.7	-6.7	-6.7
TRIG	-25.6	**-32.8**	**-44.4**	-8.5	-7.8	-34.6
TPROT	1.5	3.1	3.1	-4.3	0.0	**-5.7**
ALB	2.3	0.0	**-11.4**	-2.0	-5.9	**-15.7**
GLOB	0.0	4.5	**22.7**	-15.8	15.8	21.1
A/G	5.0	-5.0	**-25.0**	7.1	-21.4	**-32.1**
CA	1.1	-1.1	-2.2	**-2.5**	-1.1	**-3.9**

PHOS	2.6	-0.7	7.2	-5.2	-9.7	-1.5
NA	0.7	0.0	-0.7	-0.7	-0.7	-1.4
K	2.1	0.0	2.1	-4.5	-6.8	0.0
CL	1.0	2.0	1.0	0.0	1.0	1.0

Urinalysis

There was a dose-related increase in specific gravity in high dose males. Other changes of potential relationship to treatment are indicated in Table 37.

Table 37: Changes in mean urine chemistry parameters in the rat (%Δ vs. concurrent control)

Parameter	2M	3M	4M*	2F	3F	4F
PROT	**174.8**	45.9	**300.0**	-59.6	-30.1	277.9
PROT/CREAT	46.2	21.7	97.0	**-38.3**	-23.6	172.4
GLUC	50.0	0.0	100.0	0.0	0.0	**150.0**
GLUC/CREAT	16.7	16.7	0.0	33.3	16.7	50.0
CREAT	**81.0**	6.6	**95.5**	-26.0	3.5	56.0
NAG	40.7	-5.6	**94.4**	-19.0	34.5	**132.8**
NAG/CREAT	-5.3	-10.5	5.3	11.8	**35.3**	**52.9**

Gross Pathology

Gross observations of potential or equivocal relationship to treatment were observed in the skin, thymus, lymph nodes, lung, and liver.

	Males (mg/kg/Day)				Females (mg/kg/Day)			
Organ, finding	**0**	**1**	**10**	**40/20**	**0**	**1**	**10**	**20**
Adipose, Discoloration, dark						1		
Kidney, Dilatation, clear, pelvis						1		
Liver, Discoloration, dark		1						
Lung, focus		1						
-- focus, dark	1							1
-- focus, pale			1					
-- focus, dark, caudal lobe		1						
-- discoloration, dark	3	2	3	2			1	
-- discoloration, mottled			1					
--Discoloration, pale								1
Lymph node, bronchial, discoloration, dark							1	
Lymph node, lumbar, Discoloration, dark								1

	Males (mg/kg/Day)				Females (mg/kg/Day)			
Lymph node, mandibular, discoloration, dark	1	2	1	2		3	2	1
-- enlargement				1				
Lymph node, renal, discoloration, dark				1				
Skin, thin hair coat		1		2			1	
--Thin hair coat, back				1				1
--scab							1	4
--scab, back				1				1
--abnormal appearance				6			1	5
--abnormal appearance, tail				2			1	1
Thymus, discoloration				1				
--discoloration, dark			1					
--discoloration, mottled				1				1
--focus, dark	1	1		2				
--focus				1				
Uterus, dilatation, clear	--	--	--	--		1		
Peyer's patch, enlargement		1	1					
--enlargement, raised				1				

Organ Weights

Other than decreases in the absolute and/or relative weights of male and female reproductive organs (Table 38), there were no changes in organ weights that were attributed to treatment.

Table 38: Summary of treatment-related organ weight changes

Study phase	**Main**							
Dose (mg/kg/day)	**0**		**1**		**10**		**40/20**	
Number of animals	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Epididymis (No. weighed)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Absolute value (g)	1.333	-	1.298	-	1.198	-	1.127b	-
% of body weight	0.3103	-	0.3051	-	0.2824	-	0.2888	-
Prostate (No. weighed)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Absolute value (g)	0.483	-	0.472	-	0.414	-	0.327b	-
% of body weight	0.1125	-	0.1107		0.0974	-	0.0846	-
Uterus (No. weighed)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Absolute value (g)	-	0.829	-	0.767	-	0.501b	-	0.549a
% of body weight	-	0.3238	-	0.3008	-	0.1988b	-	0.2174

Significantly different from control group 1 value : a=p≤0.05, b=p≤0.01, c=p≤0.001 (Dunnett)

(Excerpted from the Applicant's NDA)

Histopathology

Adequate Battery
Yes

Peer Review
Yes. Two peer-reviews were performed – an internal peer-review by pathologists employed by the CRO, and an external peer-review by a pathologist retained by the Applicant. The peer review statement indicates that differences of opinion were discussed and that the report reflects the consensus opinion of the Applicant's pathologist and the study pathologist. The report does not indicate which findings were discussed or the manner in which the differences of opinion were resolved.

Histological Findings

Table 39: Summary of histopathological findings in the rat

	Male				Female			
Organ, Histopathological Description **Group (mg/kg/Day)**	**0**	**1**	**10**	**40/20**	**0**	**1**	**10**	**20**
Adrenal, vacuolation, cortical				1				
Adipose, hemorrhage						1		
Epididymis, infiltration, lymphocytic				2				
--decreased cellularity				1				
Esophagus, atrophy, epithelial			7	8			4	10
Eyes, atrophy, epithelial, cornea			4	8			6	10
--dysplasia, retina					1		1	
Eyelid, inflammation, mixed cell, epidermal				1				1
--inflammation, follicular/perifollicular				5				5
--crust								3
--infiltration, lymphocytic, tarsal gland							1	
Hardian gland, infiltration, lymphocytic				2	2		3	6
--pigmentation, porphyrin	1							
--degeneration/regeneration/single cell necrosis**			2	5			3	6
Heart, infiltration, myocardial				1				
Kidney, basophilia, tubular**	7			10	1			2
--infiltration, lymphocytic, cortex					1			1
--infiltration, lymphocytic, cortex, pelvis				2				
--infiltration, lymphocytic, pelvis				1				
--dilatation, pelvis								1
LN, bronchial, erythrophagocytosis							1	
LN, lumbar, erythrophagocytosis								1
LN, mandibular, erythrophagocytosis**		1				3	1	1
--plasmacytosis				2				
--cyst						1		
LN, mesenteric, erythrophagocytosis				4				3
LN, renal, erythrophagocytosis				1				1

	Male				Female			
Organ, Histopathological Description **Group (mg/kg/Day)**	**0**	**1**	**10**	**40/20**	**0**	**1**	**10**	**20**
Lungs, congestion, agonal	2	3	3	1			1	1
--macrophage aggregation, alveolar**	3	3	8	8	1	1	3	9
--inflammation, neutrophilic, perivascular				1				
Mammary, atrophy				5				
Ovary, decreased number, corpus luteum	--	--	--	--				1
Peyer's Patches, hyperplasia, lymphoid		1	1					
Skin, inflammation, mixed cell, epidermal								1
--inflammation, follicular/perifollicular				10				7
--infiltration, neutrophilic, subcutaneous tissue								1
--crust						1		1
Spleen, increased hematopoiesis**				5			2	5
Stomach, non-glandular, atrophy, epithelial, nonglandular mucosa				3				2
--ulceration, nonglandular				1				2
Testes, atrophy, tubular				1	--	--	--	--
--sperm stasis, rete testis		1			--	--	--	--
--spermatid retention			5	5	--	--	--	--
Thymus, hemorrhage, agonal**	3	1		5				1
Tongue, atrophy, epithelial			2	7			3	5
Uterus, epithelial thinning	--	--	--	--			5	5
Vagina, diestrus	--	--	--	--	2	2	1	1
--estrus	--	--	--	--	2	2	1	1
--metestrus	--	--	--	--	1	3	1	1
--proestrus	--	--	--	--	5	3	2	2
--epithelial thinning	--	--	--	--			5	6
--infiltration, lymphocytic	--	--	--	--			1	

**dose-related increase in severity score

Special Evaluation

All males were paired with an undosed female beginning on the evening of Day 65 or 71. Vaginal lavages were taken each morning until mating was documented by detection of a copulatory plug. The day of detection was designated as Gestation Day 0. The pairing period for each pair was a maximum of 7 nights. If evidence of mating was not observed, the pair was separated and the female was treated as if mating had occurred. For each female, the time taken to show positive signs of mating and the number of failed opportunities (estrus passed without signs of mating) was recorded.

As indicated in Table 40, treatment with AZD9291 was associated with a decrease in indices of male fertility, particularly decreased numbers of live fetal implants at sacrifice. This observation is explained by the observed increase in percent preimplantation loss.

Table 40: Summary of individual pregnancy data

	Male	Female	Corpora	# Impl.	Pre-impl.	Early embr.	Late embr.		% Post-	% impl.
	1001	1701	(b) (4)							
	1002	1702								
	1003	1703								
	1004	1704								
	1005	1705								
	1006	1706								
	1007	1707								
	1008	1708								
	1009	1709								
	1010	1710								
Group 1	**Mean**		**11.8**	**11.7**	**0.8**	**0.6**	**0.0**	**12.4**	**4.1**	**4.8**
	2001	2701	(b) (4)							
	2002	2702								
	2003	2703								
	2004	2704								
	2005	2705								
	2006	2706								
	2007	2707								
	2008	2708								
	2009	2709								
	2010	2710								
Group 2	**Mean**		**12.0**	**12.0**	**0.0**	**0.4**	**0.0**	**12.9**	**3.6**	**3.6**
	3001	3701	(b) (4)							
	3002	3702								
	3003	3703								
	3004	3704								
	3005	3705								
	3006	3706								
	3007	3707								
	3008	3708								
	3009	3709								
	3010	3710								
Group 3	**Mean**		**12.8**	**12.8**	**0.0**	**0.5**	**0.0**	**12.3**	**3.9**	**3.9**
	4001	4701	(b) (4)							
	4002	4702								
	4003	4703								
	4004	4704								
	4005	4705								
	4006	4706								
	4007	4707								
	4008	4708								
	4009	-4709								
	4010	4710								
Group 4	**Mean**		**9.1**	**7.9**	**16.2**	**0.3**	**0.0**	**8.4**	**3.9**	**19.7**

Toxicokinetics

Three animals/sex were assigned for toxicokinetic analysis. Plasma samples were collected during Week 4 (2 and 4 hours post-dose), Week 9 (2 and 4 hours post-dose, Group 4 males only) and Week 13 (1, 2, 4, 8 and 24 hours post-dose). Toxicokinetic analyses were for the profile collected during Week 13.

Incurred sample reanalysis (ISR) was performed to verify the reproducibility of all analytical methods. The ISR for AZD9291 and AZ1397550 met pre-specified acceptance criteria; however, the method for the AZ13575104 metabolite did not meet

acceptance criteria; therefore the results were reported but toxicokinetic analyses were not performed.

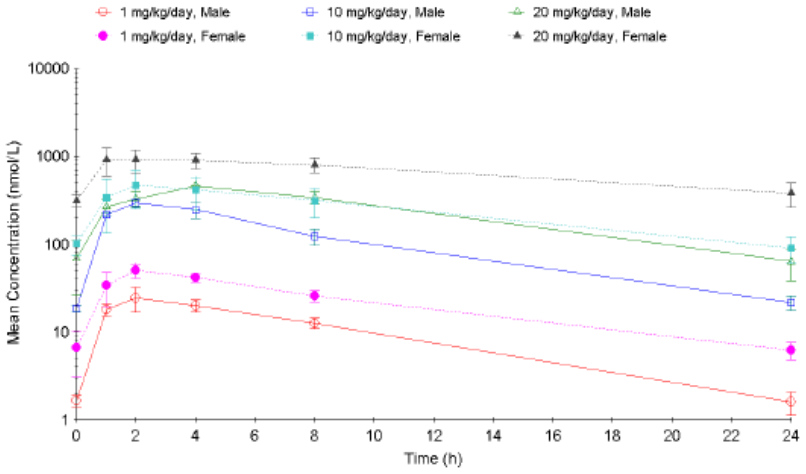
Neither AZD9291 nor its metabolites were detected in samples from control animals, and all samples collected from dosed animals contained evidence of exposure. On Day 91, AZD9291 exposures were generally proportional with dose.

Exposure (AUC) to AZD9291 was between 16-37-fold higher than that of AZ1397550; however, the half-life of the metabolite was longer than for the parent.

Table 41: Day 91 Summary of mean toxicokinetic parameters for AZD9291 and its metabolite, AZ1397550

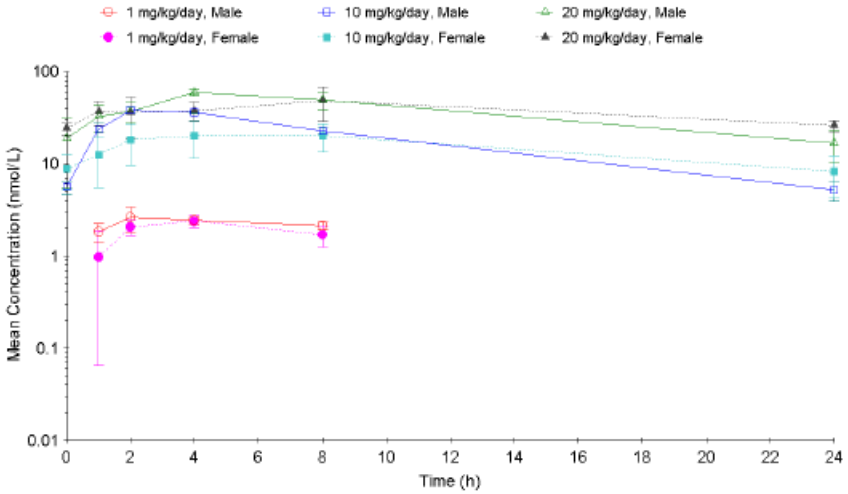
		AZD9291			AZ1397550		
Parameter	Sex	1	10	40/20	1	10	40/20
T_{max} (hours)	M	2	2	4	4	2	4
	F	2	2	2	4	4	8
C_{max} (nmol/L)	M	24.3	294	408	2.8	38	52.5
	F	50.5	472	1020	2.38	21.5	50.2
AUC_{τ} (nmol*hr/L)	M	254	2810	5880	17.3	460	870
	F	543	6190	16200	14.5	371	909
AUC_{0-t}	M	254	2810	5880	17.3	460	870
	F	543	6190	16200	14.5	371	909

Figure 50: Mean concentration-time profiles for AZD9291 in male and female rats following oral administration of AZD9291 on Day 91



(Excerpted from the Applicant's NDA)

Figure 51: Mean concentration-time profiles for AZ13597550 in male and female rats following oral administration of AZD9291 on Day 91



(Excerpted from the Applicant's NDA)

Dosing Solution Analysis

Formulation samples were analyzed during Weeks 1, 4 and 13. Results of these analyses met pre-specified acceptance criteria (±10% or ±15% for individual samples) for concentration and/or homogeneity.

6.2.2 AZD9291: Three Month Oral (Gavage) Toxicity Study in the Dog

Study no.:	526253
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	14 November 2013 (1st dose)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Drug: AZD9291 mesylate salt Lot: 02-kcwg806-87 Purity: 95.6% (HPLC % area

Key Study Findings

- All animals survived to scheduled termination.
- Dosing was limited at the high dose level (10 mg/kg) by adverse ocular findings (redness, discharge, squinting) resulting in intermittent dose holidays from Days 6-25 as described in Table 42.

Methods

Doses:	0, 1, 3, 10/6 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	Milli-Q water pH adjusted with methane sulfonic acid (final pH 3 to pH 3.5)
Species/Strain:	*Canis familiaris* (Beagle dog; animals were supplied to the CRO by the Applicant)
Number/Sex/Group:	4/sex/group
Age:	Males: 24 to 28 months Females: 25 to 28 months
Weight:	Males: 11.2 – 13.8 kg Females: 8.3 – 13.4 kg
Satellite groups:	None
Unique study design:	❖ Dose reduction in high dose animals on Day 23.
Deviation from study protocol:	In general, the reported deviations were considered unlikely to have affected the outcome of the study; however, there were numerous intermittent dose holidays granted for poor tolerability to high dose animals (due to ocular lesions and GI effects) during Days 9-25 of the study, after which animals were dose-reduced. Given the number of animals involved and the number of reported dose holidays, these events are likely to have underrepresented the toxicity of AZD9291 at the 10/6 mg/kg dose level when administered over a 3 month duration.

Observations and Results

Mortality

There were no preterm deaths.

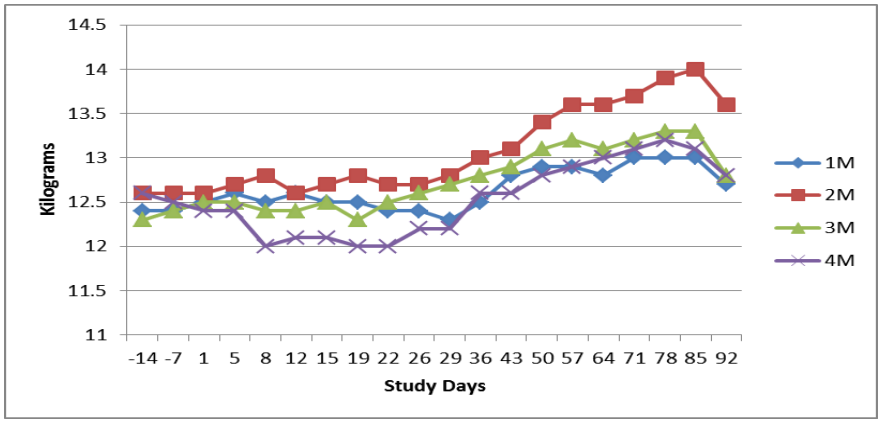
Clinical Signs

Treatment-related clinical signs in mid- and high-dose animals included: eye discharge; eye closed or partially closed; eye red; emesis; soft/yellow/mucoid feces; and skin lesions.

Body Weights

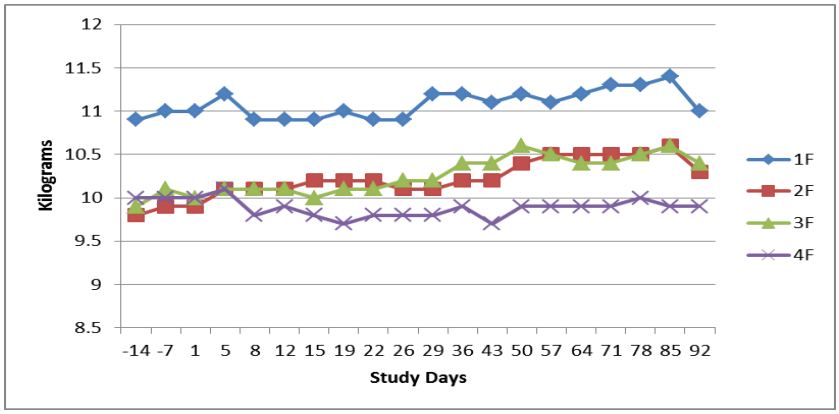
A decrease in mean body weight was observed in high dose males and females during the first week of the dosing period, which showed signs of recovery after the dose reduction on Day 23.

Figure 52: Mean male body weights



(Excerpted from Applicant's submission)

Figure 53: Mean female body weights



(Excerpted from Applicant's submission)

Feed Consumption

Sporadic decreases in mean feed consumption were noted in high dose females. These changes were intermittent and small in magnitude. By the end of the study, feed consumption was comparable to controls. There were no effects of treatment on feed consumption in males.

Ophthalmoscopy

There was no signed ophthalmology report; however, individual ophthalmoscopy findings were provided in tabular form. The ophthalmic findings consisted of corneal opacities.

A number of ocular clinical signs were recorded by the attending veterinarian during the course of the study. These included: red conjunctiva, ocular discharge, bulging eyes, moderate to severe blepharospasm, moderate to severe conjunctivitis, light intolerance, fluorescein-positive corneal lesions; and lachrymation.

The frequency and severity of these findings necessitated dose-holidays and dose-reductions, as indicated below.

Table 42: Dose holidays in high dose animals

Animal	Day of study off-dosing										
	9	10	11	12	13	15	21	22	23	24	25
4001	X	X	X	X	X						
4003		X	X	X	X			X	X	X	X
4004		X	X	X							
4501		X	X	X							
4503						X					
4504							X	X			

(Excerpted from the applicant's NDA)

ECG

Unremarkable

Hematology

Generally unremarkable. Increased in fibrinogen was noted in high-dose animals at the 4 and 13-week timepoints. Increased neutrophils were observed in high dose males during Week 13. Other changes were transient and/or lacked a relationship with dose and are therefore of questionable biological significance.

Clinical Chemistry

Decreases were observed in serum albumin particularly in high dose males and/or females. The decrease in serum calcium was likely attributable to the effect on serum albumin. Other changes, which lacked a relationship with dose and/or were transiently observed, are considered the result of normal variation and are unlikely to be treatment-related.

Table 43: Summary of mean clinical chemistry changes (% change vs. concurrent controls) in male and female dogs

Parameter	Week	Groups (Males)			Groups (Females)		
		2M	3M	4M	2F	3F	4F
TPROT (g/L)	-2	-6.8	-1.7	-1.7	1.7	-1.7	-3.4
	-1	-5.0	-1.7	-3.3	1.7	1.7	-1.7
	4	-6.8	-5.1	-6.8	-1.7	-5.2	-3.4
	13	-6.5	-3.2	-1.6	-1.7	-1.7	-1.7
ALB (g/L)	-2	-8.1	-2.7	-2.7	2.8	5.6	-2.8
	-1	-5.4	0.0	0.0	2.8	8.3	-2.8
	4	-8.3	-8.3	**-16.7**	0.0	-2.9	-14.3
	13	-5.1	-2.6	**-10.3**	-7.5	-5.0	**-10.0**

Parameter	Week	Groups (Males)			Groups (Females)		
		2M	3M	4M	2F	3F	4F
GLOB (g/L)	-2	-4.5	-4.5	4.5	4.8	-9.5	0.0
	-1	-8.7	-4.3	-4.3	0.0	-9.1	0.0
	4	0.0	0.0	8.7	-4.3	-8.7	13.0
	13	-8.3	-8.3	8.3	5.0	0.0	15.0
A/G	-2	6.2	6.2	0.0	0.0	17.6	0.0
	-1	6.2	6.2	0.0	0.0	11.8	-5.9
	4	-6.3	-6.3	-25.0	6.7	6.7	-20.0
	13	5.9	5.9	-17.6	-10.0	-5.0	**-20.0**
CA (mmol/L)	-2	-3.3	-2.2	-3.0	1.1	0.4	0.4
	-1	-3.3	-2.6	-3.7	-0.4	0.0	-1.5
	4	-3.1	-5.4	**-8.4**	-2.3	-3.8	-5.0
	13	-4.1	-5.2	-7.4	-4.1	-4.1	**-5.5**

Urinalysis

Unremarkable

Gross Pathology

The following gross observations were recorded

	Males				Females			
Organ, observation	1M	2M	3M	4M	1F	2F	3F	4F
Lung, focus, dark			1	1				
--focus, pale		2	1	3			2	1
--abnormal appearance								1
--abnormal consistency								1
--cyst, clear								1
Lymph node, axillary; discoloration, dark				1	1	1		
Lymph node, mandibular; discoloration, dark				1		1		1
Lymph node, mediastinal; discoloration, dark		2			1			
--discoloration, dark, red							1	
Lymph node, mesenteric; discoloration, dark	2	3	2	2	2	1	2	1
--discoloration, dark, red								1
Skin, scab				1				
--scab, forefoot								1
--scab, scrotum			1	1				
--thick, scrotum		1						
small intestine, duodenum; discoloration, dark, mucosa			1	1				
small intestine, ileum; discoloration, dark, mucosa			1	1				
small intestine, jejunum; discoloration, dark, mucosa		1	1					
spleen; abnormal appearance; green				1				
--focus, pale					1		1	
Stomach; abnormal content; red						1		
Tonsil; discoloration; dark	1			1				
Urinary bladder; discoloration; dark				1				
--discoloration; dark, mucosa			1				1	
--abnormal appearance; mucosa				1				

Organ Weights

Unremarkable

Histopathology

Adequate Battery

Yes

Peer Review

Yes

Histological Findings

	Male				Female			
Organ; Histopathological Description Dose (mg/kg)	0	1	3	10/6	0	1	3	10/6
Aorta; mineralization		1	1					
Brain; infiltration, mononuclear, perivascular							1	1
--infiltration, mononuclear; meninges	1	1						
Epididymis; decreased cellularity; sperm				2	--	--	--	--
--infiltration, lymphocytic			1					
Eyes; cornea; atrophy				4				3
Heart; mineralization, arterial						1		
--inflammation, mononuclear cell; arterial				1				
--fibrosis		1	1					
--infiltration mononuclear; myocardial						1		
Ileum; congestion, agonal			1	1				
Jejunum; congestion, agonal		1						
Kidney; infiltration, mixed cell; pelvic			1					
--pigmentation; cortical**	3	2	4	4		2	2	2
Lacrimal gland; inflammation, mixed cell	1							
Liver; pigmented macrophage	2		1		3	1		3
--infiltration, mixed cell		1				1		
--fibrosis, capsular							1	
LN, Mandibular; pigmentation		1		1		1		1
LN, Medistinal; erythrophagocytosis‡**		2			1		1	
LN, Mesenteric; erythrophagocytosis**	2	3	2	3	2	1	2	1
Lungs; mineralization, alveolar**	1	1			1			
--inflammation, chronic		2		3	2		2	1
--infiltration, mononuclear		1						1
--infiltration, mononuclear; peribronchiolar				1				
--pigmented macrophage	1		1					
--inflammation, mixed cell			1				1	
--macrophage aggregation								1
--infiltration, mixed cell						2		
Marrow, Sternum; decreased hematopoiesis			1					
Oviduct; cyst						1	1	
Pituitary; inflammation, lymphocytic		1						

	Male				Female			
Organ; Histopathological Description Dose (mg/kg)	0	1	3	10/6	0	1	3	10/6
Prostate; infiltration, mononuclear			1					
Salivary Gland, mandibular; infiltration, lymphocytic			1					
Salivary Gland, parotid; infiltration, lymphocytic**	3	2			2	1		
Skin; hyperplasia, epidermal								1
--ulceration**		1	1	1				
--inflammation, neutrophilic‡**		1	1	2				
--inflammation, mixed cell								1
Spleen; fibrosis				1				
Testes; atrophy, seminiferous tubule**				2				
--hypoplasia		1						
Thymus; involution				1				
Thyroid; infiltration, lymphocytic					1	1		
Trachea; inflammation, lymphocytic; lamina propria								1
Urinary bladder; congestion, agonal			1	1			1	
Uterus; cyst							1	

** = dose-related increase in severity

‡ = highest severity score (marked) noted in some or all animals

Toxicokinetics

No test article was detected in control animals. All dosed animals showed evidence of exposure to AZD9291 and its metabolites, AZ13575104 and AZ13597550. ISR successfully demonstrated that the method of quantitation for AZD9291 and AZ13597550 was reproducible, but ISR results for AZ13575104 did meet acceptance criteria and were, therefore, not reported.

Exposures to AZD9291 and AZ131597550 were generally proportional with dose on Day 91. There was no clear evidence of a gender-effect on exposure. Exposure to the metabolite was approximately 10% of the parent.

Table 44: 13-Week Dog TK

Analyte	AZD9291			AZ13597550		
Dose level (mg/kg/day)	1	3	10/6*	1	3	10/6*
Day	91	91	91	91	91	91
Males						
Median t_{max} (range) (h)	2 (2, 4)	2 (2, 4)	4 (2, 4)	3 (2, 4)	4 (4, 4)	4 (2, 4)
Mean C_{max} (SD) (nmol/L)	64.3 (35.4)	217 (79.6)	459 (145)	11.1 (5.28)	23.1 (18.5)	41.2 (10.5)
Mean $AUC_{(0-t)}$ (SD) (nmol.h/L)	704 (499)	2590 (607)	6150 (1910)	70.1 (35.8)	309 (289)	615 (180)
Females						
Median t_{max} (range) (h)	2 (2, 2)	2 (2, 4)	2 (2, 2)	3 (2, 4)	4 (2, 4)	4 (2, 4)
Mean C_{max} (SD) (nmol/L)	73.8 (32.1)	175 (63.6)	390 (48.8)	10.6 (7.50)	32.8 (12.9)	40.8 (12.1)
Mean $AUC_{(0-t)}$ (SD) (nmol.h/L)	818 (318)	1820 (746)	4690 (478)	97.8 (118)	399 (143)	542 (157)
Males and Females						
Median t_{max} (range) (h)	2 (2, 4)	2 (2, 4)	2 (2, 4)	3 (2, 4)	4 (2, 4)	4 (2, 4)
Mean C_{max} (SD) (nmol/L)	69.1 (31.7)	196 (70.4)	425 (107)	10.8 (6.02)	27.9 (15.6)	41.0 (10.4)
Mean $AUC_{(0-t)}$ (SD) (nmol.h/L)	761 (392)	2210 (754)	5420 (1510)	83.9 (82.0)	354 (217)	579 (161)

N = 4 for each group per sex; N = 8 for males and females combined.
*Due to the severity of findings in Group 4 animals, the dosage was reduced from 10 mg/kg/day to 6 mg/kg/day from Day 23 onwards.
Note: For AZD9291 Tlast was 24 h in all animals, except for M2003 and where tlast was 8 h.
For AZ13597550 tlast was 8 h in all animals at 1 mg/kg/day, except for F2504 where tlast was 24 h. Tlast was also 8 h for animal M3001 at 3 mg/kg/day. In all other animals tlast was 24 h.

(Excerpted from the Applicant's NDA)

Dosing Solution Analysis

Samples were analyzed at Weeks 1, 4, and 13, and all analyses met pre-specified acceptance criteria of ±10% for homogeneity and concentration.

7 Genetic Toxicology

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

Study title: AZD9291: Genetic Toxicity Evaluation Using the Bacterial Reverse Mutation Test in Salmonella typhimurium TA 1535, TA 100, TA 1537 and TA 98 and Escherichia coli WP2uvrA (pKM101)

Study no.: 793061
Study report location: 4.2.3.3.1
Conducting laboratory and location: (b) (4)
Date of study initiation: 08 April 2013 (dosing)
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Drug: AZD9291 (mesylate salt)

Lot: BNG-LSL-12089
Purity: 98.36%

Methods

Strains:	*S. typhimurium* strains : TA1535, TA1537, TA98, TA100; *E. coli* strain: WP2 strain uvrA/pKM101
Concentrations in definitive study:	With and without S9 metabolic activation: 0.3, 1.0, 3.3, 10, 33.3, 100, 333.3 µg/plate
Basis of concentration selection:	Significant cytotoxicity at 500 µg/plate
Negative control:	DMSO
Positive control:	TA100 : 1µg sodium azide (-S9) TA1535: 1 µg sodium azide (-S9) TA1537 : 80 µg 9-AA (-S9) TA98 : 1 µg 2-NF (-S9) WP2 uvrA/pKM101: 25 µg potassium (-S9)dichromate All strains (+S9): 2-20 µg/mL 2-AA
Formulation/Vehicle:	DMSO
Incubation & sampling time:	3 days

Study Validity

Yes, negative values (Table 45) fell within historical ranges. Positive control colony counts were at least 3-fold above vehicle control treatments for each strain and condition tested.

Table 45: Historical solvent control data

TA 1535		TA 1537		TA 98		TA 100		*E. coli* WP2*uvr*A (pKM101)	
-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
8-22	8-22	6-23	7-26	15-43	20-53	71-123	73-131	99-137	170-223

(Excerpted from the Applicant's NDA)

Results

Under the conditions of this assay, AZD9291 was non-mutagenic.

Table 46: (b) (4): Plate Incorporation Method - Without Metabolic Activation

						(b) (4)
WP2uvrA (pKM101)	**AZD9291 Mesylate**	0.3 µg	*123.7*	*18.5*	*1.0*	
	Salt	1 µg	*135.7*	*6.4*	*1.1*	
		3.3 µg	*129.0*	*12.1*	*1.1*	
		10 µg	*137.3*	*15.5*	*1.2*	
		33.3 µg	*122.0*	*3.6*	*1.0*	
		100 µg	*140.3*	*12.0*	*1.2*	
		333.3 µg	*94.7*	*15.5*	*0.8*	
	DMSO	-	*119.3*	*19.3*	-	
TA 1535	**NaN_3**	1 µg	*80.0*	*16.5*	*5.5*	
TA 1537	**9AA**	80 µg	*1387.7*	*254.1*	*101.5*	
TA 98	**2NF**	1 µg	*224.3*	*31.9*	*8.9*	
TA 100	**NaN_3**	1 µg	*339.3*	*13.4*	*3.6*	
WP2uvrA (pKM101)	**$K_2Cr_2O_7$**	25 µg	*780.0*	*26.9*	*6.5*	

Key to Positive Controls		Key to Plate Postfix Codes	
NaN_3	Sodium Azide	TL	Thin Lawn
9AA	9-Aminoacridine		
2NF	2-Nitrofluorene		
$K_2Cr_2O_7$	Potassium dichromate		

Strain	Test item	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**AZD9291 Mesylate**	0.3 µg	*16.3*	*5.5*	*1.1*	(b) (4)
	Salt	1 µg	*13.3*	*1.5*	*0.9*	
		3.3 µg	*15.3*	*8.0*	*1.0*	
		10 µg	*21.7*	*3.2*	*1.5*	
		33.3 µg	*12.7*	*8.0*	*0.9*	
		100 µg	*16.0*	*5.6*	*1.1*	
		333.3 µg	*14.0*	*3.5*	*1.0*	
	DMSO	-	*14.7*	*10.0*	-	
TA 1537	**AZD9291 Mesylate**	0.3 µg	*16.7*	*1.5*	*1.2*	
	Salt	1 µg	*18.7*	*2.3*	*1.4*	
		3.3 µg	*20.0*	*3.0*	*1.5*	
		10 µg	*8.3*	*2.1*	*0.6*	
		33.3 µg	*16.3*	*4.2*	*1.2*	
		100 µg	*16.7*	*1.2*	*1.2*	
		333.3 µg	*6.7*	*2.9*	*0.5*	
	DMSO	-	*13.7*	*1.2*	-	
TA 98	**AZD9291 Mesylate**	0.3 µg	*34.7*	*5.5*	*1.4*	
	Salt	1 µg	*28.7*	*3.1*	*1.1*	
		3.3 µg	*32.7*	*6.5*	*1.3*	
		10 µg	*24.7*	*2.3*	*1.0*	
		33.3 µg	*32.0*	*8.7*	*1.3*	
		100 µg	*31.7*	*4.0*	*1.3*	
		333.3 µg	*18.7*	*2.1*	*0.7*	
	DMSO	-	*25.3*	*5.8*	-	
TA 100	**AZD9291 Mesylate**	0.3 µg	*85.3*	*2.1*	*0.9*	
	Salt	1 µg	*95.7*	*3.8*	*1.0*	
		3.3 µg	*99.3*	*12.7*	*1.1*	
		10 µg	*110.7*	*10.8*	*1.2*	
		33.3 µg	*104.0*	*8.9*	*1.1*	
		100 µg	*111.3*	*9.0*	*1.2*	
		333.3 µg	*24.3*	*3.5*	*0.3*	
	DMSO	-	*94.0*	*1.7*	-	

(Excerpted from the Applicant's NDA)

Table 47: (b) (4): Plate Incorporation Method - With Metabolic Activation

Strain	Test item	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	AZD9291 Mesylate Salt	0.3 µg	18.0	7.8	1.1	(b) (4)
		1 µg	13.0	2.6	0.8	
		3.3 µg	14.7	8.1	0.9	
		10 µg	12.3	5.0	0.8	
		33.3 µg	15.7	9.0	1.0	
		100 µg	13.7	8.0	0.9	
		333.3 µg	13.3	6.4	0.8	
	DMSO	-	16.0	2.6	-	
TA 1537	AZD9291 Mesylate Salt	0.3 µg	18.7	1.5	1.2	
		1 µg	19.0	2.6	1.2	
		3.3 µg	17.0	4.6	1.1	
		10 µg	18.7	2.1	1.2	
		33.3 µg	20.3	4.9	1.3	
		100 µg	21.0	2.0	1.4	
		333.3 µg	14.3	2.5	0.9	
	DMSO	-	15.3	2.9	-	
TA 98	AZD9291 Mesylate Salt	0.3 µg	33.0	4.6	0.8	
		1 µg	38.0	9.5	1.0	
		3.3 µg	43.0	5.0	1.1	
		10 µg	35.3	2.3	0.9	
		33.3 µg	55.3	3.2	1.4	
		100 µg	47.3	8.0	1.2	
		333.3 µg	40.0	14.7	1.0	
	DMSO	-	39.0	6.2	-	
TA 100	AZD9291 Mesylate Salt	0.3 µg	117.0	14.1	1.1	
		1 µg	99.0	7.8	0.9	
		3.3 µg	97.0	1.7	0.9	
		10 µg	95.3	16.3	0.9	
		33.3 µg	104.0	11.1	0.9	
		100 µg	106.3	7.5	1.0	
		333.3 µg	91.7	17.6	0.8	
	DMSO	-	111.3	0.6	-	
WP2uvrA (pKM101)	AZD9291 Mesylate Salt	0.3 µg	249.3	20.3	1.2	
		1 µg	227.3	15.9	1.1	
		3.3 µg	226.3	14.7	1.1	
		10 µg	235.0	22.1	1.1	
		33.3 µg	232.7	19.5	1.1	
		100 µg	234.3	18.5	1.1	
		333.3 µg	199.3	10.7	0.9	
	DMSO	-	212.3	3.8	-	
TA 1535	2AAN	2 µg	187.0	19.1	11.7	
TA 1537	2AAN	2 µg	147.0	15.4	9.6	
TA 98	2AAN	2 µg	1056.0	71.9	27.1	
TA 100	2AAN	2 µg	2222.0	88.7	20.0	
WP2uvrA (pKM101)	2AAN	20 µg	2134.0	28.0	10.1	

Key to Positive Controls		Key to Plate Postfix Codes	
2AAN	2-Aminoanthracene	ST	Slightly Thin Lawn

(Excerpted from the Applicant's NDA)

7.2 *In Vitro* Assays in Mammalian Cells

Study title: AZD9291: Genetic Toxicity Evaluation using the Mouse Lymphoma Cell Thymidine Kinase Locus Assay

Study no.:	793056
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	15 January 2013 (experimental start)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Drug: AZD9291 (mesylate salt) Batch: BNG-LSL-12089 Purity: 98.6%

Methods

Cell line:	L5175Y $TK^{+/-}$
Concentrations in definitive study:	Minus S9: 1, 2, 3, 4, 5, 6, 7, 8 µM Plus S9: 2.5, 4, 5.5, 7, 8.5, 10, 11.5, 13 µM
Basis of concentration selection:	Cytotoxicity
Negative control:	Solvent (DMSO)
Positive control:	Minus S9: MMS Plus S9: 3-methylcholanthrene
Formulation/Vehicle:	DMSO
Incubation & sampling time:	3 hours±S9; 24 hours + S9; cloning efficiency assessed on Day 2

Study Validity

Yes, with the following exception, all positive and negative controls were within historical range provided:

- The mutant frequency for 3-MC-treated cultures was 2491 X 10^{-6} in the culture treated at a concentration of 10µM. While this value exceeded previous control range provided, the result was considered sufficient to demonstrate the sensitivity of the assay as it demonstrated a statistically and biologically significant increase in revertants compared with the concurrent control. There is no impact of this exception on the interpretation of the data.

While a few small increases in mutant frequency were observed at some concentrations in the 3-hour incubation performed in the presence of S9 activation, the increases were not considered biologically significant.

Table 48: Mouse lymphoma LY5178Y cell historical control data from August 2006-December 2011

Vehicle Control Data

Vehicle Control	S9	Exposure Time	n†	Mutant Fraction x 10^{-6}			No-effect Maximum (Induced Mutant Fraction)	Mean Colony Size Ratio (Small/Large)		
				Mean	SD	Range		Mean	SD	Range
All, pooled	-	4 h	59	81	24	49-155	49	1.40	0.41	0.73-2.64
All, pooled	-	24 h	50	89	32	47-194	60	1.36	0.50	0.60-2.94
All, pooled	+	4 h	109	93	27	47-188	79	1.42	0.38	0.64-2.75

† = Each value is the mean of 4 replicate cultures
The No-effect Maximum represents the maximum difference recorded between the 2 pairs of vehicle control cultures in any experiment. That is, the lower mean mutant fraction (x 10^{-6}) is subtracted from the higher. This difference, when applied to the response from a mutagen, is termed the induced mutant fraction (IMF).

Positive Control Data

Positive Control	S9	Concen-tration (µg/mL)	Exposure Time	n†	Mutant Fraction x 10^{-6}			RTG %		Mean Colony Size Ratio (Small/Large)		
					Mean	SD	Range	Mean	Range	Mean	SD	Range
EMS	-	250	4 h	59	722	261	433-1560	59	38-79	0.54	0.11	0.29-0.84
MMS	-	10	4 h	59	1063	320	543-2094	38	22-75	2.22	0.58	1.39-3.79
EMS#	-	150	24 h	48	2226	833	1197-4475	33	8-92	0.35	0.10	0.14-0.63
EMS#	-	100	24 h	2	2882	-	2637-3126	31	24-38	0.41	-	0.32-0.49
MMS	-	5	24 h	50	1938	460	1115-3204	33	17-91	1.65	0.44	0.86-2.94
3-MC	+	2.5	4 h	109	917	411	403-2277	60	14-96	1.27	0.35	0.67-3.61
3-MC	+	10	4 h	54	1086	458	515-2251	47	6-80	1.34	0.28	0.73-2.18

‡ = Each value is the mean of 2 replicate cultures
EMS = Ethyl methanesulphonate
MMS = Methyl methanesulphonate
3-MC = 3-Methylcholanthrene
After many years of use, 150 µg EMS/mL became too toxic in the 24 h test system, resulting in a recent reduction to 100 µg/mL.

(Excerpted from the Applicant's NDA)

Results

Under the conditions of this assay, AZD9291 was non-mutagenic.

Table 49: AZ19291: In Vitro Mouse Lymphoma TK Assay Mutation Test: 3 Hour Exposure in the absence of S9

Chemical	Concentration (µmol/L)	Relative Total Growth %	Mutant Fraction (x 10^{-6})	IMF (Induced Mutant Fraction x 10^{-6})	Ratio of Small to Large Colonies
DMSO	(100 µL added)	100	109	N/A	1.19
MMS	(10 µg/mL)	53	920	810	2.83
AZD9291	1	105	84	-	1.24
	2	107	105	-	1.11
	3	69	97	-	1.29
	4	33	99	-	1.35
	5	15	144	35	1.93
	6	NPT	NPT	-	-
	7	NPT	NPT	-	-
	8	NPT	NPT	-	-

IMF = Mutant frequency of treatment minus mutant frequency of vehicle control group
N/A = Not Applicable
NPT = Not Plated - Toxic
- = IMF ≤ 0

(Excerpted from the Applicant's NDA)

Table 50: AZD9291: In Vitro Mouse Lymphoma TK Assay Mutation Test: 3 Hours Exposure in the presence of S9

Chemical	Concentration (µmol/L)	Relative Total Growth %	Mutant Fraction (x 10^{-6})	IMF (Induced Mutant Fraction x 10^{-6})	Ratio of Small to Large Colonies
DMSO	(100 µL added)	100	110	N/A	1.29
3-MC	(2.5 µg/mL)	27	2122	2012	1.45
	(10 µg/mL)	10	2491	2380	1.71
AZD9291	2.5	NPS	NPS	-	-
	4.0	90	103	-	1.30
	5.5	57	142	32	0.87
	7.0	38	204	94	2.26
	8.5	24	271	161	1.04
	10.0	15	195	85	2.20
	11.5	NPT	NPT	-	-
	13.0	NPT	NPT	-	-

IMF = Mutant frequency of treatment minus mutant frequency of vehicle control group
N/A = Not Applicable
NPS = Not Plated - Surplus
NPT = Not Plated - Toxic
- = IMF ≤ 0

(Excerpted from the Applicant's NDA)

Table 51: AZ19291: In Vitro Mouse Lymphoma TK Assay Mutation Test: 24 Hour exposure in the absence of S9

Chemical	Concentration (µmol/L)	Relative Total Growth %	Mutant Fraction (x 10^{-6})	IMF (Induced Mutant Fraction x 10^{-6})	Ratio of Small to Large Colonies
DMSO	(200 µL added)	100	105	N/A	1.21
MMS	(5 µg/mL)	49	1931	1825	1.47
AZD9291	0.5	NPS	NPS	-	-
	1.0	91	112	7	0.74
	2.0	77	109	4	1.25
	3.0	54	123	18	1.26
	4.0	37	169	64	0.78
	5.0	17	194	89	2.16
	6.0	NPT	NPT	-	-
	7.0	NPT	NPT	-	-

IMF = Mutant frequency of treatment minus mutant frequency of vehicle control group
N/A = Not Applicable
NPT = Not Plated - Toxic
- = IMF ≤ 0

(Excerpted from the Applicant's NDA)

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

Study title: AZD9291: Genetic Toxicity Evaluation Using the Rat Micronucleus Test After Two Oral Doses

Study no:	793538
Study report location:	4.2.3.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	07 May 2013 (1st dose)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Drug: AZD9291 (mesylate salt) Batch: BNG-LSL-12089 Purity: 98.6%

Key Study Findings

Under the conditions of this assay, AZD9291 was considered negative for induction of micronucleated immature erythrocytes in the Han Wistar rat.

Methods

Doses in definitive study:	0, 30, 150, 300 mg/kg
Frequency of dosing:	Daily (2 days)
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% HPMC (3500-5600 cps)
Species/Strain:	*Rattus norvegicus* / Han Wistar (CRL:WI(Han))
Number/Sex/Group:	7M/Group
Satellite groups:	None
Basis of dose selection:	MTD
Negative control:	Vehicle
Positive control:	Cyclophosphamide (20 mg/kg)

Study Validity

In Test 1, the formulation analysis results indicated that the test solutions were sub-potent. Because the results failed to meet pre-specified acceptance criteria of ±10%; the formulations were considered out of speciation, and the data from Test 1 were not analyzed. The formulations used in Test 2 were considered acceptable.

Because the positive control demonstrated the sensitivity of the system using a known inducer of micronucleus formation, and the negative control data fell within historical ranges, the study met the criteria for a valid test.

Table 52: Summary of control group mean and range of micronucleus counts in 2000 IE in male Han Wistar rats

n[1]=	Animals per group	Number of MIE/2000 IE counted	Mean historical control range	Range of micronuclei[2]
6	7	2.8	2.43 to 3.14	0 to 7

[1] n Number of animal groups (7 animals per group).
[2] for an individual animal.

Natural process limits will be calculated when at least 10 studies have been completed

(Excerpted from the Applicant's NDA)

Results

- There were no preterm deaths and no adverse clinical findings noted.
- As illustrated in Table 53, there were no meaningful increases in the number of micronucleated immature erythrocytes in any treatment group, and no effects were observed on the mean percentage of immature erythrocytes.
- A clear increase in the incidence of MIE was observed in animals treated with cyclophosphamide.
- Under the conditions of this assay, AZD9291 was negative for induction of MIE in the Han Wistar rat.

Table 53: Mean incidence of micronucleated immature erythrocytes (MIEs) in rat bone marrow and percentages of immature erythrocytes (IE)

Group	Treatment	Dose level (mg/kg/day)	Mean number of MIE in 2000 immature erythrocytes	Mean percentage of IE
1	Vehicle	0	2.0	59.8
2	AZD9291	30	2.4	58.6
3	AZD9291	150	2.4	55.4
4	AZD9291	300	1.7	58.4
5[a]	Cyclophosphamide	20	55.7***	52.1

*** Fisher's Exact Test p<0.001.
[a] Group 5 (positive control) animals from Test 1

(Excerpted from the Applicant's NDA)

8 Carcinogenicity

Carcinogencity studies were not conducted and are not required to support a drug intended for the treatment of patients with advanced cancer.

9 Reproductive and Developmental Toxicology

9.2 Embryonic Fetal Development

Study title: AZD9291: Oral (Gavage) Investigative Dose Range Finding Embryofetal Development and Pre and Post Natal Study in the Rat

Study no.:	496800
Study report location:	4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	28 March 2014 (1st dose)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Drug: AZD9291 mesylate salt Lot: 02-kcwg806-87 Purity: 95.6% (HPLC % area)

Key Study Findings

- **Embryofetal and neonatal death**:
  - Administration of AZD9291 from GD 2-20 led to increased post-implantation loss and early embryonic death.
  - When administered during organogenesis through Day 6 of lactation (GD6-LD6), AZD9291 exposure led to an increase in postnatal death and an increase in total litter loss, as well as a reduction in mean pup weight.

- **Effects on fetal weight**:
  - When administered during organogenesis (GD 6-16), AZD9291 exposure led to reduced fetal weight at maternal doses of ≥20 mg/kg/Day.

- **Malformations**:
  - Visceral abnormalities and malformations were observed in some pups of treated dams; however, the incidences were of low frequency and did not exhibit a clear relationship to dose. Due to the small number of litters used and the absence of historical control data, the relationship of these findings to maternal AZD9291 exposure cannot be excluded.

- **Placental and lactational transfer**:
  - Toxicokinetic exposures were evaluated in dams and fetuses and/or nursing pups. Fetal exposure at the end of gestation (GD20) was approximately 36% of those observed in dams on GD16. Fetal exposure to the AZ13597550 metabolite was also demonstrated; however, fetal metabolite levels were relatively low - less than 7% of maternal levels.
  - A low level of exposure to AZD9291 and its metabolite was demonstrated in nursing pups. At the maternal Cmax (2 hours post-dose), neonatal exposure to AZD9291 was approximately 2% of the maternal exposure

levels. Peak neonatal exposure to the AZ13597550 metabolite, however, was approximately 12% of maternal levels at 2 hours post-maternal-dose.

Methods

Doses:	See below (Study Design)
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	MilliQ water pH adjusted with methane sulfonic acid (final pH 3.0 to 3.5).
Species/Strain:	Species: *Rattus norvegicus* Strain: Han Wistar (Crl: WI(Han) rat)
Number/Sex/Group:	6 pregnant dams/Group
Satellite groups:	None
Study design:	❖ Phase 1: 0, 20 mg/kg/Day from Gestation Days 2-20 ❖ Phase 2: 0, 1, 10, 20, or 30 mg/kg/Day from GD 6 to GD 16 (necropsy on Day 21) ❖ Phase 3 (Batch 1): 0, 1 or 30 mg/kg/Day from GD 6 to PND 6 (lactation); necropsy on PNDs 7-9 ❖ Phase 3: (Batch 2): 0, 1 or 20 mg/kg/Day from GD6 to PND6 (lactation; necropsy on PNDs 7-9)
Deviation from study protocol:	Reported deviations appear unlikely to have affected overall study interpretation.

Observations and Results

Mortality

Two females (one Phase 3, Batch1 female (30 mg/kg, GD 23), and one Phase 3, Batch 2 female (20 mg/kg, PND 4) were humanely euthanized due to adverse clinical signs, which differed between the two animals.

Group/Sex (dose level)	Animal reference number	Terminal day	Important findings
10F (30 mg/kg/day)	57	Day 23 gestation	Adverse clinical observations: subdued, thin, hunched body, laboured respiration, skin cold to touch, pale discoloured skin, encrusted lids in both eyes and eyes partially closed. Necropsy: small and large intestines contained yellow watery fluid. Microscopically: mild epithelial atrophy in the cornea and in the tongue.
13F (20 mg/kg/day)	74	Day 4 lactation	Adverse clinical observations: fast and irregular respiration, subdued behaviour and staining on fur around the muzzle, weight loss and reduced food consumption. Necropsy: pale discolouration of the spleen, liver and lung, a small thymus, enlargement of all the intestines by thickening of the intestinal wall, with abnormal dark contents in the caecum, colon and rectum. Microscopically: epithelial atrophy in the cornea and in the tongue.

(Excerpted from the Applicant's NDA)

Clinical Signs

Treatment-related clinical signs in animals that survived to scheduled termination included ploughing, piloerection, irregular/labored respiration, excess salivation, soft feces, hunching, and subdued behavior.

Body Weight

Figure 54: Mean maternal body weights (Phase 1)

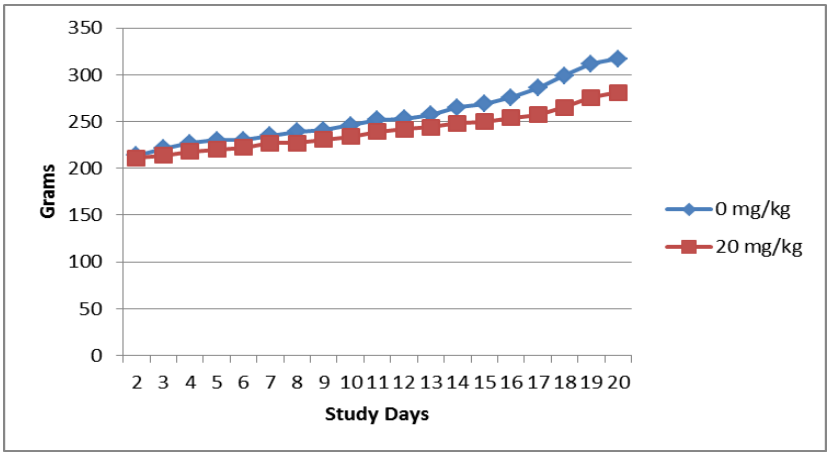


Figure 55: Mean Maternal Body Weights (Phase 2)

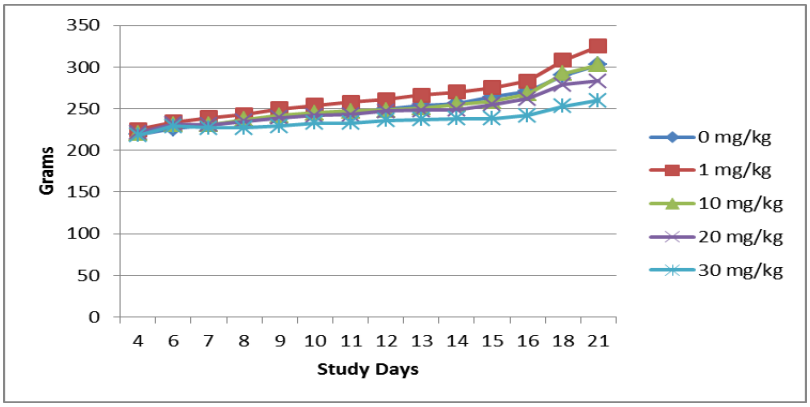


Figure 56: Mean Maternal Body Weights (Phase 3 Batch 1)

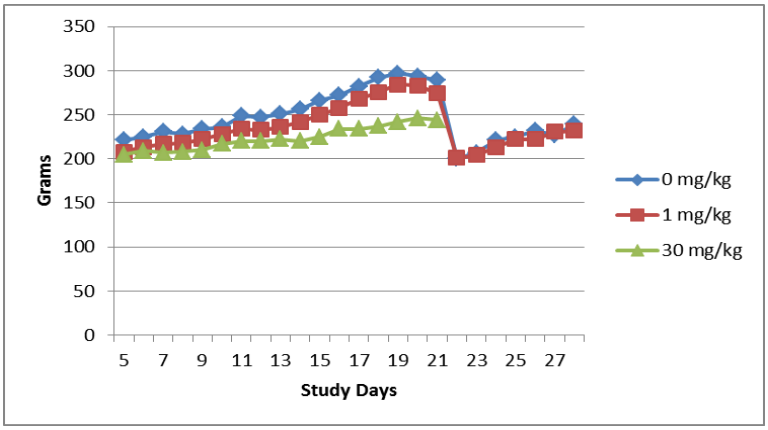
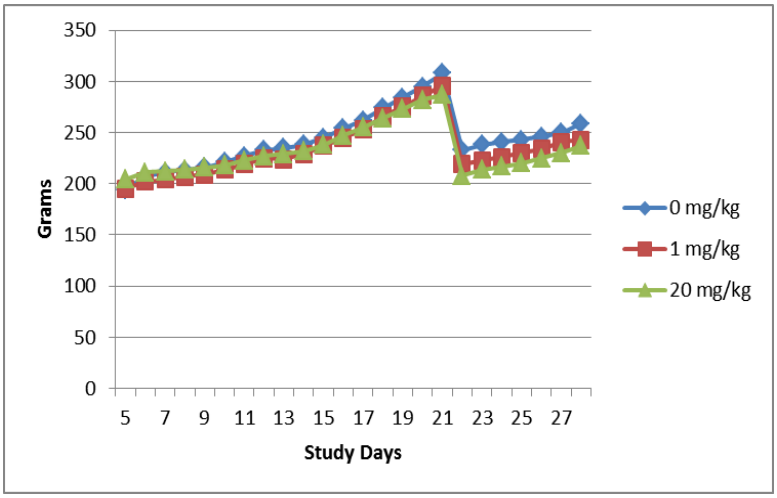


Figure 57: Mean Maternal Body Weights (Phase 3 Batch 2)



Feed Consumption

An apparently dose-related decrease in feed consumption was observed during Phase 2, in animals treated at doses of between 10-30 mg/kg. Statistical analyses were not performed; however, based on the corresponding decreases in mean body weight in high-dose dams (20 mg/kg in Phase 1 and 30 mg/kg in Phase 2), the decreased feed consumption appears to have been biologically significant.

Toxicokinetics

Terminal TK collection was performed in dams (Phase 2 and Phase 3), pups (Phase 3) and in fetuses (Phase 1), and samples were analyzed for the presence of AZD9291 and/or its metabolite, AZ13597550. Quantitation of the metabolite, AZ13575104, was also performed; however, because the ISR for this metabolite failed, the assay was deemed unreliable. Plasma concentrations were reported (data not shown) but TK assessments were not conducted.

Exposures to AZD9291 and AZ13597550 (peak and overall) in dams were approximately proportional with dose over the 30-fold dose range on GD 16 (Table 54 and Table 55). Overall exposure to the metabolite was approximately 5% of the parent.

At the end of gestation (GD20), fetal concentrations of AZD9291 were approximately 36% of maternal levels measured on GD16, and circulating concentrations of AZ19579550 were about 7% of maternal concentrations measured on GD16 (Table 56).

AZD9291 appears to be excreted in the milk of rats, as concentrations in pups whose mothers received an oral dose of 20 mg/kg on lactation Day 6 were approximately 2% of maternal levels at 2 hours post-dose. At the same maternal dose level, plasma concentrations of the AZ13597550 metabolite in pups were 12% and 31% of maternal levels at 2 and 24 hours post-dose, respectively.

Table 54: Summary of mean AZD9291 TK parameters in dams (Phase 2)

Dose level (mg/kg)	**1**	**10**	**20**	**30**
Day	**16**	**16**	**16**	**16**
Females				
Median t_{max} (range) (h)	4 (2, 8)	2 (2, 4)	4 (2, 8)	6 (2, 8)
Mean C_{max} (SD) (nmol/L)	86.7 (32.9)	759 (282)	990 (84.8)	1430 (400)
Mean $AUC_{(0\text{-}t)}$ (SD) (nmol.h/L)	1130 (352)	10200 (4570)	16600 (1470)	21200 (4580)

N is 6 females per dose.

(Excerpted from the Applicant's NDA)

Table 55: Summary of mean metabolite (AZ13597550) TK parameters in dams (Phase 2)

Dose level (mg/kg)	1	10	20	30
Day	16	16	16	16
Females				
Median t_{max} (range) (h)	8 (1, 8)	4 (4, 4)	8 (4, 8)	6 (2, 8)
Mean C_{max} (SD) (nmol/L)	2.61 (1.10)	25.1 (7.23)	43.7 (8.17)	58.9 (13.4)
Mean $AUC_{(0-t)}$ (SD) (nmol.h/L)	31.1 (25.1)	380 (115)	756 (119)	1020 (219)

N is 6 females per dose.

(Excerpted from the Applicant's NDA)

Table 56: Summary of mean plasma concentrations of AZD9291 and metabolite AZ13597550 in dams (Phase 2) and fetuses (Phase 1)

Analyte	Phase	Animal	Group	Dose (mg/kg/day)	Day (of gestation)	Time (h) 2*	24
AZD9291	1	Fetuses	2	20	20	280 (55.6)	-
AZD9291	2	Dams	6	20	16	774 (94.5)	266 (61.6)
AZ13597550	1	Fetuses	2	20	20	1.98 (0.506)	-
AZ13597550	2	Dams	6	20	16	28.7 (6.88)	17.4 (1.22)

*Sample taken from fetuses at termination, approximately 2 h post dose of the dam.
NC Not calculated.

(Excerpted from the Applicant's NDA)

Table 57: Summary of mean AZD9291 and metabolite AZ13597550 plasma concentrations in rat pups and dams (Phase 3)

Analyte	Group	Animal	Dose (mg/kg/day)	Day (of lactation)	Time (h) 2	24*
AZD9291	9	Dams	1	6	40.3 (18.8)	2.62 (0.945)
AZD9291	9	Pups	1	6	BLQ	BLQ
AZD9291	13	Dams	20	6	1040 (389)	221 (153)
AZD9291	13	Pups	20	6	18.3 (24.3)	18.4 (6.50)
AZ13597550	9	Dams	1	6	1.35 (0.384)	BLQ
AZ13597550	9	Pups	1	6	BLQ	BLQ
AZ13597550	13	Dams	20	6	30.1 (7.43)	15.0 (7.32)
AZ13597550	13	Pups	20	6	3.56 (3.54)	4.58 (1.31)

*Sample taken from pups at termination, approximately 24 h post dose of the dam.
BLQ Below lower limit of quantification of 1.00 nmol/L.

(Excerpted from the Applicant's NDA)

Dosing Solution Analysis

Met pre-specified acceptance criteria of ±10% of nominal.

Necropsy

Epithelial atrophy was noted in the corneas and tongues of dams exposed during Phases 1 and 3 at doses of 20 and 30 mg/g/day. Endothelial vacuolation and corneal edema was noted in high dose dams.

Cesarean Section Data

Table 58: Summary of Pregnancy Data (Phase 1)

	Dose (mg/kg/day)	
Parameter Evaluated	**0**	**20**
Number of females mated	6	6
Number pregnant on GD 20 (%)	6 (100)	6 (100)
Number of preterm decedents	0	0
Total corpora lutea graviditatis	81	76
Total number of implants	73	73
Mean pre-implantation loss (%)	9.5	3.7
Total live implants (%)	61	44
Total dead implants (%)	12	29
Total early embryonic deaths (%)	12	29
Total late embryonic deaths (%)	0	0
Total dead fetuses (%)	0	0
Mean post-implantation loss (%)	15	40
Total mean uterine weight (g)	59±13	41±9

Table 59: Summary of Pregnancy Data (Phase 2)

	Dose (mg/kg/day)				
Parameter Evaluated	**0**	**1**	**10**	**20**	**30**
Number of females mated	6	6	6	6	6
Number pregnant	6	6	6	6	6
Number of preterm decedents	0	0	0	0	0
Number pregnant on GD 21	6	6	6	6	6
Pregnancy frequency (%)	100	100	100	100	100
Corpora lutea graviditatis	71	77	72	75	76
Total number of implants	66	73	68	73	75
Mean pre-implantation loss (%)	7.6	5.0	5.8	3.2	1.5
Live implants (%)	62	70	68	72	72
Dead implants (%)	4	3	0	1	3

	Dose (mg/kg/day)				
Parameter Evaluated	**0**	**1**	**10**	**20**	**30**
Early embryonic deaths (%)	4	3	0	1	3
Late embryonic deaths (%)	0	0	0	0	0
Dead fetuses (%)	0	0	0	0	0
Mean implantation loss (%)	5.6	4.0	0	1.2	3.0
Mean total uterus weight (g)	72±10	82±10	75±10	73±12	66±6
Live male fetuses (%)	29	33	35	36	30
Live female fetuses (%)	33	37	33	36	42
Live fetal sex ratio (male:female)	1:1.14	1:1.12	1:0.94	1:1.0	1:1.40
Mean uterus weight (g)	72	82	75	73	66
Mean fetal weight (g)	4.90±.039	4.98±0.35	4.86±0.34	4.4±030	4.07±0.26
Mean litter weight (g)	50±6.82	58.0±7.89	54.7±7.36	52.6±9.22	48.6±4.24

Table 60: Survival Indices – Phase 3 Batch 1

	Dose (mg/kg/day)		
Parameter Evaluated	**0**	**1**	**30**
Birth Index			
Mean litter index (%)	96	91	71
Dams losing > 2 pups	0	1	3
Number of litters	6	6	5
Live Birth Index			
Mean litter index (%)	95	92	48
Dams losing > 1 pup	1	2	3
Number of Litters	6	6	5
Viability Index			
Mean Litter Index	45	67	0
Number losing > 3 pups	3	2	3
Number of litters	6	6	3

Table 61: Survival Indices – Phase 3 Batch 2

	Dose (mg/kg/day)		
Parameter Evaluated	**0**	**1**	**20**
Birth Index			
Mean litter index (%)	98	95	99
Dams losing > 2 pups	0	0	0
Number of litters	6	6	6
Live Birth Index			
Mean litter index (%)	99	87	100

	Dose (mg/kg/day)		
Parameter Evaluated	**0**	**1**	**20**
Dams losing > 1 pup	0	1	0
Number of Litters	6	6	6
Viability Index			
Mean Litter Index	99	83	65
Number losing > 3 pups	0	0	2
Number of litters	6	6	6

Table 62: Litter performance – Phase 3, batch 1

	Dose (mg/kg/day)		
Parameter Evaluated	0	1	30
Number of females mated	6	6	6
Number pregnant	6	6	6
Number of preterm decedents	0	0	1
Number of females producing a live litter	6	6	3
Gestation Index (%)	100	100	50
Mean number of implantation sites per pregnancy	11.3±1.5	11.3±1.0	--
Mean number of corpora lutea per pregnancy	12.3±1.2	14.5±2.4	--
Mean pre-implantation loss (%)	8.2	21.0	--
Mean total number of pups born per litter	10.7±2.1	10.5±1.3	--
Mean number of live pups per litter on LD0	9.7±2.5	10.5±1.3	--
LD1	9.3±3.1	10.5±1.3	--
LD4	9.0±3.6	10.5±1.3	--
LD6	9.0±3.6	10.5±1.3	--
Total number of males on LD1	17(61)	22(52)	--
Total number of females on LD1	11(39)	20(48)	--
Total litter loss (by Day 3-4 of lactation)	3/6	2/6	5/5

Table 63: Litter performance – Phase 3, batch 2

	Dose (mg/kg/day)		
Parameter Evaluated	**0**	**1**	**20**
Number of females mated	6	6	6
Number pregnant	6	6	6
Number of preterm decedents	0	0	0
Number of females producing a live litter	6	6	6
Gestation Index (%)	100	100	100
Mean number of implantation sites per pregnancy	10.7±1.5	10.6±1.5	12.3±0.5
Mean number of corpora lutea per pregnancy	12.3±1.4	13.2±0.8	13.8±1.7
Mean pre-implantation loss (%)	13.5	19.9	10.2
Mean total number of pups born per litter	10.5±1.6	10.4±1.7	12.3±0.5

	Dose (mg/kg/day)		
Parameter Evaluated	**0**	**1**	**20**
Mean number of live pups per litter on LD0	10.3±1.5	10.4±1.7	12.3±0.5
LD1	10.2±1.3	10.4±1.7	12.3±0.5
LD4	10.2±1.3	10.4±1.7	12.0±0.8
LD6	10.2±1.3	10.4±1.7	12.0±0.8
Total number of males on LD1	39(49)	30(58)	24(49)
Total number of females on LD1	31(51)	22(42)	25(51)

Table 64: Litter and pup weight - Phase 3(Batch 1)

	Dose (mg/kg/day)	
Parameter Evaluated	**0**	**1**
Litter		
LD1	47±18	54±8
LD4	66±29	73±6
LD6	88±34	94±9
Males		
LD1	4.5±0.4	5.3±0.3
LD4	7.4±0.3	7.1±1.1
LD6	9.9±0.4	9.1±1.6
Females		
LD1	4.7±0.6	5.1±0.3
LD4	7.3±1.4	7.0±1.1
LD6	9.9±0.1	9.0±1.7

Table 65: Table 40: Litter and pup weight - Phase 3(Batch 2)

	Dose (mg/kg/day)		
Parameter Evaluated	**0**	**1**	**20**
Litter			
LD1	69±10	66±9	67±4
LD4	107±16	101±14	106±17
LD6	160±24	155±20	106±15
Males			
LD1	6.9±0.3	6.6±0.3	5.5±0.3
LD4	10.6±0.6	9.9±0.5	7.2±0.2
LD6	15.8±0.6	15.2±0.9	8.9±0.9
Females			
LD1	6.7±0.2	6.1±0.2	5.3±0.2
LD4	10.2±0.3	9.4±0.2	6.3±1.2
LD6	15.6±0.5	14.6±0.9	8.8±1.0

Offspring Malformations

Table 66: Summary of fetal malformations - Phase 2*

Malformation	0	1	10	20	30
❖ Anencephaly, absent lower jaw. ❖ Bilateral eyelids absent; bilateral pinna malpositioned ❖ Mouth opening absent ❖ Small tongue present ❖ Single median nostril. ❖ Small fetus		1(1)			
❖ Lower jaw shortened, mouth present as small opening ❖ High arched palate, tongue markedly reduced in size				1(1)	
❖ Thoracic situs inversus ❖ Left and right single lung lobe present ❖ Accessory lung lobe absent		1(1)			
Number with major abnormality		2(1)		1(1)	
Total number examined	62(6)	70(6)	68(6)	72(6)	72(6)

Offspring Variations

Table 67: Summary of minor fetal abnormalities and variations: Phase 2

	Dose (mg/kg/day)				
Abnormality/Variation	**0**	**1**	**10**	**20**	**30**
❖ Cervical remnant of thymus	2(2)	2(2)	1(1)	0	0
❖ Additional liver lobe with median cleft	2(1)	4(2)	4(3)	1(1)	4(4)
❖ Right median liver lobe bifurcated	0	0	1(1)	0	0
❖ Umbilical artery left sided	7(3)	15(5)	14(5)	7(7)	8(5)
Number with minor abnormality/variation	8(3)	18(5)	20(6)	8(5)	11(6)
Total number examined	62(6)	70(6)	68(6)	72()46	72(6)

*Incidences reported as #affected fetuses (#litters)

Table 68: Summary of minor pup abnormalities and variations - Phase 3 batch 1

	mg/kg/Day	
Abnormality/Variant	**0**	**1**
❖ Cervical remnant of thymus	0	3(3)
❖ Additional liver lobe with median cleft	0	1(1)
❖ Umbilical artery left sided	0	4(2)
Number with minor abnormality/variant	0	8(4)
Total number examined	27(3)	42(4)

*Incidences reported as #affected fetuses (#litters)

Table 69: Summary of minor pup abnormalities and variations - Phase 3 batch 2

	Dose (mg/kg/day)		
Abnormality/Variant	**0**	**1**	**20**
❖ Cervical remnant of thymus	0	1(1)	1(1)
❖ Additional liver lobe with median cleft	1(1)	7(4)	1(1)
❖ Umbilical artery left sided	2(2)	3(1)	4(3)
Number with minor abnormality/variant	3(3)	8(4)	6(3)
Total number examined	61(6)	52(5)	48(4)

10 Special Toxicology Studies

10.1 AZD9291: Evaluation of in vitro phototoxicity on Balb/c 3T3 fibroblasts using the Neutral Red Uptake assay (Study Report #8273754)

Phototoxicity was evaluated by neutral red uptake in Balb/c 3T3 cells. Although the compound was toxic to treated cells, there was no meaningful difference in toxicity to Balb/c 3T3 cells following incubation with AZD9291 at a test concentration of 150.5 µg/mL when the cells were exposed to either UV-A-irradiated or un-irradiated; therefore, under the conditions of this assay, the drug was considered negative for phototoxicity. The positive control (chlorpromazine) met the acceptance criteria for photoirritation and demonstrated assay validity.

10.2 Ultrastructural Evaluation of Lungs Taken from the AZD9291 3-Month Rat Study (Report # EM-14.148)

An electron microscopy study was performed to assess the ultrastructural features of macrophage infiltrates observed in the lungs of animals in the 13-week rat study. The Applicant states that least 5 micrographs from each animal were evaluated at magnifications from 10,400X to 35,500X. The samples evaluated, are summarized in Table 70.

Table 70: Design of the rat lung ultrastructure study

Group No.	AZD9291 Dose (mg/kg/day)	Number of animals per group in the 3 month rat study	No. of Animals examined by EM		Animal identification numbers for animals examined by EM	
			Males	Females	Males	Females
1	0	10M +10F	2	2	1005, 1007	1502, 1507
4	40/20[a]	10M +10F	3	5	4002, 4003, 4009	4501, 4502, 4505, 4507, 4508

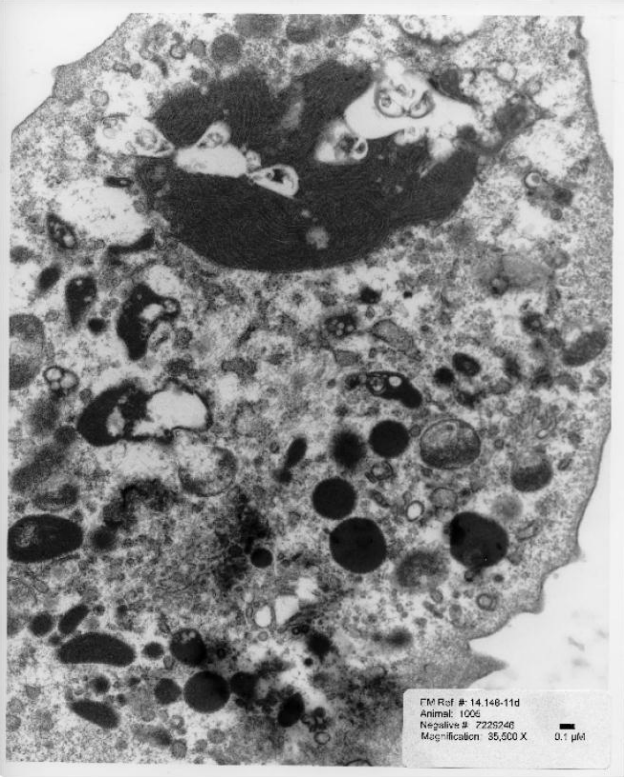
[a] Males received 40 mg/kg/day from Day 1 to 55; due to the severity of clinical signs these animals were taken off-dose from Day 56 and recommenced dosing at 20 mg/kg/day from Day 62. Females received 20 mg/kg/day.

(Excerpted from the Applicant's NDA)

The findings were characterized by light microscopy as aggregates of foamy alveolar microphages that had increased amounts of cytoplasm. Ultrastructurally, this was characterized as an accumulation of secondary lysosomes in various stages of digestion, and was considered consistent with early phospholipidosis in females and with lipofuscinosis or multivesicular bodies in males.

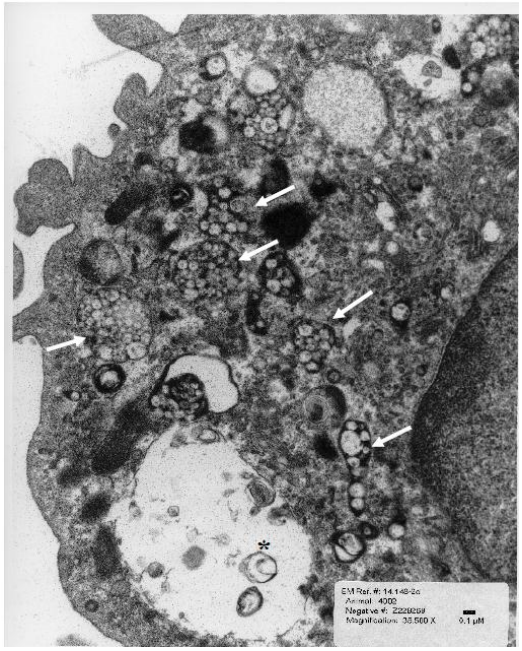
While the findings were present in treated and control animals (Figure 58); the finding was more prominent in treated animals (Figure 59 and Figure 60).

Figure 58: High magnification of alveolar macrophage in a control male that contained multiple secondary lysosomes



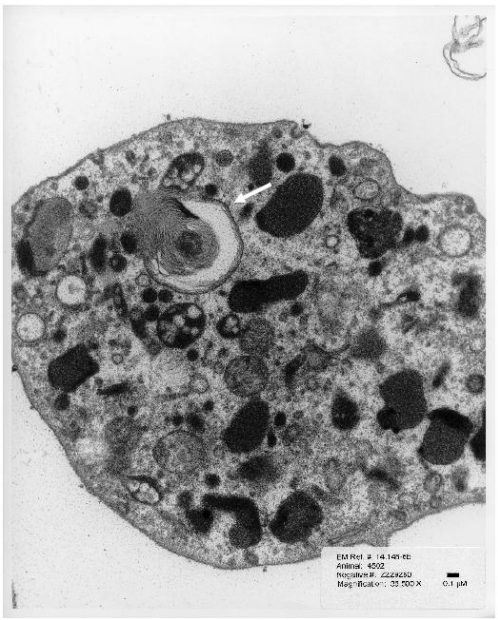
(Excerpted from the Applicant's NDA)

Figure 59: High magnification of alveolar macrophages with lysosomes containing clusters of electron-lucent circular structures in a high dose male



(Excerpted from the Applicant's NDA)

Figure 60: High magnification of alveolar macrophages with lysosomes containing whorls of membranes and electron-lucent circular structures in a high dose female



(Excerpted from the Applicant's NDA)

10.3 AZ13575104 and [redacted]: One Month Investigatory Toxicity Study in the Rat (Report # 3500KR)

The purpose of this non-GLP study was to assess the toxicity of the active metabolite, AZ13575104 when administered orally for 28 days in the rat. While the compound [redacted] was also investigated in this study, the report states that a decision to not progress further development with the compound was made, and therefore only data on the 104 metabolite will be reviewed.

The study was conducted in two parts: an initial dose-ranging phase and a repeat-dose phase. Only the repeat-dose phase will be reviewed.
Each [redacted] was tested at three dose levels, using 8 female animals per group. High dose recovery cohorts (N = 3 females/compound; 28 days) were also included for both test agents. A summary of the repeat-dose phase is provided in Table 71.

Table 71: Study Design of the repeat-dose toxicology study of AZ13575104 and [redacted] in the rat

Group	Compound / Vehicle	Animal reference numbers	Dosing days	Dose levels mg/kg/day	Dose volume mL/kg/day	Concentration of dosing formulation mg/mL
Main test Groups						
1	Control	1-8	1-28	0	10	0
2	AZ13575104	9-16	1-28	5	10	0.5
3	AZ13575104	17-24	1-28	10	10	1
4	AZ13575104	25-32	1-28	15	10	1.5
[redacted]						
Recovery Groups						
8	Control	69-72	1-28	0	10	0
9	AZ13575104	73-76	1-28	15	10	1.5
[redacted]						

(Excerpted from the Applicant's NDA)

There were two preterm deaths in Group 4 (15 mg/kg AZ13575104). These animals were terminated on Days 8 and 16 for adverse clinical signs, including altered feces, thin appearance, piloerection, anogenital staining, and palpebral closure.

In animals that survived to scheduled termination, treatment-related clinical signs included skin lesions, piloerection, staining around the eye, and increased salivation.

While there was no effect of AZ13575104 on group mean body weights in the repeat-dose phase of the study, two AZ13575104-treated females in the 15 mg/kg dose group were terminated prematurely due to inappetence and body weight loss.

No clear pattern of changes suggestive of target organ toxicity following AZ5104 administration was observed in clinical pathology parameters.

A summary of histopathological findings following AZ5104 administration is presented in Table 72. There were no notable findings in recovery –cohort animals.

Table 72: Summary of histopathological findings in the 4-week rat metabolite qualification study

	Female (mg/kg/Day)			
Organ; Histopathological Description	0	5	10	15
Adrenal; cortical cell degranulation				2
--inflammatory infiltrates				1
Duodenum; villous blunting				1
Eyes; cornea; epithelium atrophy**		3	8	8
Heart; inflammatory infiltrates				1
Ileum; inflammatory infiltrates				1
Jejunum; inflammatory infiltrates				1
Liver; decrease glycogen				2
--inflammatory infiltrates**	3	5	4	5
LN, Mandibular; lymphoid hyperplasia			2	
LN, Mesenteric; sinus erythrocytes			1	6
Lungs; hemorrhage/congestion	1			3
Pancreas; single-cell necrosis				2
Skin; erosion/ulceration‡			1	
--follicular/epithelial degeneration‡**		5	5	6
--inflammatory infiltrates‡**	1	4	2	6
Stomach; edema				1
--inflammatory infiltrates				1
Thymus; hemorrhage/congestion**	1		1	4

‡ = highest severity score assigned to some/all animals
**Increasing severity with dose

Table 73: Summary of mean AZ13575104 toxicokinetic parameters in the rat metabolite qualification study

Group	Dose (mg/kg)	n	Day	t_{max} range(h)	C_{max} (±SD) µmol/L	$AUC_{(0-24)}$ (±SD) µmol·h/L
Main Test Phase						
2	5	4	28	3	0.142 (0.0494)	1.10 (0.180)
3	10	4	28	3–6	0.268 (0.0875)	2.61 (0.525)
4	15	2	28	3–6	0.312	4.06

(Excerpted from the Applicant's NDA)

10.4 AZD9291: One Month Oral (Gavage) Toxicity Study in the Rat (Report #528622)

The purpose of this study was to compare the toxicity profiles of two batches of AZD9291 produced using different manufacturing processes, for the purpose of impurity qualification. The study design is summarized in Table 74.

Table 74: Design of the 1-month oral impurity qualification toxicology study in the rat

Group	Animals	Animal reference number	Test item	Daily dose levels mg/kg/day
Main study groups				
1	10M + 10F	1001-1010, 1501-1510	Control	0
2	10M + 10F	2001-2010, 2501-2510	AZD9291 Batch 1	10
3	10M + 10F	3001-3010, 3501-3510	AZD9291 Batch 2	10
Toxicokinetic groups				
1	3M + 3F	1101-1103, 1601-1603	Control	0
2	3M + 3F	2101-2103, 2601-2603	AZD9291 Batch 1	10
3	3M + 3F	3101-3103, 3601-3603	AZD9291 Batch 2	10

AZD9291 Batch 1 correction factor 1.06
AZD9291 Batch 2 correction factor 1.07

Table 75: Summary of impurities contained in Batch 1 and Batch 2 test articles

Impurity	Batch 1 (AA-152-5, 14-1 Bx 1) Purity: 94.4%	Batch 2 (BNG-LSL-12025)† Purity: 93.7%
(b) (4)		

Impurity	Batch 1 (AA-152-5, 14-1 Bx 1) Purity: 94.4%	Batch 2 (BNG-LSL-12025)† Purity: 93.7%
(b) (4)		

† Batch 2 was previously tested in the 1-month rat study submitted at the time of IND filing (see Appendix)

Treatment with AZD9291 at a daily oral dose of 10 mg/kg was well-tolerated. Compound-related histopathological changes were observed in the male mammary gland, prostate, seminal vesicles, uterus, and vagina of treated animals with both batches of AZD9291. There were no apparent differences in body weight, hematology, clinical chemistry or histopathological parameters between animals treated with Batch 1 and Batch 2, suggesting that there was no exacerbation of toxicity attributable to the differences in impurity profiles. There were no meaningful differences in toxicokinetic exposures with the two sources of material (Table 76).

Table 76: Summary of AZD9291 Day 28 group mean TK parameters

Test Item	AZD9291 Batch 1	AZD9291 Batch 2
Dose level (mg/kg/day)		(b) (4)
Mean C_{max} (SD) (nmol/L)		
Mean $AUC_{(0-t)}$ (SD) (nmol.h/L)		

N is 6 for males and females combined

(Excerpted from the Applicant's NDA)

10.5 (b) (4) Genetic Toxicity Evaluation Using the Bacterial Reverse Mutation Test in Salmonella typhimurium TA1535, TA100, TA1537 and TA98 and Escherichia coli WP2 uvrA (pKM101) (Report #8303535)

The impurity, (b) (4)) was tested for mutagenic activity in the Ames bacterial mutagenicity assay in four auxotrophic (histidine-requiring) strains of *S. typhimurium* (TA98, TA100, TA1535, and TA1537) and one auxotrophic (tryptophan-requiring) strain of *E. coli* (WP2 *uvrA* pKM101). Compounds were tested using the plate-incorporation method and mutagenicity was assessed both in the presence and absence of metabolic activation by Aroclor-induced rat liver S9 microsome fractions. Test concentrations for the definitive assay were selected based on evidence of toxicity (diminution of the bacterial lawn) observed in the dose-ranging study.

Under the conditions of this assay, (b) (4) was positive for mutagenicity (revertant counts exceeded 2X the concurrent DMSO controls) in TA1537 strain of S. typhimurium

when tested in the presence and absence of S9 activation. (b) (4) was negative in all other strains tested.

The study met the criteria for a valid test, as the mean number of revertant colonies for positive and negative control cultures fell within the historical control ranges established by the testing facility.

Table 77: Individual plate counts from the dose-ranging mutagenicity study of (b) (4) (-S9)

Strain	Compound	Conc. Level (µg/plate) [µmol/plate]	Revertant Numbers			
			Mean	Standard Deviation	Fold Increase *	Revertant Numbers per Plate
TA98	(b) (4)	(b) (4)	25.0	11.3	1.1	(b) (4)
			20.0	5.0	0.9	
			20.3	1.2	0.9	
			26.0	10.8	1.1	
			21.0	2.0	0.9	
			13.7	0.6	0.6	
	DMSO		23.0	8.5		
TA100	(b) (4)		125.3	31.1	1.1	
			113.7	4.5	1.0	
			118.7	0.6	1.1	
			115.0	2.6	1.0	
			58.7	10.3	0.5	
	DMSO		112.0	12.4		
TA1535	(b) (4)		18.7	5.1	1.0	
			24.3	9.0	1.3	
			25.3	10.1	1.3	
			12.0	5.2	0.6	
			10.3	1.2	0.5	
	DMSO		19.0	5.5		
TA1537	(b) (4)		12.7	4.7	1.5	
			17.7	3.5	2.2	
			72.0	11.5	8.8	
			193.0	29.3	23.5	
			79.3	66.0	9.7	
	DMSO		8.2	3.8		
WP2 *uvrA* pKM101	(b) (4)		189.7	13.1	1.0	
			195.3	4.7	1.0	
			209.3	18.5	1.1	
	DMSO		196.8	7.9		
TA98	2NF		938.0	148.8	40.8	
TA100	NaN_3		768.0	105.0	6.9	
TA1535	NaN_3		678.7	21.2	35.7	
TA1537	AAC		397.0	44.7	48.4	
WP2 *uvrA* pKM101	NQO		1169.0	39.9	5.9	

* Calculated as a fold increase compared to the concurrent vehicle controls

T = toxicity (no revertant colonies); U = no data obtained; V = very thin bacterial background lawn; M = plate counted manually; S = slight thinning of background lawn; B = Bubbles or split in agar

(Excerpted from the Applicant's NDA)

Table 78: Individual plate counts from the dose-ranging mutagenicity study of (b) (4) (+S9)

Strain	Compound	Conc. Level (µg/plate) [µmol/plate]	Revertant Numbers			
			Mean	Standard Deviation	Fold Increase [a]	Revertant Numbers per Plate
TA98	(b) (4)	(b) (4)	38.7	4.0	1.0	(b) (4)
			46.7	8.6	1.2	
			38.7	7.8	1.0	
			41.3	3.2	1.1	
			32.0	8.5	0.8	
	DMSO		38.8	11.7		
TA100	(b) (4)		102.3	8.6	0.8	
			108.3	16.9	0.8	
			118.0	6.0	0.9	
			120.7	19.8	0.9	
			27.7	6.8	0.2	
	DMSO		135.4	10.3		
TA1535	(b) (4)		15.7	2.5	0.8	
			21.0	9.6	1.1	
			20.7	4.6	1.0	
			23.3	7.0	1.2	
			16.0	4.4	0.8	
	DMSO		20.0	6.7		
TA1537	(b) (4)		21.0	3.5	1.1	
			21.7	7.1	1.1	
			48.7	9.0	2.5	
			193.0	63.6	9.8	
			74.7	5.7	3.8	
	DMSO		19.6	1.3		
WP2 *uvrA* pKM101	(b) (4)		229.0	16.5	1.1	
			185.7	59.5	0.9	
			207.3	15.5	1.0	
			172.7	67.3	0.8	
			189.7	8.7	0.9	
			32.0	1.7	0.2	
	DMSO		212.2	21.4		
TA98	B[a]P		322.0	26.7	8.3	
TA100	AAN		1606.7	64.6	11.9	
TA1535	AAN		298.3	4.0	14.9	
TA1537	AAN		140.0	11.8	7.1	
WP2 *uvrA* pKM101	AAN		816.7	40.0	3.8	

[a] Calculated as a fold increase compared to the concurrent vehicle controls

(Excerpted from the Applicant's NDA)

Table 79: Plate counts from the confirmatory mutagenicity study with (b) (4) (-S9)

Strain	Compound	Conc. Level (µg/plate) [µmol/plate]	Revertant Numbers			
			Mean	Standard Deviation	Fold Increase [a]	Revertant Numbers Per Plate
WP2 *uvrA* pKM101	(b) (4)	(b) (4)	211.3	23.0	0.9	(b) (4)
			211.3	19.3	0.9	
			208.7	21.1	0.9	
			139.0	115.4	0.6	
			198.5	50.2	0.9	
	DMSO		231.8	16.8		
WP2 *uvrA* pKM101	NQO		892.3	75.1	3.8	

[a] Calculated as a fold increase compared to the concurrent vehicle controls

(Excerpted from the Applicant's NDA)

Table 80: Plate counts from the confirmatory mutagenicity study with (b) (4) (-S9)

Strain	Compound	Conc. Level (µg/plate) [µmol/plate]	Revertant Numbers			
			Mean	Standard Deviation	Fold Increase [a]	Revertant Numbers Per Plate
TA1537	(b) (4)	(b) (4)	9.7	5.0	1.9	(b) (4)
			9.7	4.2	1.9	
			15.7	5.9	3.0	
			39.3	9.6	7.6	
			22.0	8.2	4.2	
	DMSO		5.2	1.9		
TA1537	AAC		246.3	36.7	47.4	

[a] Calculated as a fold increase compared to the concurrent vehicle controls

(Excerpted from the Applicant's NDA)

10.5 (b) (4) Genetic Toxicity Evaluation Using the Bacterial Reverse Mutation Test in Salmonella typhimurium TA1535, TA100, TA1537 and TA98 and Escherichia coli WP2 uvrA (pKM101) (Report #8303536)

The impurity, (b) (4) was tested for mutagenic activity in the Ames bacterial mutagenicity assay in four auxotrophic (histidine-requiring) strains of *S. typhimurium* (TA98, TA100, TA1535, and TA1537) and one auxotrophic (tryptophan-requiring) strain of *E. coli* (WP2 *uvrA* pKM101). (b) (4) was tested using the plate-incorporation method and mutagenicity was assessed both in the presence and absence of metabolic activation by Aroclor-induced rat liver S9 microsome fractions. Test concentrations for the definitive assay were selected based on evidence of toxicity (diminution of the bacterial lawn) observed in the dose-ranging study.

Under the conditions of this assay, (b) (4) was negative for mutagenicity as the number of revertants did not exceed 2X those of the concurrent controls when tested in the presence and absence of S9 activation.

The study met the criteria for a valid test, as the mean number of revertant colonies for positive and negative control cultures fell within the historical control ranges established by the testing facility (Table 81 and Table 82). The negative control used in this study was acetonitrile:purified water: acetic acid, at a v/v/v ratio of 67:23:10.

Table 81: Individual plate counts from the dose-ranging mutagenicity study of (b) (4) (-S9)

Strain	Compound	Conc. Level (µg/plate) [µmol/plate]	Revertant Numbers			
			Mean	Standard Deviation	Fold Increase[a]	Revertant Numbers per Plate
TA98	(b) (4)	(b) (4)	22.3	3.2	1.2	(b) (4)
			19.3	1.5	1.1	
			16.0	4.4	0.9	
			22.3	1.2	1.2	
			20.0	8.5	1.1	
	Vehicle Control ^		18.2	6.5		
	Untreated Control		21.6	5.9		
TA100	(b) (4)		113.0	7.9	0.9	
			121.0	5.3	1.0	
			113.7	16.9	0.9	
			113.3	8.5	0.9	
			48.3	11.6	0.4	
	Vehicle Control ^		122.2	9.1		
	Untreated Control		231.6	19.2		
TA1535	(b) (4)		27.0	1.7	1.1	
			26.7	7.8	1.1	
			25.0	1.0	1.0	
			32.7	6.5	1.3	
			27.0	8.2	1.1	
	Vehicle Control ^		24.2	4.3		
	Untreated Control		169.6	16.3		
TA1537	(b) (4)		9.5	6.4	1.3	
			7.7	2.5	1.0	
			6.3	2.5	0.8	
			8.0	3.5	1.1	
			7.3	2.1	1.0	
	Vehicle Control ^		7.6	2.3		
	Untreated Control		8.6	3.9		
WP2 *uvrA* (pKM101)	(b) (4)		155.0	64.4	1.0	
			187.7	18.2	1.2	
			124.3	31.8	0.8	
			295.7	62.9	1.8	
			180.7	9.5	1.1	
			28.3	2.9	0.2	
	Vehicle Control ^		160.8	8.6		
	Untreated Control		214.2	15.9		

^ Acetonitrile:Purified Water:Acetic Acid (67:23:10 v/v/v)

[a] Calculated as a fold increase compared to the concurrent vehicle controls

T = toxicity (no revertant colonies); U = no data obtained; V = very thin bacterial background lawn; S = slight thinning of background lawn;

(Excerpted from the Applicant's NDA)

Table 82: Individual plate counts from the dose-ranging mutagenicity study of (b) (4) (+S9)

Strain	Compound	Conc. Level (µg/plate) [µmol/plate]	Revertant Numbers			
			Mean	Standard Deviation	Fold Increase[a]	Revertant Numbers per Plate
TA98	(b) (4)	(b) (4)	36.0	7.9	1.1	(b) (4)
			29.7	4.0	0.9	
			33.0	7.0	1.0	
			35.7	8.3	1.0	
			41.7	7.8	1.2	
			12.3	6.4	0.4	
	Vehicle Control ^		34.0	9.9		
	Untreated Control		28.6	4.0		
TA100	(b) (4)		107.3	14.6	0.9	
			89.3	14.4	0.7	
			109.3	12.5	0.9	
			96.3	7.5	0.8	
			101.7	22.2	0.8	
	Vehicle Control ^		122.2	18.2		
	Untreated Control		121.0	10.3		
TA1535	(b) (4)		23.0	3.0	1.1	
			21.0	6.6	1.0	
			26.3	9.9	1.3	
			27.7	3.2	1.3	
			36.0	9.2	1.7	
	Vehicle Control ^		21.0	1.9		
	Untreated Control		30.6	11.3		
TA1537	(b) (4)		22.7	1.5	1.6	
			18.3	5.0	1.3	
			21.0	8.2	1.5	
			20.7	2.1	1.5	
			14.5	6.4	1.1	
	Vehicle Control ^		13.8	5.1		
	Untreated Control		19.0	3.8		
WP2 *uvrA* (pKM101)	(b) (4)		203.3	23.2	1.1	
			185.7	31.8	1.0	
			201.3	12.7	1.1	
			161.7	26.3	0.9	
			218.7	25.1	1.2	
			88.7	5.5	0.5	
	Vehicle Control ^		178.4	24.0		
	Untreated Control		275.6	18.2		

^ Acetonitrile:Purified Water:Acetic Acid (67:23:10 v/v/v)
[a] Calculated as a fold increase compared to the concurrent vehicle controls

(Excerpted from the Applicant's NDA)

10.6 (b) (4): Genetic Toxicity Evaluation Using the Bacterial Reverse Mutation Test in Salmonella typhimurium TA1535, TA100, TA1537 and TA98 and Escherichia coli WP2 uvrA pKM101

The impurity, (b) (4) was tested for mutagenic activity in the Ames bacterial mutagenicity assay in four auxotrophic (histidine-requiring) strains of *S. typhimurium* (TA98, TA100, TA1535, and TA1537) and one auxotrophic (tryptophan-requiring) strain of *E. coli* (WP2 *uvrA* pKM101). Compounds were tested using the plate-incorporation method and mutagenicity was assessed both in the presence and absence of metabolic activation by Aroclor-induced rat liver S9 microsome fractions. Test concentrations for the definitive assay were selected based on evidence of toxicity (diminution of the bacterial lawn) observed in the dose-ranging study.

Under the conditions of this assay, (b) (4) was negative for mutagenicity as the number of revertants did not exceed 2X those of the concurrent controls when tested in the presence and absence of S9 activation.

The study met the criteria for a valid test, as the mean number of revertant colonies for positive and negative control cultures fell within the historical control ranges established by the testing facility (Table 83 and Table 84). The vehicle used in this study was dimethylformamide (DMF).

Table 83: Individual plate counts from the dose-ranging mutagenicity study of (b) (4) (-S9)

Strain	Compound	Conc. Level (µg/plate) [µmol/plate]	Revertant Numbers			
			Mean	Standard Deviation	Fold Increase [a]	Revertant Numbers Per Plate
TA98	(b) (4)	(b) (4)	23.3	1.2	1.3	(b) (4)
			7.7	4.0	0.4	
			18.0	1.7	1.0	
			11.3	2.5	0.6	
			7.7	6.4	0.4	
	DMF		18.4	2.5		
TA100	(b) (4)		97.0	12.5	0.9	
			86.3	8.1	0.8	
			72.3	18.9	0.7	
			13.7	4.6	0.1	
			7.3	2.5	0.1	
	DMF		105.6	10.9		
TA1535	(b) (4)		14.3	7.0	0.9	
			15.7	1.5	1.0	
			16.7	9.0	1.0	
			7.7	3.1	0.5	
			1.3	0.6	0.1	
	DMF		16.0	4.7		
TA1537	(b) (4)		7.0	5.0	0.6	
			11.7	0.6	1.1	
			15.3	1.5	1.4	
			7.0	4.4	0.6	
	DMF		11.0	1.4		
WP2 *uvrA* pKM101	(b) (4)		219.7	4.9	1.0	
			193.3	28.4	0.8	
			193.0	13.5	0.8	
			192.0	6.6	0.8	
			169.7	12.1	0.7	
			124.0	6.1	0.5	
	DMF		230.8	17.5		
TA98	2NF		709.0	146.7	38.5	
TA100	NaN_3		541.3	18.6	5.1	
TA1535	NaN_3		543.7	24.0	34.0	
TA1537	AAC		383.0	42.2	34.8	
WP2 *uvrA* pKM101	NQO		1015.0	96.7	4.4	

[a] Calculated as a fold increase compared to the concurrent vehicle controls

(Excerpted from the Applicant's NDA)

Table 84: Individual plate counts from the dose-ranging mutagenicity study of (b) (4) (+S9)

Strain	Compound	Conc. Level (µg/plate) [µmol/plate]	Revertant Numbers			
			Mean	Standard Deviation	Fold Increase [a]	Revertant Numbers Per Plate
TA98	(b) (4)	(b) (4)	32.3	6.4	1.0	(b) (4)
			26.0	8.7	0.8	
			31.7	2.5	1.0	
			27.0	7.2	0.9	
			18.3	3.8	0.6	
	DMF		30.8	9.4		
TA100	(b) (4)		119.0	18.4	1.0	
			86.0	25.5	0.7	
			96.0	11.0	0.8	
			31.3	6.8	0.3	
			5.7	1.2	0.0	
	DMF		115.8	10.8		
TA1535	(b) (4)		18.7	5.5	1.1	
			17.3	5.7	1.0	
			17.7	1.2	1.0	
			14.0	4.4	0.8	
			4.3	2.5	0.3	
	DMF		17.0	8.0		
TA1537	(b) (4)		18.7	1.5	1.2	
			16.0	5.6	1.0	
			20.7	5.7	1.3	
			15.3	2.9	0.9	
	DMF		16.2	3.9		
WP2 *uvrA* pKM101	(b) (4		270.7	15.3	1.2	
			231.3	21.1	1.0	
			259.3	21.4	1.2	
			248.7	4.5	1.1	
			217.3	18.9	1.0	
			159.7	22.6	0.7	
			23.7	2.9	0.1	
	DMF		225.2	38.8		
TA98	B[a]P		435.0	34.0	14.1	
TA100	AAN		1492.7	50.4	12.9	
TA1535	AAN		326.0	30.8	19.2	
TA1537	AAN		206.7	6.4	12.8	
WP2 *uvrA* pKM101	AAN		558.3	52.4	2.5	

[a] Calculated as a fold increase compared to the concurrent vehicle controls

(Excerpted from the Applicant's NDA)

11 Integrated Summary and Safety Evaluation

Osimertinib (AZD9291) is an oral, small-molecule, irreversible inhibitor of the epidermal growth factor receptor (EGFR) that blocks growth-promoting signals that emanate downstream of the receptor following ligand-binding. Inhibitory activity is greater against EGFR isoforms bearing the T790M mutation. The Applicant also demonstrated that osimertinib blocks other EGFR-family members, including HER2 and HER3 in cellular assays.

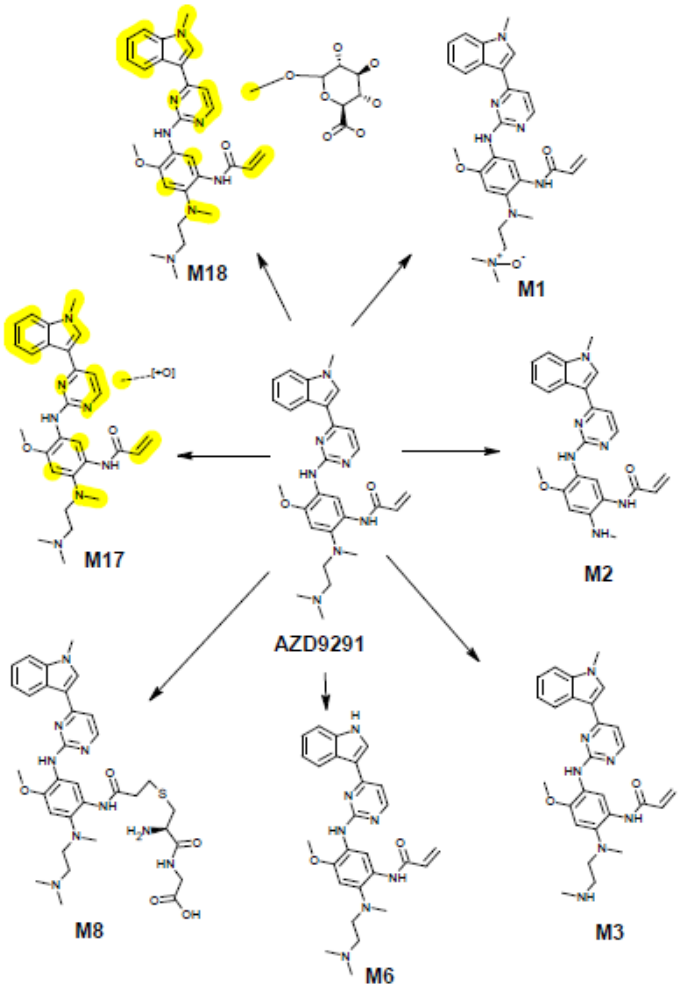
The mechanism of action for osimertinib was evaluated in a panel of in vitro and in vivo studies to assess its target engagement, binding affinity/binding kinetics, target selectivity, and anti-tumor effects. Using mass spectrometry, osimertinib was found to (b) (4) (Cross, DA, et al. 2014; Schwartz, PA, et al., 2014). Target

engagement was found to be a time-dependent process, and to persist for at least 48 hours after washout in cultured cell models.

Figure 61: Structural model of Osimertinib binding to the EGFR T790M mutant

The Applicant also evaluated the cross-species metabolic profile of osimertinib in an in vitro panel and demonstrated that osimertinib is extensively metabolized, but that there are no unique human metabolites. The proposed human metabolic scheme for osimertinib is given in Figure 62. Two of its metabolites (AZ13575104 (denoted as M6) and AZ13597550 (denoted as M3)) exhibit a similar degree of pharmacological activity and selectivity as the parent, and are present at approximately 10% of the parent AUC; thus, the pharmacology and toxicity of these compounds were evaluated in tandem throughout the development program. The structures of M3, and M6, are provided below.

Figure 62: Osimertinib proposed metabolic scheme in humans



(Excerpted from the Applicant's submission)

(b) (4)

, the data were deemed to be unreliable, and Applicant opted to characterize their IC_{50}s using cell-based functional assays, including blockade of EGFR receptor activation (phosphorylation of EGFR) and downstream signaling, such as ERK activation, in (b) (4)

As assessed by both inhibition of cell proliferation and inhibition of pEGFR production, osimertinib and its metabolites exhibited a moderate degree of selectivity (up to 30-fold) toward the mutant isoforms of EGFR (T790M, L858R, and exon 19 deletion mutants) relative to the wild-type isoform. Both metabolites showed a similar increased activity against the EGFRm isoforms.

The Applicant provided data from several murine tumor models demonstrating the anti-tumor activity of osimertinib and its metabolite, AZ13575104. Consistent with its in vitro properties, both osimertinib and AZ13575104 exhibited greater suppression of Ex19del and T790M/L858R xenografts than were observed in WT tumor models.

The Applicant evaluated off-target binding-selectivity and demonstrated that osimertinib and/or its metabolites significantly inhibited the following kinases at potentially physiologically-relevant concentrations (i.e. IC_{50}s≤ 100nM): HER2, HER4, ACK-1, and BLK. Osimertinib demonstrated some potential for inhibition of IGF1R based on IC_{50} concentrations approaching the clinical Cmax. While inhibition of this receptor seemed unlikely at IC_{50}s that did not factor in free versus bound protein, the Applicant submitted follow-up studies to demonstrate this point. Effects on IGF1R were evaluated in cultured cells that overexpress exogenous human IGF1R; based on these studies, effects of osimertinib and AZ13575104 on IGF1R signaling are likely outside of the physiological range.

In receptor binding and enzyme inhibition studies, osimertinib and its metabolites, AZ13575104 and AZ13597550, also inhibited a number of neurotransmitter pathways and/or cardiac ion channels at low multiples (<3-5X) of the observed clinical Cmax. These compounds were found to be less potent than predicted on the basis of receptor binding and enzyme inhibition assays, when they were tested in in electrophysiology assays in whole cells. IC_{50}s in these assays ranged upwards of 30μM, suggesting that osimertinib and its metabolites are unlikely to mediate cardiac toxicity via the $hK_v1.5$, $hCa_v3.2$, or hHCN4 channels. The hERG IC_{50} for osimertinib was 690 nM; therefore, some inhibition is expected at the clinical C_{max} of 501 nM, though the free levels of osimertinib are unlikely to reach this level.

Osimertinib and/or its metabolites were evaluated in a complete panel of safety pharmacology studies to evaluate their effects on vital organ function, including cardiac, respiratory, CNS, visual, and GI parameters. In the anesthetized Guinea pig, osimertinib administration increased diastolic, mean arterial, and left ventricular systolic pressures; PR, QRS, and QTcB durations, and was associated with a reduction in heart rate and dP/dtmax. When tested in the conscious telemetered beagle dog, osimertinib was also associated with an increase in heart-rate-corrected QTcR, and decreases in dP/dt+ and heart rate. Effects were also observed in the rat telemetry study, particularly during the hours of rest. Administration of osimertinib in rats led to increased blood pressure without a change in heart rate at oral doses of 50 and 100 mg/kg.

In the combined visual, respiratory and GI transit study, there were no effects on CNS activity as measured by the functional observational battery. A decrease in pupil size and visual acuity was observed at the mid- or mid- and low-dose levels, respectively. In the respiratory assessment, a decrease in minute volume and expiratory rate were also observed by whole body plethysmography, and in the GI transit study, a decrease in gastric emptying and GI transit was observed.

The general toxicology studies for osimertinib consisted of dose-ranging and repeat-dose GLP toxicology studies in the rat and dog. The selection of toxicology species was made on the basis of pharmacodynamic and metabolic similarity to humans, and dose-selection for pivotal toxicology studies (3-month rat and dog studies) on the basis of MTDs determined in studies of shorter durations.

In the 13-week toxicity study in the rat, there were no preterm deaths, but target organs included the eyes (atrophy of the corneal epithelium and retinal dysplasia), skin (inflammatory cell infiltration), lung (inflammatory cell infiltration), kidneys (tubular basophilia and inflammatory cell infiltration) and GI tract (soft feces/hunching/salivation). Hematology changes included decreased red blood cell indices, which correlated with increased incidences of erythrophagocytosis in high dose animals; increased WBC indices; increased fibrinogen and decreased APTT. Clinical chemistry changes included mildly increased ALT and AST; decreased triglycerides, albumin, and total protein. Decreased calcium was also observed; however, this was likely secondary to the observed decreases in albumin. Mating treated males with untreated dams on Days 65-71 indicated a treatment-related decrease in male fertility, demonstrated by decreased numbers of live fetal implants and increased preimplantation loss at the high dose level. Exposures in this study were approximately 0.5-1X the observed clinical AUC in males and females, respectively. While levels of AZ13575104 were measured, they were not reported, due to technical problems with the bioanalytical assay, involving the presence of a co-eluting peak that interfered with the quantitation of this metabolite. In the 3-month rat study, relative exposure to AZ1397550 in high dose animals was approximately 0.77-0.81X those observed in humans at the 80 mg/day dose level. In a separate metabolite qualification study performed with AZ13575104 no unique toxicities were identified compared with those observed with osimertinib. The AUCs achieved at the AZ13575104 maximum tolerated dose of 10 mg/kg were 2.3-fold higher than those expected to occur in humans; thus, the toxicity of both metabolites is considered adequately characterized for use in patients with advanced cancer.

In the 13-week dog study, ocular toxicity (corneal atrophy) was dose-limiting, and necessitated a dose-reduction from 10 mg/kg/Day to 6 mg/kg/Day after Day 25. Other histological target organs included the lung (mononuclear infiltration/aggregation), skin (hyperplasia, inflammatory cell infiltration), bone marrow (decreased hematopoiesis) and testes (atrophy). Achieved exposures at the highest dose level in this study were 0.42X of the human AUCs for osimertinib, and 0.51X the human AUC for AZ13597550.

Osimertinib was negative in the bacterial and mammalian cell mutagenicity assays, both in the presence and absence of S9 metabolic activation, and negative for induction of structural chromosome aberrations in primary peripheral blood mononuclear cells and in the rat in vivo micronucleus assay. One impurity, (b) (4) was identified as mutagenic in the in vitro reverse mutation assay in bacteria (Ames). The specification for this impurity is no more than (b) (4) %, which, though high for a genotoxic impurity, is below the impurity qualification threshold of 0.15% described in ICH Q3A. At the clinical dose of osimertinib of 80 mg daily, this specification would result in clinical exposures of this impurity of no more than (b) (4) µg/day. Given that this drug is intended for the

treatment of patients with advanced cancer, this specification is acceptable for the current indication, (b) (4) All other impurities that are specified above the levels in ICH Q3A or Q3B were qualified in animal studies.

Administration of osimertinib to pregnant dams from Gestation Day 2 to 20 resulted in decreases in live implants, increases in dead implants, and increases in post-implantation loss, including increased rates of early and late embryonic death at the high dose of 20 mg/kg (approximately 1.5 times the clinical Cmax at 80 mg/day). When administered from Gestation Day 6 through Lactation Day 6 (GD6-LD6), osimertinib resulted in an increase in postnatal death and an increase in total litter loss, as well as a reduction in mean pup weight at doses of ≥20 mg/kg/Day. A non-dose-related increase in visceral abnormalities and malformations were observed in pups from some dams treated with osimertinib from GD6-20 at doses of 20 mg/kg/day, or AUCs of approximately 1.5-times those observed in patients at dose of 80 mg/Day.

References

1. Schwartz, P.A., et al., 2014. Covalent EGFR inhibitor analysis reveals importance of reversible interactions to potency and mechanisms of drug resistance. PNAS. 111(1):173-78
2. Cross, D.A. et al. 2014. AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. Cancer Discovery. 4(9): 1046–1061.

12 Appendix/Attachments

From the Review of IND 117,879

6.2 Repeat-Dose Toxicity

Study title: AZD9291: One Month Oral Toxicity Study with Assessment of Recovery in the Rat

Study no.:	3416AR
Study report location:	4.2.3.2
Conducting laboratory and location:	Safety Assessment UK, AstraZeneca R&D Alderley Alderley Park Macclesfield, SK10 4TG, England UNITED KINGDOM
Date of study initiation:	24 April 2012 (first dose)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AZD9291 free base, BNG-LSL-12025, 93.8%

Key Study Findings

There were no preterm deaths. Target organs were the GI organs, eyes, liver, lungs, reproductive organs, skin, tongue, kidney, and lymphoid organs.

Methods

Doses:	4, 10, 40 mg/kg/Day (Males) 4, 10, 20 mg/kg/Day (Females)
Frequency of dosing:	Daily
Route of administration:	Oral Gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Water containing 0.5% w/v hydroxypropyl methylcellulose
Species/Strain:	Rat, Wistar Hannover substrain RccHan:WIST
Number/Sex/Group:	10M / 10F (Main Cohorts) / 5/Sex (Control/High)
Age:	Approximately 10 weeks old
Weight:	307-316g (M) / 201-208g (F) on Day 1 of Dosing
Satellite groups:	3M / 3F Toxicokinetic
Unique study design:	Sex-specific high dose; P450 analysis from liver samples
Deviation from study protocol:	The Sponsor stated that there were no protocol deviations that affected overall study interpretation. *N.b.* protocol was not attached to report.

Observations and Results

Mortality

None

Clinical Signs

Piloerection, periocular staining, salivation

Body Weights

Mean body weights and body weight gains in high dose males and females were non-significantly lower than controls at the end of the treatment interval (Figure 63 and Figure 64).

Figure 63: Body Weights of Main and Recovery Cohort Males

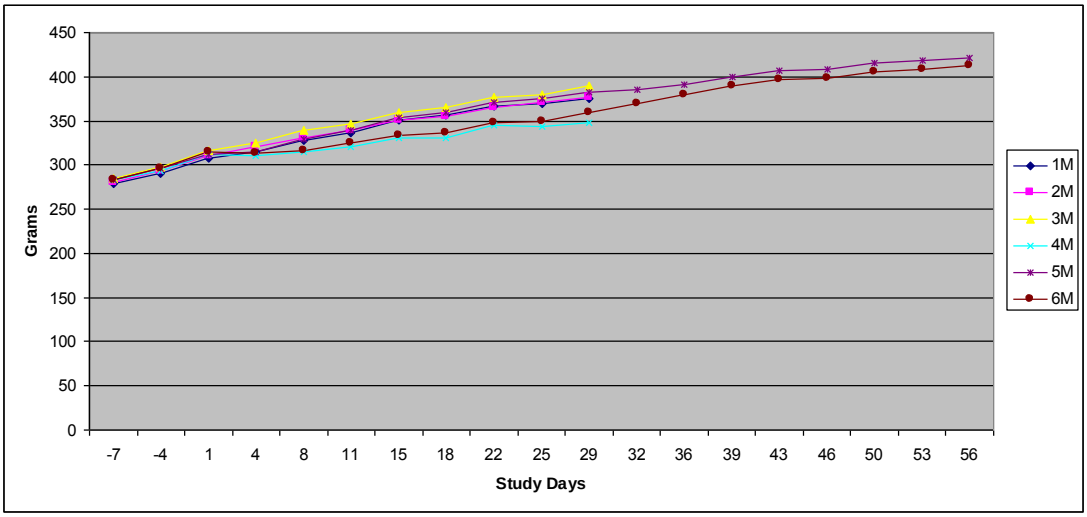
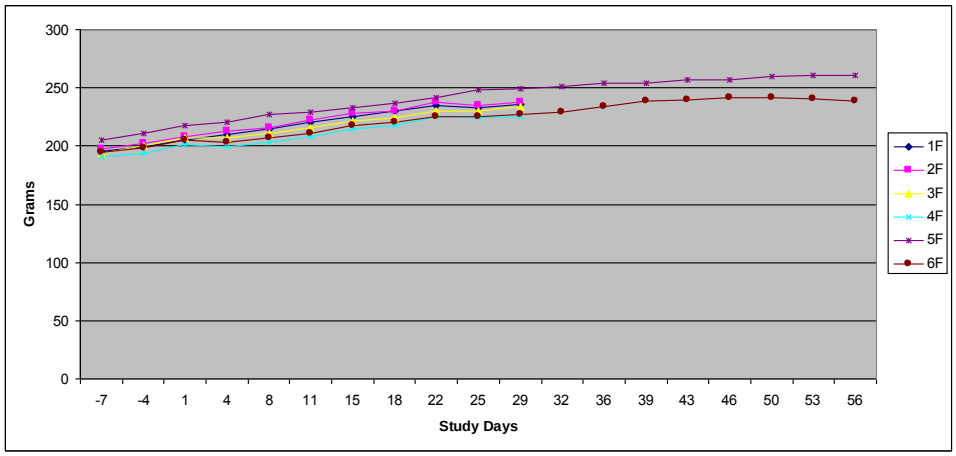


Figure 64: Body Weights of Main and Recovery Cohort Females



Feed Consumption

Unremarkable – note that animals were group-housed, so individual feed consumption data are not available.

Ophthalmoscopy

Unremarkable; however, no veterinary ophthalmology report was provided.

ECG

N/A

Hematology

Table 85: Treatment-Related Hematology Changes (%) in Treated Groups vs. Concurrent Controls

	Hb g/dL	RBC 10^{12}/mL	HCT L/L	MCV fL	RDW %	WBC 10^9/L	NEUT 10^9/L	LYM 10^9/L	MONO 10^9/L	PLT 10^9/L	MCH pg
1M											
5M											
2M											
3M	↓4.61		↓4.26	↓3.94							↓3.35
4M	↓7.24		↓6.38	↓4.12		↑45.18	↑153.19	↑25.93	↑142.86	↑16.37	↓3.91
6M		↓5.11			↑7.81						
1F											
5F											
2F											
3F						↑26.81	↑41.94				
4F						↑24.26	↑82.26		↑38.46		
6F							↑58.57				

Values Reflect % Change vs. Concurrent Controls
Note that Group 5 = Control Recovery Cohort; Group 6 = Recovery High Dose Cohort

Clinical Chemistry

Table 86: Treatment-Related Clinical Chemistry Changes (%) in Treated Groups vs. Concurrent Controls

	GLDH	GLU	TG	CHOL	TP	ALB	GLOB	CA	PHOS
1M									
2M									
5M	↓37.5								
3M	↓25.0					↓4.7			
4M	↓50.0	↓17.7	↓33.9	↓25.0	↓6.1	↓7.0	↓4.3	↓3.7	
6M									↑13.3
1F									
5F									
2F									
3F									
4F						↓6.579	↓8		
6F									

Values Reflect % Change vs. Concurrent Controls
Note that Group 5 = Control Recovery Cohort; Group 6 = Recovery High Dose Cohort
GLDH = glutamate dehydrogenase (IU/L); GLU = glucose (mM); TG = triglycerides (mM); CHOL = cholesterol (mM); TP = total protein (mM); ALB = albumin (g/L); GLOB = globulin (g/L); CA = calcium (mM); PHOS = phosphorous (mM)

Urinalysis:

Treatment-related changes in urine parameters are provided in Table 87. None of the changes was indicative of toxicity, and as there were relatively few histological changes in the kidneys, these changes are likely not clinically meaningful. Urinary glucose changes were likely potentially pharmacodynamic, given the effect on insulin receptors; however, given that the serum glucose was not elevated and the effect was not present in both sexes, the toxicological relevance is unclear. Similarly, the effect on urinary creatinine, which was alsopresent in only one sex, was not corroborated by histological findings.

Table 87: Summary of Changes (Expressed as % Change of Control) in Rat Urine Chemistry Following Treatment with AZ9291

	UVOL mL	USG	UPHS	UCRE mM	UGLU mM	UNAG IU/L	UTPC mg/mM	UGLUC
2M								
3M	↓34.7	↑0.3	↑5.3				↓38.0	↑42.9
4M	↓55.8	↑1.2	↑5.3	↑118.2	↑150.0	↑79.7		
6M								
2F								
3F								
4F			↑11.3					
6F								

Group 2 = 4 mg/kg ; Group 3 = 10 mg /kg ; Groups 4 and 6 = 40 mg/kg (males) and 20 mg/kg (females). Groups 1 and 5 = controls. Note that Group 5 = Control Recovery Cohort; Group 6 = Recovery High Dose Cohort
Values Reflect % Change vs. Concurrent Controls
UVOL = urinary volume; USG = urinary specific gravity; UPHS = urine pH; UCRE = urinary creatinine; UGLU = urinary glucose; UNAG = urinary N-acetylglucosamine; UTPC = urinary total protein/creatinine ratio; UGLUC = urinary glucose/creatinine ratio

Gross Pathology

Skin discoloration and/or scabbing (mammary, muzzle); lymph node discoloration

Organ Weights

Statistically-significant reductions in absolute epididymis, prostate, thymus, and liver weights were observed in high dose males. A reduction in relative thymus and prostate weight as percent of mean body weight was also observed in HD males. No organ weight changes were observed in recovery males.

Reduced thymus weights (absolute and % of mean body weight) were observed in high dose females. Reduced liver weights (absolute and as percent of mean body weight) were observed in high dose females. Increased absolute mean lung weight was observed in high dose recovery females vs. concurrent controls.

Histopathology

Adequate Battery
Yes

Peer Review
No

Histological Findings

Group (mg/kg/Day)	0	4	10	40	0	4	10	20
Sex	Male				Female			
Organ, Histopathological Description								
Adrenals, increased vacuolation				1				
Duodenum, Brunner's gland degeneration				1				
Epididymis, reduced sperm		1		3+1R				
--cellular debris in lumena		1		3+1R				
--mononuclear cell infiltration				1				
Eyes, cornea, epithelial atrophy*		10	10	10		10	10	10
Eyelids, inflammatory cell infiltration*								1
Harderian Glands, mononuclear cell infiltration	1			3				2
--glandular degeneration with inflammation								1
Heart, inflammatory cell infiltration				1				1
Jejunum, focal inflammatory cell infiltration				1				
Kidney, hydronephrosis*	1				2		1	
Liver, degreased glycogen				1				
--inflammatory cell infiltration	4			5				
LN, Mandibular, lymphoid hyperplasia*		1	1	2			1	
LN, Mesenteric, sinus erythrocytes with erythrophagocytosis				10				1
Lungs, focal congestion/hemorrhage		1						
--focal inflammatory cell infiltration		1		1				
--focal macrophages	3			4	2		1	3
Muzzle, focal/follicular inflammatory cell infiltration*				3				4
Ovaries, corpora luteal degeneration/inflammation							4	10
Skin, focal/follicular inflammatory cell infiltration*				2				1
-- follicular dysplasia								2
Testes, segmental tubular degeneration		1		1R				
--spermatid retention			3	5				
--tubular degeneration				6				
Tongue, epithelial atrophy				10			8	10

Special Evaluation

Concentrations of P450 enzymes were evaluated in treated and control animals. AZD9291 induced expression of CYPs 1A, 3A and 4A in males and females (Sponsor-Table 88). Decreased CYP2B levels were observed in treated males and females. The changes in absolute concentrations of these enzymes were relatively small; therefore, the clinical relevance of these effects is unclear.

Table 88: Summary of CYP Metabolic Enzyme Levels Measured in Rat Livers Following Treatment with AZD9291

Group	CYP1A		CYP2B		CYP3A		CYP4A	
	Conc. pmol/mg	Fold Change	Conc. pmol/mg	Fold Change	Conc. pmol/mg	Fold Change	Conc. pmol/mg	Fold Change
Male Animals								
1	17.71	1.00	179.78	1.00	35.88	1.00	16.72	1.00
4	21.28	1.20	137.28	0.76	41.92	1.17	19.68	1.18
Female Animals								
1	30.29	1.00	195.26	1.00	41.63	1.00	15.29	1.00
4	33.83	1.12	141.16	0.72	49.58	1.19	17.71	1.16

Fold change compared to the concentration seen in the relevant controls (no change = 1.00).

Toxicokinetics

The toxicokinetics profile of AZD9291 in the rat was evaluated on Days 1 and 28 of dosing (Sponsor-Table 89). Peak and overall (AUC) exposures were generally proportional with dose over the dose range on Day 1. There was a gender effect on exposure, but the effect was weak (<2X), which may have explained the gender effect on toxicity. There was little evidence for accumulation by either C_{max} or AUC and although exposures were higher in some groups on Day 28 than on Day 1, the pattern was not consistent across dose levels; indeed, in some cases, exposures were lower on Day 28 than on Day 1 (e.g. AUCs for high-dose males and mid-dose females) but the magnitude was not sufficient to be considered indicative of metabolic induction.

Toxicokinetic analysis of AZD9291 and its two metabolites, AZ13597550 and AZ13575104 was performed using a validated method which involved sample extraction by protein precipitation and analyte detection by MS following separation by liquid chromatography (LC-MS/MS). The LLOQ was 2.5 nM for AZD9291 and AZ13597550. AZ13575104 assessment was non-quantitative due to the presence of a co-eluting peak; thus, results were recorded as analyte present/absent.

The method was found to perform satisfactorily (8/8 batches met acceptance criteria), and no samples from control animals exceeded the LLOQ for AZD9291 or AZ13597550. ISR was performed and reproducibility was demonstrated. Data from long-term plasma stability assessments indicated that concentrations of AZD9291 and AZ13597550 may have declined by 23 and 20%, respectively, due to temporal instability under the conditions of storage.

The primary impact of this observation is to the interpretation of the toxicokinetic results, leading to an under-estimation of dose/exposure parameters such as dose-linearity, half-life, etc.

Table 89: Summary of AZD9291 Toxicokinetic Parameters in the Rat on Study Days 1 and 28

Study Day	Sex	Dose (mg/kg)	C_{max} (nmol/L)		T_{max} (Hours)	$AUC_{(0-24)}$ (nmol.Hours/L)		AUC/Dose		C_{max}/Dose		n
			Mean	S.D.	range	Mean	S.D.	Mean	S.D.	Mean	S.D.	
1	Male	4	116	26.5	2-4	1310	422	169	54.5	15.0	3.44	3
		10	245	52.3	2-4	2540	607	132	31.4	12.7	2.72	3
		40	945	121	2-4	14000	3750	181	49.0	12.2	1.56	3
	Female	4	140	33.9	2-4	1560	356	202	46.0	18.1	4.41	3
		10	398	60.1	2-4	4050	266	210	13.9	20.6	3.09	3
		20	607	50.4	2-4	8030	815	209	21.3	15.8	1.29	3
28	Male	4	129	39.0	2-4	1660	545	216	70.5	16.7	5.06	3
		10	218	44.5	2	2500	314	130	15.9	11.3	2.29	3
		40	546	84.5	2-4	9530	867	124	11.7	7.08	1.10	3
	Female	4	200	47.6	2-4	2470	491	320	64.1	25.9	6.18	3
		10	329	47.4	2-4	3410	373	177	19.1	17.0	2.42	3
		20	615	105	4-8	9980	2910	259	75.2	16.0	2.76	3

Table 90: Summary of AZ13597550 Exposures in the Rat Following Oral Administration of AZD9291 on Study Days 1 and 28

Study Day	Sex	Dose (mg/kg)	C_{max} (nmol/L)		T_{max} (Hours)	$AUC_{(0-24)}$ (nmol.Hours/L)		AUC/Dose		C_{max}/Dose		n
			Mean	S.D.	range	Mean	S.D.	Mean	S.D.	Mean	S.D.	
1	Male	4	11.0	0.493	4-8	NC	NC	NC	NC	1.43	0.0608	3
		10	29.2	3.23	4	394	73.3	20.4	3.81	1.51	0.170	3
		40	106	27.4	4-8	2090	576	27.1	7.48	1.38	0.357	3
	Female	4	7.77	1.80	4	NC	NC	NC	NC	1.01	0.236	3
		10	23.8	1.84	2-4	339	22.6	17.6	1.19	1.24	0.0971	3
		20	30.3	4.82	2-4	512	110	13.3	2.81	0.787	0.125	3
28	Male	4	14.2	4.57	4	NC	NC	NC	16.1	1.84	0.595	3
		10	25.9	2.00	4	343	20.8	17.7	1.04	1.34	0.104	3
		40	70.9	19.1	4-8	1340	279	17.4	3.61	0.918	0.247	3
	Female	4	11.2	2.29	4	NC	NC	NC	NC	1.45	0.293	3
		10	22.2	5.24	2-4	278	14.0	14.4	0.721	1.15	0.271	3
		20	31.0	3.23	4	554	29.5	14.4	0.721	0.804	0.0843	3

NC Not calculated, limited data to define $AUC_{(0-24)}$ for all animals

Dosing Solution Analysis

The Sponsor states that the analyses conformed with pre-specified acceptance criteria; however, no DFA report was provided. An IR was sent, requesting the report.

6.2 Repeat-Dose Toxicity

Study title: AZD9291: One Month Oral Toxicity Study with Assessment of Recovery in the Dog - Report Amendment

Study no.:	1351AD
Study report location:	4.2.3.2
Conducting laboratory and location:	Safety Assessment UK AstraZeneca R&D Alderley Alderley Park, Macclesfield. SK10 4TG. England UNITED KINGDOM
Date of study initiation:	15 May 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AZD9291, BNG-LSL-12025, 93.8% purity

Key Study Findings

There were no preterm deaths. GI toxicity at 20 mg/kg required dose suspension between Days 8-10 of study, followed by reduction on Day 11 of study. Other target organs included the liver, lymphoid organs, lungs,testes/ovaries, accessory sex organs, skin, kidneys, tongue and urinary bladder. Ocular toxicity was observed in 20/12 mg/kg/Day animals.

Methods

Doses:	0, 2, 6, 20/12 mg/kg/Day
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	Suspension in water containing 0.5% w/v hydroxypropyl methylcellulose
Species/Strain:	Dog, Beagle
Number/Sex/Group:	3M /3F (Main) + 2M/2F high dose animals (no vehicle control recovery cohort)
Age:	17.8-21.8 months (male) 17.6-21.5 months (female)
Weight:	9.8-15.5 kg (male) at start of dosing 7.3-14.7 kg (female) at start of dosing
Satellite groups:	None
Unique study design:	Insulin and glucose measurements
Deviation from study protocol:	Suspension of dosing on Day 8 in high dose cohorts due to toxicity; resumption of dosing on Day 11 at a lower dose level.

Observations and Results

Mortality

There were no preterm deaths; however, due to poor tolerability, dosing in the high dose (20 mg/kg/Day) cohorts was suspended between Days 8-10, and reinitiated at a lower dose level (12 mg/kg/Day) on Day 11.

Clinical Signs

Emesis, diarrhea (including red feces),

Body Weights

Body weight reduction was observed in some treated male groups and all treated female groups.

Figure 65: Male Body Weights

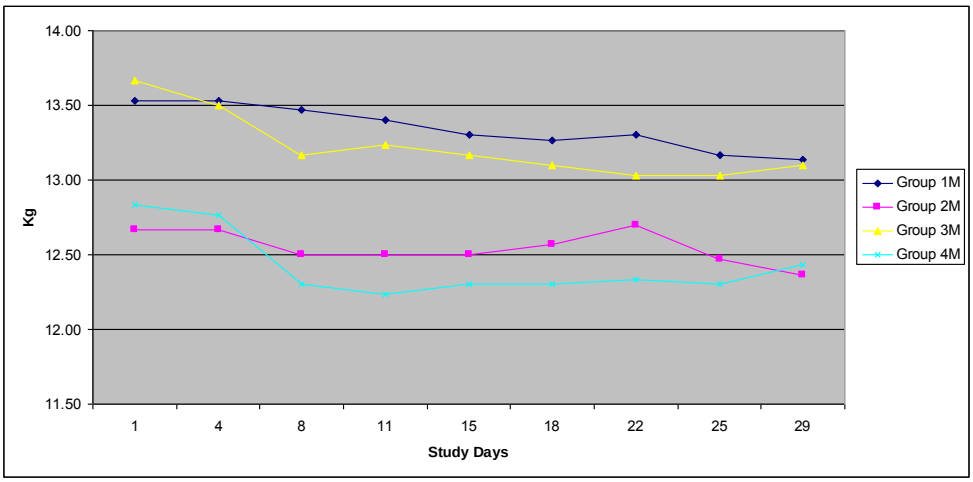
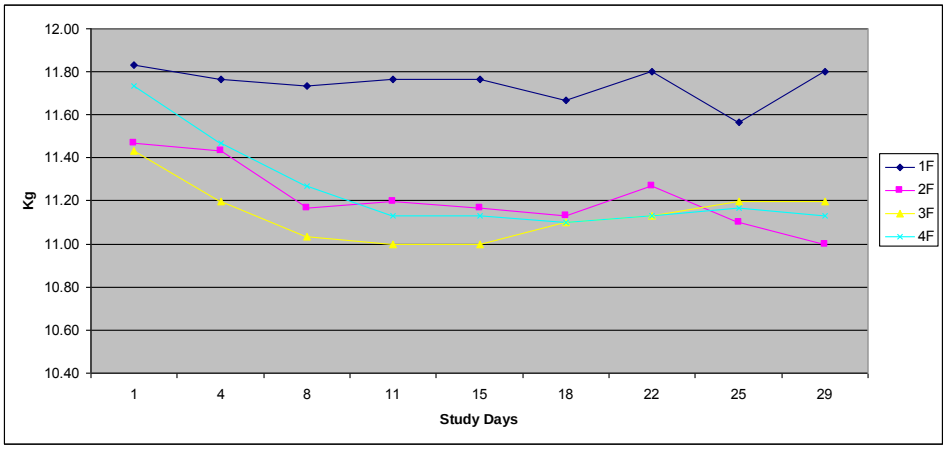


Figure 66: Female Body Weights



Feed Consumption

A reduction in feed consumption was observed in treated females.

Figure 67: Male Feed Consumption

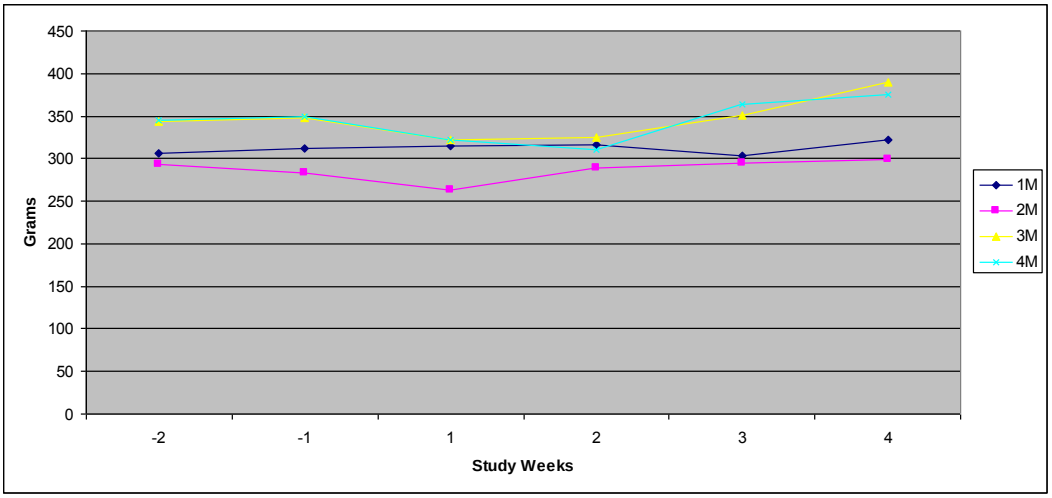
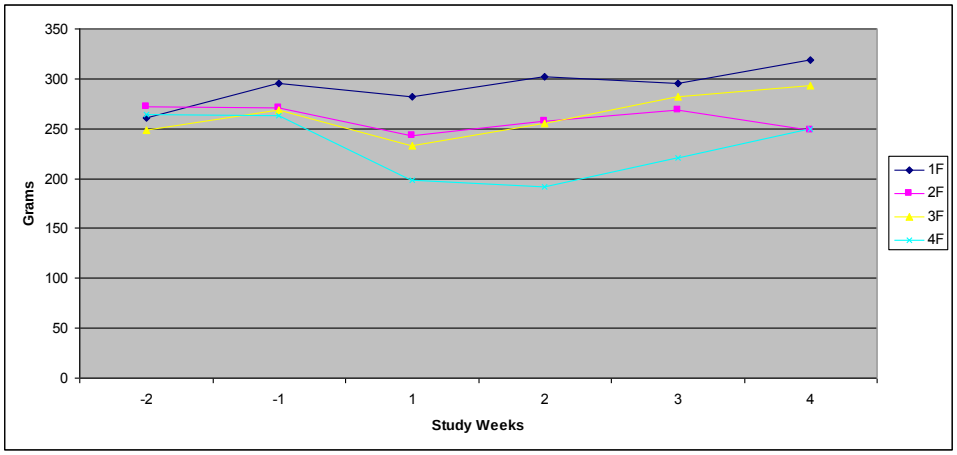


Figure 68: Female Feed Consumption



Ophthalmoscopy

The eye is a target organ of AZD9291. Treatment-related findings observed in the eyes are summarized in Table 91. Corneal findings appeared to partially regress in high dose animals following the dose-holiday and resumption at a lower dose. Corneal opacity observed in Recovery Cohort animals was not fully reversible.

Table 91: Incidence of Ophthalmology Findings Associated with AZD9291 Administration in Dogs

Dose (mg/kg/Day)	0	2	6	20 / 12
Findings	Incidence = # animals affected (M / F)			
Corneal Translucency			1 / 1	3 / 3
Fluoroscein Staining (Days 8-10)				3/3
Conjunctival Reddening				1 / 2
Palpebral Closure				1 / 1
Watery Discharge				0 / 1

ECG

There was no treatment-related effect on mean diastolic blood pressure, heart rate, ECG parameters or waveform morphology. Note that data from males and females were combined in the analysis. While a Phase Report was not provided for this portion of the study, a stand-alone GLP ECG study in the dog was conducted and the data are described in Section 4.3.

Hematology

Unremarkable

Coagulation

An apparent increase in fibrinogen was observed in mid- and high-dose males and females. Other coagulation parameters were unaffected.

Clinical Chemistry

Increased cholesterol and triglycerides in mid- and high-dose males and females at the end of study. Values exceeded both mean pre-dose and mean concurrent control values.

Urinalysis

Increase in mean urinary n-acetylglucosamine in Group 2 and Group 4 females on Day 25.

Gross Pathology

Treatment-related gross necropsy observations included discolorations (red, patchy-red, or pale) of the gastrointestinal organs, thyroid, thymus and lymph nodes, lung, and skin.

Organ Weights

The following organ weight differences were observed: an apparent decrease in the absolute and relative weights of the thymus and spleen in treated males and females. An apparent increase in absolute and relative lung weight in high dose males. Statistical analyses were not performed; therefore, it is unclear whether these groups differed from controls.

Histopathology

Adequate Battery
Yes

Peer Review
No

Histological Findings

Group (mg/kg/Day)	0	2	6	20/12	0	2	6	20/12
Sex	Male				Female			
Organ, Histopathological Description								
Duodenum, atrophy				1				2
Epididymis, round cells present		1		1				
--reduced sperm	1	1						
--lymphoid aggregates		1						
Eyes, corneal atrophy				3			1	3
Heart, coronary artery subintimal degeneration							1	
Kidney, depressed capsule			1	1				1
Lacrimal glands, inflammatory cell infiltrates			1					
Liver, increased periportal glycogen*		1						
--increased hepatocellular glycogen			1		1			
LN, axillary, sinus histiocytes			1					
--sinus erythrocytes				2				
LN, Mesenteric, sinus erythrocytes*	1	1	3	2	1	1	2	1
Lungs, capsular fibrosis			1					
--alveolar inflammatory cell/protein exudate		1		1				
--vascular congestion		1					1	
--alveolar macrophages							2	1
--chronic inflammatory cell infiltrates			1					2
--inflammation*			1					2
--subpleural fibrosis/fibroplasia*	1			1		1		1
Ovaries, no corpora lutea						1	1	1
Skin, chronic folliculitis			1					
--parakeratosis/scab			1					
--epidermal atrophy				3				3
Spleen, siderofibrosis		1						
--red pulp, congestion								1
Sternum, increased cellularity							1	
Submandibular salivary gland, inflammatory cell infiltrates*				1		1		
Testes, seminiferous tubule atrophy		1		2				
Thymus, decreased lymphocytes*	1	1	1	1	1	1	1	1
--lymph node sinus erythrocytes				1				
Thyroid, agonal fat hemorrhage			1					
--lymphocytic thyroiditis*		2			1		1	
Tongue, epidermal atrophy			1	3			2	3
Urinary bladder, vascular congestion				1				1

*denotes increasing severity with dose

Special Evaluation

The Sponsor evaluated the effect of AZD9291 on serum insulin and glucose. Consistent with its known inhibition of the insulin receptor, increased insulin blood levels were observed. On Day 28 (and to a lesser extent, on Day 1), AZD9291 increased serum insulin in males and females, but had little effect on the concentration of circulating glucose.

Figure 69: Effects of AZD9291 on Insulin Levels on Study Day 1 in the Male Dog

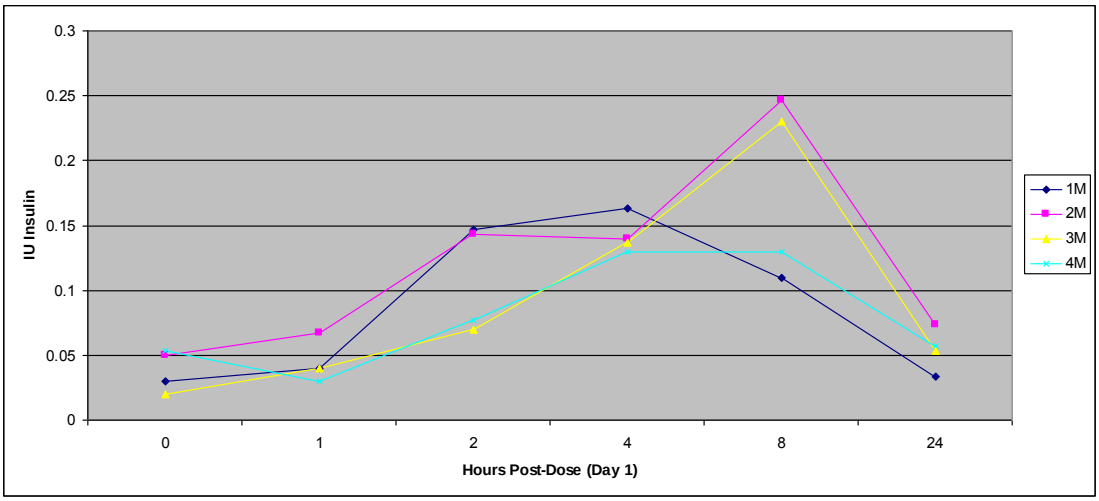


Figure 70: Effects of AZD9291 on Insulin Levels on Study Day 1 in the Female Dog

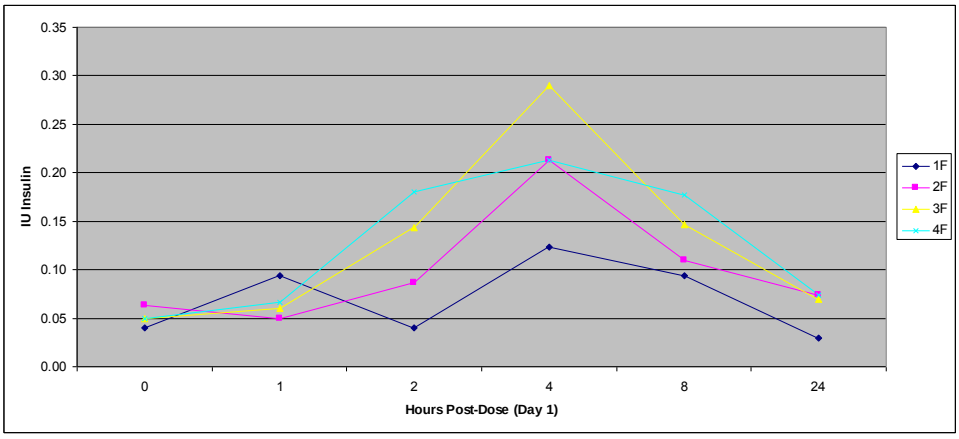


Figure 71: Effects of AZD9291 on Insulin Levels on Study Day 28 in the Male Dog

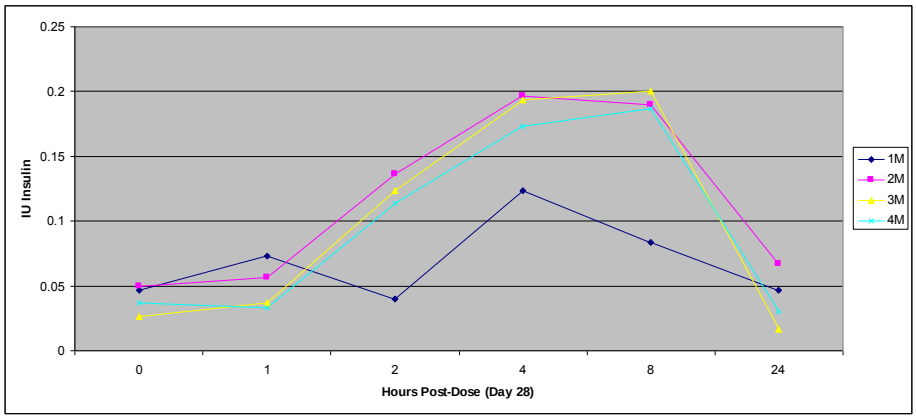


Figure 72: Effects of AZD9291 on Insulin Levels on Study Day 28 in the Female Dog

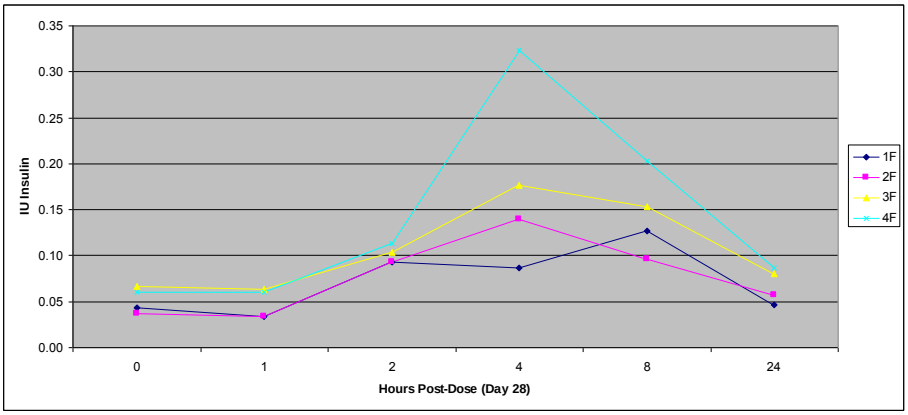


Figure 73: Effect of AZD9291 on Plasma Glucose in Male Dogs (Day 1)

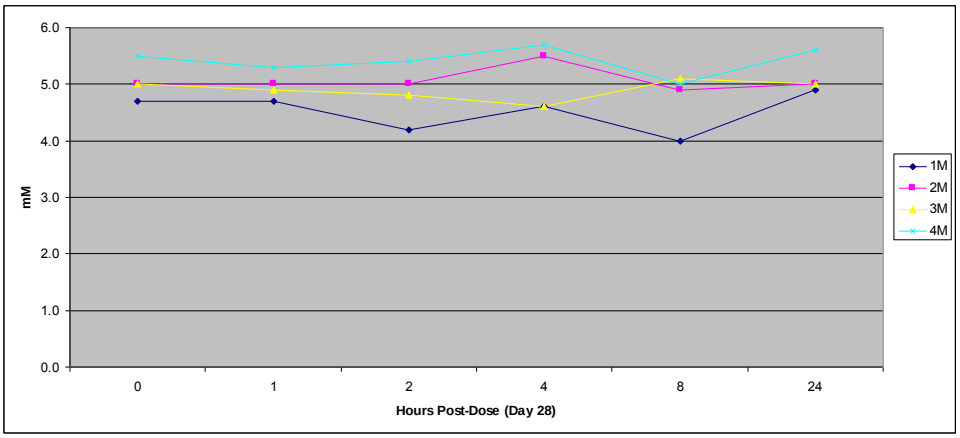


Figure 74: Effect of AZD9291 on Plasma Glucose in Female Dogs (Day 1)

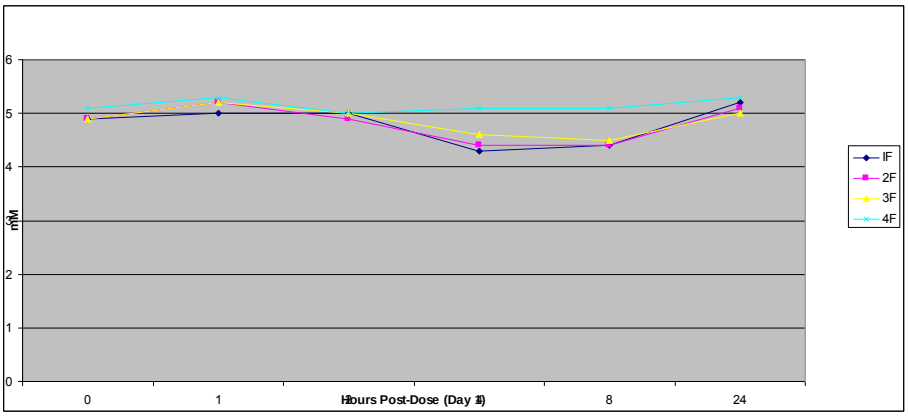


Figure 75: Effect of AZD9291 on Plasma Glucose in Male Dogs (Day 28)

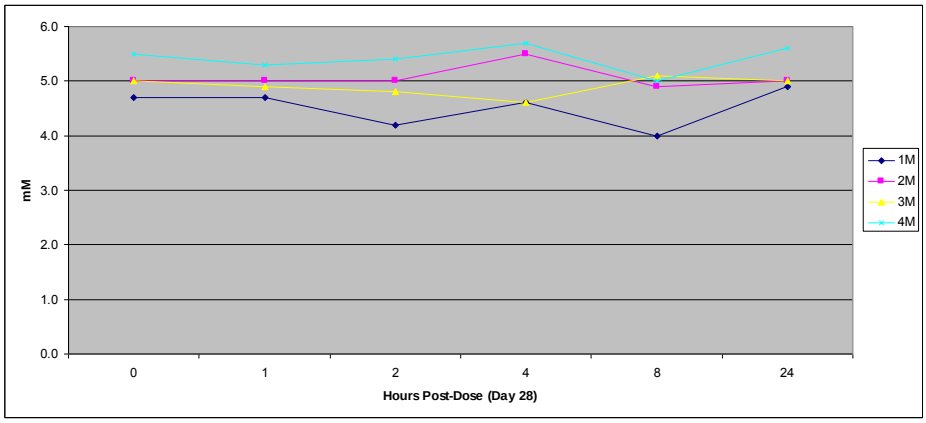
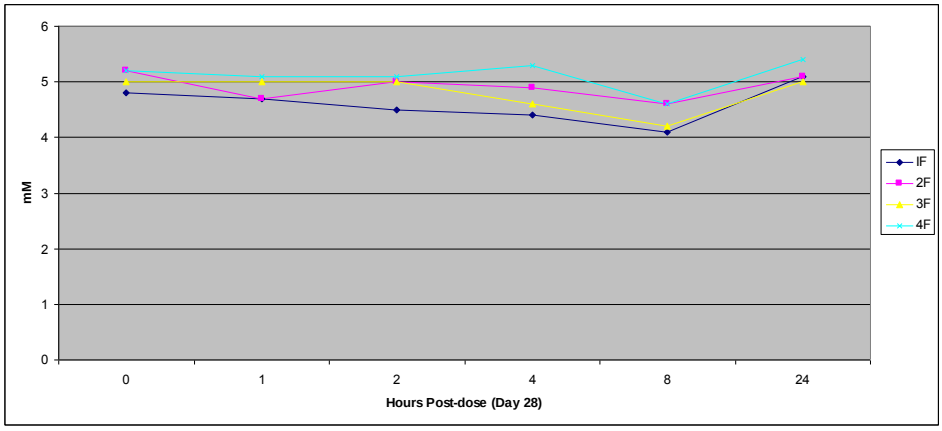


Figure 76: Effect of AZD9291 on Plasma Glucose in Female Dogs (Day 28)



Toxicokinetics

Exposures (peak and AUC) in the dog were essentially linear with dose over the dose range evaluated on Day 1 (Sponsor-Table 92). There was no consistent evidence for accumulation, and no gender-effect on exposure.

Due to ongoing toxicity, a dosing holiday was given to males and females in the high dose groups (20 mg/kg/Day) between Days 8-10, after which, dosing was resumed at 12 mg/kg/Day on Day 11. Peak and overall (AUC) exposures to AZ13597550 were approximately proportional with dose, and overall mean exposure to AZ12597550 was approximately 15-20% of parent (Sponsor-Table 93).

Table 92: Summary AZD9291 TK Parameters in the Dog Following Administration of AZD9291

Study Day	Dose (mg/kg)	Gender	T_{max} Range (Hours)	C_{max} (nmol/L) Mean	S.D.	$AUC_{(0-24)}$ (nmol*h/L) Mean	S.D.	n
1	2	3M + 3F	2-4	179	120	2050	1120	6
	6	3M + 3F	2-4	336	171	3810	1580	6
	20	6M + 6F	2-8	1380	709	16600	7450	12
11	12	6M + 6F	2-4	1310	471	14300	5320	12
15	2	3M + 3F	2-4	225	81.8	2660	928	6
	6	3M + 3F	2-4	632	485	7140	4720	6
	12	6M + 6F	2-4	1340	398	15900	4430	12
28	2	3M + 3F	2-4	230	111	2670	1150	6
	6	3M + 3F	2-4	692	346	7270	3100	6
	12	6M + 6F	2-8	1060	555	12200	6050	12

Table 93: Summary AZ13597550 TK Parameters in the Dog Following Administration of AZD9291

Study Day	Dose (mg/kg)	Gender	T_{max} Range (Hours)	C_{max} (nmol/L) Mean	S.D.	$AUC_{(0-24)}$ (nmol*h/L) Mean	S.D.	n
1	2	3M + 3F	4	31.3	24.1	501	315	6
	6	3M + 3F	4	54.4	34.7	657	410	6
	20	6M + 6F	2-4	245	149	3070	1740	12
11	12	6M + 6F	4	212	94.9	2690	1250	12
15	2	3M + 3F	4	32.2	16.3	399	158	6
	6	3M + 3F	4	97.3	97.4	1260	1320	6
	12	6M + 6F	4-8	208	75.8	2790	999	12
28	2	3M + 3F	4	33.5	17.9	439	183	6
	6	3M + 3F	2-4	115	92.3	1410	1140	6
	12	6M + 6F	4-8	199	116	2450	1380	12

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/s/

SHAWNA L WEIS
10/08/2015

WHITNEY S HELMS
10/08/2015

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 208065 **Applicant: AstraZeneca Pharmaceuticals, LP** **Stamp Date: 05 June 2015**

Drug Name: AZD9291 **NDA Type: Original NDA**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		The formulations differed between animal studies (oral liquid) and that proposed for marketing (an oral capsule); however, the formulations used in animal studies appear to have been adequate to achieve the requisite exposures.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)			Impurity qualification studies have been performed. Whether these are adequate to qualify the relevant impurities or whether they address all of the relevant impurities will be a review issue.
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_____

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

At this time, there are no review issues.

Shawna L. Weis, PhD	16 June 2015
Reviewing Pharmacologist	Date
Whitney S. Helms, PhD	16 June 2015
Team Leader/Supervisor	Date

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

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/s/

SHAWNA L WEIS
06/18/2015

WHITNEY S HELMS
06/19/2015