

CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-399

Pharmacology Review(s)

Division of Oncology Drug Products
Review and Evaluation of Pharmacology and Toxicology Data
Filename 21399 Iressa for Non-Small Cell Lung Cancer.doc
Review number 1

NDA 21-399

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Submitted August 2, 2002

Information to sponsor: YES

Sponsor: AstraZeneca Pharmaceuticals LP.

Reviewer name: W. David McGuinn, Jr., Ph. D., D.A.B.T.
HFD 150

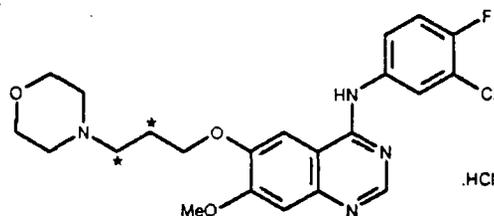
Review completion date: August 16, 2002, edited October 9, 2002

Drug:

Trade name: IRESSA
Generic name: gefitinib
Code name: ZD1839
Chemical name: 4-(3-Chloro-4-fluorophenylamino)-7-methoxy-6-(3-(4-morpholinyl)propoxy) quinazoline
CAS registry number: Not Assigned
Mole file number: None
Molecular formula: C₂₂H₂₄ClFN₄O₃
Molecular weight: 446.91

Structure:

*Denotes the position of radiolabelled carbons in most experiments requiring labeled compound.



Relevant INDs/NDAs/DMFs:

IND [redacted]
IND [redacted]

Drug class: Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor
Indication: Locally advanced or metastatic Non-Small Cell Lung Cancer (NSCLC)

Clinical formulation: Brown round tablet containing ZD1839, 250 mg
Tablet core: Lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, povidone, sodium lauryl sulfate and magnesium stearate.
Coating: Hydroxypropyl methylcellulose, polyethylene glycol 300, titanium dioxide and yellow ferric oxide.

Route of administration:

PO

Dose:

250 mg/kg (about 140 mg/m² or 3.12 mg/kg)

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on Approvability

The toxicology information in this application supports the approval of Iressa for use against locally advanced or metastatic non-small cell lung.

The pharmacology information in the application does not support all of the sponsor's claims for the mechanism of action.

B. Recommendation for Non-clinical Studies

No further toxicology information is necessary to support the indication of locally advanced or metastatic non-small cell lung.

C. Recommendations on Labeling

1) The company has not submitted enough information to establish the following MECHANISM OF ACTION statement:

DRAFT

The pharmacology studies do not establish that gefitinib selectively inhibits EGFR. The evidence demonstrates it inhibits other receptor tyrosine kinases at concentrations similar to those that inhibit EGFR. Neither do the studies establish a biochemical link between the inhibition of EGFR and inhibition of tumor growth.

The pharmacology and toxicology support the following change:

The following wording is by Dr. Morse.

The mechanism of the clinical antitumor action of gefitinib is not adequately characterized. gefitinib inhibits the intracellular phosphorylation of tyrosine kinases which are associated with many transmembrane cell surface receptors.

DRAFT

I propose the following:

2) The sponsor proposes the following wording for the "Pregnancy" section:

DRAFT

The reproductive toxicology studies support the following statement.

IRESSA may cause fetal harm when administered to a pregnant woman. A single dose study in rats showed that Gefitinib crosses the placenta after an oral dose of 5 mg/kg (30 mg/m², about 1/5 the recommended human dose on a mg/m² basis). When pregnant rats treated with 5 mg/kg from the beginning of organogenesis to the end of weaning gave birth, there was a reduction in the number of offspring born alive. This effect was more severe at 20 mg/kg and was accompanied by high neonatal mortality soon after parturition. In this study a dose of 1 mg/kg caused no adverse effects

In rabbits, a dose of 20 mg/kg/day (240 mg/m², about twice the recommended dose in humans on a mg/m² basis) caused reduced fetal weight.

3) In the section, **Carcinogenesis, Mutagenesis, Impairment of Fertility**, the sponsor proposes the following:

Carcinogenicity studies have not been with gefitinib.

Changes:

Gefitinib has been tested for genotoxicity (mutagenicity) in a series of *in vitro* (bacterial mutation, mouse lymphoma, and human lymphocyte) assays and an *in vivo* rat micronucleus test. Under the conditions of these assays gefitinib did not cause genetic damage.

Carcinogenicity studies have not been with gefitinib.

4) Under "Adverse Reactions" the statement about QT prolongation needs information on species and dose. The sponsor's proposed text reads.

Changes:

5) The sponsor proposes the following statement in the "Overdose Section":

However, in phase I clinical trials, a limited number of patients were treated with daily doses of up to 1000 mg. An increase in frequency and severity of some adverse reactions was observed, mainly diarrhea and skin rash. Adverse reactions associated with overdose should be treated symptomatically; in particular, severe diarrhea should be managed appropriately.

The section should read:

Half this dose caused no mortality in mice.

II. Summary of Non-clinical Findings

A. Brief Overview of Non-clinical Findings

Scientists at AstraZeneca selected Gefitinib for its ability to inhibit the epidermal growth factor cell surface receptor (EGFR). They have postulated that the compound will be cytostatic, that it will delay the growth of tumors, and increase the rate of apoptosis. Gefitinib appears to bind competitively at ATP sites.

Mice tolerate single doses of gefitinib as high as 6000 mg/m² (about 40 times the clinical dose) with few signs of clinical toxicity. Twice this dose, 12,000 mg/m², killed four of ten rats by day five. Death was preceded by hunched posture, loss of skin tone, piloerection, subdued behavior, and trembling and shaking. Daily oral doses of 750 mg/m²/day (about five times the clinical dose) killed three of five female rats; 800 mg/m² killed one of three dogs. Across species, chronic dosing at the highest tolerated doses (usually about 2 to 3 times the clinical dose on a mg/m² basis) causes body weight loss, increased platelet and white cell counts (predominantly neutrophils), decreased red cell parameters, increased hepatic enzymes, decreased serum protein, and increased blood urea. Microscopic changes (primarily atrophy and vacuolization) occur in the adrenals, lymph nodes, small intestine, liver, kidney, skin, lungs and thymus. The dose response curve appears to be rather steep but the response of individuals is variable, that is, some individuals appear considerably more sensitive than others. Clinically, diarrhea, asthenia, and acne-form rash are dose-limiting.

Gefitinib caused no genetic damage in the standard battery of tests. Carcinogenicity studies have not been done. In rabbits, a maternally toxic dose of 240 mg/m² caused reduced fetal weights. Gefitinib crosses the placenta and is excreted in milk. It does not appear to cross the blood brain barrier. *In vitro*

studies suggest a potential to inhibit cardiac repolarization and dogs given a single dose of 2000 mg/m² develop bradycardia that persists for at least two half-lives. Non-clinical studies also show that gefitinib has the potential to inhibit important pharmacological sites not involved with its primary mode of action at concentrations that may be achieved clinically. These sites include the monoamine transporter and dopaminergic, adrenergic and serotonin sites.

Oral bioavailability is about 65% in humans and is limited by metabolism. Bioavailability in humans is somewhat higher than in rodents. Metabolism is extensive and is mediated primarily by cytochrome P450 3A4 in humans. At least five metabolites are active. More than 90% of the drug and its metabolites are excreted in the feces. Distribution is rapid and the volume of distribution is considerably greater than total body fluid across species. Across species, 90% or more of the drug is plasma bound.

B. Pharmacological Activity

Epidermal growth factor (EGF) stimulates the growth of epithelial cells, including epidermal cells. Epidermal growth factor receptor (EGFR) was the first receptor protein recognized to be a tyrosine-specific protein kinase. When two EGFR molecules in close proximity bind EGF, they form a dimer. The intracellular components of the dimer phosphorylate each other at multiple sites. Intracellular signaling proteins then bind to the various phosphorylated receptor sites, which in turn phosphorylate the signaling proteins. These phosphorylated proteins initiate a cascade of signals within the cellular cytoskeleton and the nucleus that begins the process of cell division. Inhibition of this process at different points in the cascade can prevent cell division.

Many human solid tumors overexpress EGFR. This overexpression correlates with a poor prognosis. Blockade of the EGFR signal can prevent or delay cell division *in vitro*. AstraZeneca extrapolated these observations to hypothesize that inhibition of EGFR might slow the growth of human tumors. They developed gefitinib by screening for activity against EGFR. Gefitinib probably inhibits tyrosine kinase activity by binding competitively at the ATP site. The compound is an ATP mimetic.

The data does not support the claim that gefitinib is specific for the EGF Receptor. It significantly, inhibits other tyrosine kinase receptor proteins such as vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF). Gefitinib also inhibits numerous other pharmacologically significant sites such as dopamine D₃, muscarinic M₁, adrenergic α_{1d}, muscarinic M₄, tachykinin NK₂, 5-HT_{2b}. The inhibitory constants for these sites range from 1.4 to 4 μM. The trough concentration of gefitinib in humans at steady state is 0.5 μM; C_{max} values are 1 μM or greater.

C. Non-clinical Safety Issues Relevant to Clinical Use

The sponsor does not need to do any further non-clinical studies to support the proposed indication.

III. Administrative

A. Reviewer signature: _____

B. Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

C. cc: list:

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PREVIOUS CLINICAL STUDIES

AstraZeneca began phase I trials of Iressa in 1997. They have completed at least nine trials in healthy male volunteers to determine the tolerability and pharmacokinetics of oral Iressa. AstraZeneca has done at least five clinical pharmacology studies in cancer patients. Numerous other trials in various phases are currently ongoing in patients with advanced malignant disease. In at least seven of these, Iressa is given as singular therapy and in at least five in combination with other cancer drugs. These various trials established that patients tolerated 250 or 500 mg of oral Iressa per day given as continuous therapy with acceptable toxicity, though it has been given at considerably higher doses – a gram or more – in a few of the initial Phase I studies. The most frequent clinical manifestations of toxicity include skin rash and skin disorders, diarrhea, nausea, vomiting, asthenia, anemia, leukopenia, neutropenia and thrombocytopenia.

PREVIOUS REVIEWS

AstraZeneca, then Zeneca Pharmaceuticals, originally submitted information about gefitinib under IND [redacted] Dr. Hua Zheng reviewed much of this information in three reviews:

Safety Review (number 1) December 16, 1997
Original Review (number 2) January 29, 1998
Second Review (sic) (number 3) April 9, 1998

I have included the results of these reviews in my summaries and conclusions, and appended Dr. Zheng's reviews.

**APPEARS THIS WAY
ON ORIGINAL**

PHARMACOLOGY AND TOXICOLOGY REVIEW

Pharmacology:

- 1) O'Brien DP, Nelson LA, Williams JL, Kemp CJ, Erwin CR, Warner BW. Selective inhibition of the epidermal growth factor receptor impairs intestinal adaptation after small bowel resection. *J Surg Res* 2002 Jun 1;105(1):25-30.

DP O'Brien *et al.* (*J Surg Res* 2002 Jun 1;105(1):25-30) tested the hypothesis that EGFR signaling is required for post-resection intestinal adaptation. They did a 50% small bowel resection (SBR) or sham surgery on mice (C57B1/6, n = 26). They then dosed the mice orogastrically with ZD1839 (50 mg/kg/day) or vehicle. After 3 days, they killed the mice and assessed indices of adaptation (wet weight, crypt depth, and villus height) and apoptotic index (number of apoptotic bodies per crypt) in the ileum. They measured the expression of proliferating cell nuclear antigen (PCNA) and activated EGFR by Western blotting. They found that ZD1839 prevented EGFR activation and the normal post-resection increases in ileal wet weight, villus height, and crypt depth. ZD1839 reduced the enterocyte proliferation two-fold in the SBR group. Although the difference did not reach statistical significance, rates of enterocyte apoptosis were the highest in the inhibitor-treated mice. Thus after SBR, pharmacological inhibition of the EGFR attenuates proliferation and the normal adaptive response of the intestine. These results suggest that EGFR is a mediator of the post-resection adaptation response.

- 2) Magne N, Fischel J, Dubreuil A, Formento P, Poupon M, Laurent-Puig P, Milano G. Influence of epidermal growth factor receptor (EGFR), p53 and intrinsic MAP kinase pathway status of tumour cells on the antiproliferative effect of ZD1839 ("Iressa"). *Br J Cancer* 2002 May 6;86(9):1518-23

Permanent downstream activation of the mitogen-activated protein kinase pathway can theoretically bypass the upstream block of epidermal growth factor receptor-dependent mitogen-activated protein kinase activation at the epidermal growth factor receptor level. These investigators studied the effect of epidermal growth factor receptor content, p53 status and mitogen-activated protein kinase signaling status on ZD1839 sensitivity in a panel of human tumor cell lines. These included seven head and neck cancer cell lines and two colon cancer cell lines (LoVo, HT29) with derivatives differing only by a specific modification in p53 status. They evaluated the anti-proliferative activity of ZD1839 by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide test. ZD1839 concentrations ranged from 0.2-200 μ M (48-h exposure). Epidermal growth factor receptor expression, p53 status and p42/p44 (for testing a constitutively active mitogen-activated protein kinase pathway status) were determined by competition analysis (Scatchard plots), denaturing gradient cell electrophoresis and Western blot, respectively. Epidermal growth factor receptor levels ranged from 388 to 33794 fmol/mg-protein, a range that is similar to that found in head and neck tumors. The IC_{50} values for cell sensitivity to ZD1839 ranged from 6 to 31 μ M. Epidermal growth factor receptor concentration (expression) correlated inversely with IC_{50} ($P = 0.022$, $r = 0.82$). The expression of p53 in a cell line did not correlate with the sensitivity to ZD1839. In two head-and-neck cancer cell lines, with comparably elevated epidermal growth factor receptor expression, the ZD1839 IC_{50} value was two-fold higher in the one with a constitutively active mitogen-activated protein kinase. Thus, ZD1839 was active against cells with a range of epidermal growth factor receptor levels, although more so in cells with higher epidermal growth factor receptor expression. Activity was unaffected by p53 status, but was reduced in cells with an intrinsically activated mitogen-activated protein kinase pathway.

- 3) Ciardiello F, et al. ZD1839 (IRESSA), an EGFR-selective tyrosine kinase inhibitor, enhances taxane activity in bcl-2 overexpressing, multidrug-resistant MCF-7 ADR human breast cancer cells. *Int J Cancer* 2002 Mar 20;98(3):463-9.

Bcl-2 over-expression increases the potential for doxorubicin-resistant, estrogen-independent, MCF-7 ADR human breast cancer cells to form tumors *in vivo*. These investigators measured the sensitivity of two bcl-2-overexpressing MCF-7 ADR clones and control neomycin-transfected MCF-7 ADR-neo cells to three taxanes, paclitaxel, docetaxel and IDN 5109. The two bcl-2-overexpressing MCF-7 ADR clones were relatively resistant to all three taxanes, whereas the MCF-7 ADR neo cells were relatively resistant to paclitaxel and docetaxel, but sensitive to IDN 5109. They found that both MCF-7 ADR neo and bcl-2-overexpressing MCF-7 ADR clones express high levels of the epidermal growth factor receptor (EGFR) and its ligand, transforming growth factor-alpha (TGF-alpha). So they then tested the growth inhibitory effect of ZD1839 on these same clonal lines. Inhibition of cell growth on soft agar by ZD1839 increased with dose in all 3 clones (IC_{50} about 0.1 μ M). Growth inhibition was coincident with dose-dependent inhibition of EGFR tyrosine autophosphorylation and of the production of TGF-alpha, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). To determine whether the blockade of EGFR signaling might affect the sensitivity of bcl-2-overexpressing MCF-7 ADR cells to taxanes, they treated cells with ZD1839 and with paclitaxel, docetaxel or IDN 5109. They observed a dose-dependent increase in growth inhibition and increased apoptosis. Combined treatment with IDN 5109 and ZD1839 also caused a significant inhibition of bcl-2 expression in bcl-2-overexpressing MCF-7 ADR cells. Thus *in vitro* treatment with ZD1839 appears to overcome taxane resistance in a model of hormone-independent, multidrug-resistant, human breast cancer. I cannot determine the nature of this effect because the experiment lacks a ZD1839 control. The experiments do demonstrate a ZD1839 dose response.

- 4) Anderson NG, Ahmad T, Chan K, Dobson R, Bundred NJ. ZD1839 (Iressa), a novel epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, potently inhibits the growth of EGFR-positive cancer cell lines with or without erbB2 overexpression. *Int J Cancer* 2001 Dec 15;94(6):774-82.

These investigators designed their experiments to correlate EGFR over-expression with ZD1839 potency. They used EGFR-over-expressing cell lines, A431 and MDA-MB-231, *in vitro* and found IC_{50} values in the nanomolar range. They found that ZD1839 blocked autophosphorylation of EGFR and prevented activation of PLC-gamma 1, ERK MAP kinases and PKB/Akt by EGF. It also inhibited proliferation of EGFR(+) cancer cell lines over-expressing erbB2 (SKBr3, SKOV3, BT474) by between 20% and 80%, effects which correlated with inhibition of EGF-dependent erbB2 phosphorylation and activation of ERK MAP kinase and PKB/Akt in SKOV3 cells. Oral administration of ZD1839 inhibited the growth of MDA-MB-231 and SKOV3 tumor xenografts in athymic mice, by 71% and 32%, respectively. Growth inhibition coincided with reduced proliferation but no change in apoptotic index. Collectively, these results show that ZD1839, at the doses studied, inhibits the proliferation of cells overexpressing EGFR and also EGFR(+) cells that overexpress erbB2.

Safety Pharmacology:

- 1) Evaluation of effect on cardiac action potential in isolated canine Purkinje fibers. —
 Study No. 20010256 Submitted to IND [redacted] November
 EDR submission 21399 RRZ 003, November 30, 2001, Module4/pharm/tsd1212.pdf.

Animal cardiac tissue from three male beagle dogs
 Drug ZD1839, Batch ADM60517H99
 Preparation 12 preparations of Purkinje fibers (6 with ZD1839 and 6 with vehicle) perfused sequentially as follows:

Bath

First Group (n=6)	Second Group (n=6)
<ul style="list-style-type: none"> . Tyrode, . 0.1% ethanol in Tyrode (sequence 1), . 0.1% ethanol in Tyrode (sequence 2), . 0.1% ethanol in Tyrode (sequence 3), . 0.1% ethanol in Tyrode (sequence 4), . Tyrode, . Cisapride, $3 \times 10^{-7}M$. 	<ul style="list-style-type: none"> . Tyrode, . 0.1% ethanol in Tyrode, . ZD1839 at a concentration of $0.25 \times 10^{-6}M$, . ZD1839 at a concentration of $2.5 \times 10^{-6}M$, . ZD1839 at a concentration of $10 \times 10^{-6}M$, . Tyrode, . Cisapride, $3 \times 10^{-7}M$.

Investigators at the _____ did this study for AstraZeneca. The investigators dissected free-running Purkinje fibers from both ventricles of the heart. They mounted these fibers in a bath irrigated with oxygenated Tyrode's solution (36.5 + 0.5°C). They inserted electrodes in the fibers and stimulated steady pulses (2 ms duration, about 2 Volts amplitude, 1 Hz). After an hour of stabilization, the Purkinje fibers were impaled with glass microelectrodes to record the action potential. The authors established a stable baseline then perfused continuously with control solution or ZD1839 according to the table above. The concentration of ZD1839 increased sequentially and each concentration was perfused for 30 minutes. They recorded action potentials every five minutes. Lastly they added the positive control, Cisapride, and recorded action potential changes. Recorded action potential parameters included amplitude (APA), resting potential (RE), maximal rate of depolarization (V_{max} i.e. d^2V/dt^2 max) and action potential duration (APD50, APD70 and APD90 i.e. 50, 70 and 90% return to resting potential).

Under these conditions, the vehicle, 0.1% ethanol in Tyrode caused no statistically significant effect on action potential parameters. Likewise the lowest concentration ZD1839 (0.25 µM) caused no statistically significant changes in the action potential.

At concentrations of 2.5 µM and 10 µM, perfusion of ZD1839 caused an increase in APD70 (6 and 20 ms respectively) and APD90 (9 and 27 ms respectively). These change were not statistically significant for the mid-dose using Newmann-Keuls ANOVA but the dose effect is evident and the authors considered the mid-dose a threshold concentration for effects on action potential duration. The effect of ZD1839 was not reversible during the washout period (30 min) and did not appear to have reached a maximum. Higher concentrations would probably have shown still greater changes. Treatment did not cause early after-depolarization at any concentration. The positive control caused a consistent increase in the duration of the action potential consistent with its typical effects.

The sponsor chose the concentrations used in this experiment because they are clinically relevant. The clinical dose in man is 250 to 500 mg. In human studies, C_{max} after a 500 mg PO dose is about 1200 ng/ml (2.7 µM, the authors did not cite the study from which this information was derived). ZD1839 is approximately 91% bound to proteins in humans with an estimated free concentration at therapeutic levels of 0.24 µM. Thus, the concentration range in this study spans about 40 times the free concentration achieved man at therapeutic doses. Concentrations of ZD1839 higher than 10 µM were not

used because they are cytotoxic *in vitro*. Thus ZD1839 is toxic to cells of the His-Purkinje bundle at concentrations that may be clinically relevant. This toxicity is not reversible over a course of 30 minutes.

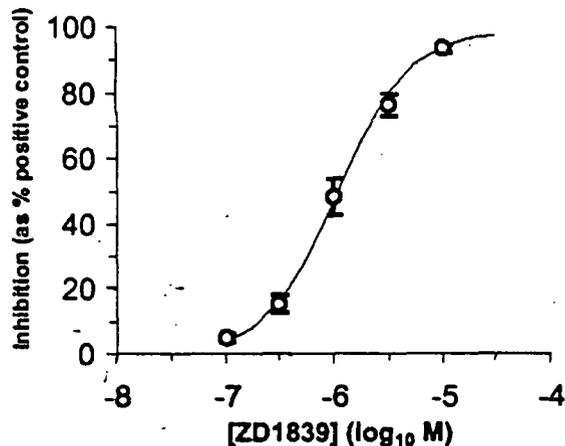
2) The Effects of Test Substances on the Activity of the Voltage-Dependent Potassium Channel Encoded by The Human Ether-A-Go-Go-Related Gene (hERG). Study No: TSZ41. EDR submission RRZ 003, November 30, 2001, Module4/pharm/tsz41.pdf.

Cells	Human embryonic kidney (HEK) cells expressing hERG
Drug	ZD1839, batch not specified
Tested concentrations	0, 0.1, 0.316, 1, 3.16, 10 μ M
Buffer	10 mM HEPS
Culture medium	MEM (Eagle) media with Earles salts with 10% Foetal Calf Serum , 0.4 mg/ml geneticin and 10% Ml supplement
Negative control	1% DMSO
Positive control	3.16 X 10 ⁻⁶ M AR-C155039XX (dofetilide)

Investigators at AstraZeneca UK Limited, Cheshire, England did this *in vitro* study non-GLP study.

The aim of the study was to evaluate the effects of ZD1839 on the voltage-dependent potassium channel encoded by the human ether-a-go-go-related gene, hERG. Human embryonic kidney cells express this gene as an active ion channel. Cardiomyocytes express this ion channel as the slow potassium rectifier, I_{Ks}, a primary channel of cardiac repolarization. Inhibition of this channel causes delayed repolarization, QT_c prolongation, and in severe cases, arrhythmias leading to Torsade-du-pointes. Thus, this test predicts cardiac toxicity.

The kidney cells were clamped at -80 mV then depolarized to 50 mV for 1000 ms at 15 second intervals. Electrodes measured the repolarization current as a function of time. For each cell recorded, data obtained in the presence of ZD1839 were expressed as a percentage of the inhibition produced by the positive control. The following graph shows the percent inhibition as a function of increasing ZD1839 concentration.



The following table shows the parameters determined from this curve.

Parameter	Explanation	Value	SEM
α	The % inhibition value at the asymptote of the concentration-effect curve	98.5	1.6
m	the slope of the concentration-effect curve at its mid-point	1.35	0.12
pIC_{50}	the negative \log_{10} of the molar concentration of test compound producing 50% inhibition of the hERG tail current	5.96	0.08
β	The baseline value for the concentration-effect curve (usually set to zero)	0	0

Thus ZD1839 causes half-maximal inhibition of this important cardiac ion channel at a concentration of 1 μ M, a concentration 2 to 10 times lower than the concentration that caused action APD70 prolongation in dog cardiac cells. Inhibition approaches 100% at 10 μ M. Physiological concentrations in humans range from about 0.5 to 1.0 μ M with chronic dosing.

- 3) Evaluation of effects on blood pressure, heart rate and electrocardiogram after a single oral dosing in male conscious dogs. Study number TZD1211, EDR submission RRZ 003, November 30, 2001, Module4/pharm/tzd1211.pdf.

Animal Six male beagle dogs weighing between 10.8 kg and 14.5 kg
 Drug ZDI839 batch No. 150000635
 Vehicle 0.5% w/s hydroxypropylmethylcellulose and 0.1% w/v polysorbate 80.
 Apparatus — computerized acquisition system by —
 Telemetric transmitters

Sequential doses:

- 1) Vehicle
- 2) ZD1839 at a dose of 5 mg/kg (200 mg/m²)
- 3) ZD1839 at a dose of 50 mg/kg (2000 mg/m²)

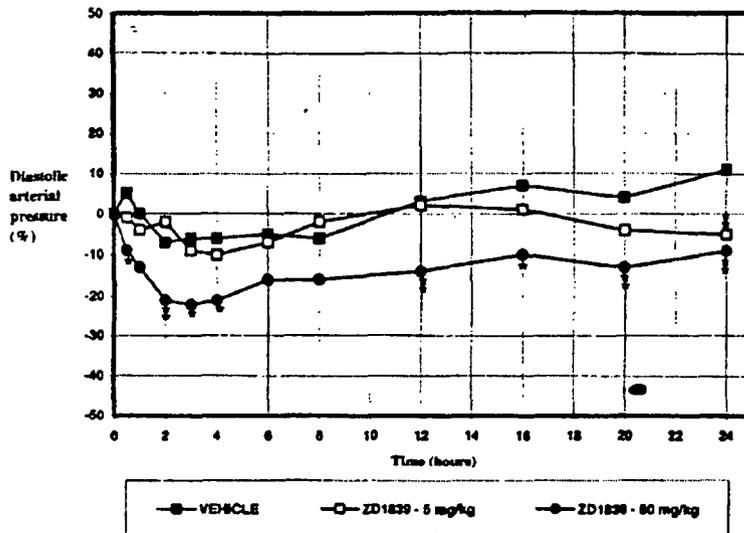
Schedule Vehicle and each dose of ZD1839 were given at least 3 days apart.
 Route PO gavage, 10 ml/kg

Investigators at the _____ did this study for AstraZeneca.

Before dosing the investigators implanted the telemetric transmitter in the peritoneal cavity under thiopental Anastasia. They placed the electrodes in the Lead II configuration. They placed the sensor catheter of transmitters via the femoral artery. They took telemetric measurements of blood pressure, heart rate and electrocardiogram (Lead II) started at least 24 hours before the administration and continued for 48 hours after dosing.

Vehicle and the 5 mg/kg dose caused no biologically significant changes in arterial blood pressure, heart rate or cardiac conduction times.

At a dose of 50 mg/kg, ZD1839 caused hypotension; the diastolic pressure decreased about 18% at three hours post dosing and recovering slowly over the next 21 hours. The following graph shows this decrease.



%: effect expressed as percentage of variation calculated in relation to predose values.

Vehicle: 0.5% hydroxypropylmethylcellulose and 0.1% polysorbate 80.

*: $P \leq 0.05$, **: $P \leq 0.01$, when compared with the control group dosed with the vehicle; analysis of variance for repeated measurements with NEWMAN-KEULS test if $P \leq 0.05$.

Systolic pressure followed a similar pattern but the changes were not as severe. These results strongly suggest a dose-related phenomenon; an intermediate dose of 25 mg/kg would have been most informative.

All the dogs had increased heart rates immediately post-dosing. Such a result is not unusual after a procedure that involves significant handling. Nevertheless, the tachycardia was more severe (about 20% above baseline) in both groups of dosed dogs compared to controls (about 10% above baseline). The low dose and control dogs rebounded to bradycardia (about 12% below baseline) within two hours, but the high dose group did not rebound to this level until about 4 hours post-dosing. This effect was probably dose related, but it did not reach the level of statistical significance (Newmann-Keuls ANOVA).

The combined data did not show a statistically significant changes in T-wave morphology at either 5 or 50 mg/kg. But, at 2 hours post-dose, 1 out of 6 dogs at 5 mg/kg and 2 out of 6 dogs at 50 mg/kg showed greater than a 10% QTc prolongation in comparison to baseline values.

The sponsor chose the doses of ZD1839 claiming that the high dose was about ten times greater than the clinical dose. It is actually about 7 times higher on a mg/m^2 basis. In an acute oral study in dogs (AstraZeneca Reference TLD/909) single doses of 25 and 50 mg/kg were well tolerated. Higher doses of 100 mg/kg and above caused emesis, soft/loose/fluid feces, some loss of skin tone, subdued behavior and transient loss or reduction of appetite. Nevertheless, doses of 25 or 75 mg/kg might have added significantly to this experiment.

This study confirms that ZD1839 does have measurable effects on heart rate and blood pressure at doses less than one order of magnitude above the clinical dose. Nevertheless, pharmacokinetic data from dogs given doses of 50 mg/kg of ZD1839 suggest that these effects occur at plasma concentrations significantly greater than those encountered clinically.

- 4) A study of pharmacological receptor binding cross-reactivity. Study number TSY/276. EDR submission RRZ 003, November 30, 2001, Module4/pharm/tsy276.pdf

Investigators at _____ did this study for AstraZenaca. The studies are standard *in vitro* studies to determine the reactivity of ZD1839 at numerous well-characterized pharmacological enzyme or receptor sites. The methods section is sparse and consists mostly of references to standard assays. The array of assays is comprehensive. The investigators offered no interpretation of the data nor did they offer any conclusions. I have inserted the investigator's tables of significant results. They show that ZD1839 interacts with numerous pharmacological sites at physiological concentrations. IRESSA may inhibit epidermal growth factor but that does not appear to be the limit of its pharmacological activity.

PRIMARY TESTS							
PRIMARY							
CAT. #	BIOCHEMICAL ASSAY	SPECIES	CONC.	% INH.	IC₅₀^a	K_i	B₂₁
106000	Acyl CoA-Cholesterol Acyltransferase.						
	Intestine		100 µM	67	49.3 µM		
132000	Leukotriene C ₄ Synthetase		100 µM	52	97.6 µM		
156000	Phosphodiesterase PDE5		100 µM	71	17.5 µM		
156100	Phosphodiesterase PDE6		100 µM	65	43.9 µM		
168000	Protein Kinase, Ca ²⁺ /Calmodulin-Dep. PK II		100 µM	57	19.9 µM		
172000	Protein Kinase, fyn (p59 ^{tyr}) Tyrosine Kinase		10 µM	50			
176000	Protein Kinase, lck (p56 ^{lck}) Tyrosine Kinase		10 µM	52	7.87 µM		
177000	Protein Kinase PKA, Non-Selective		100 µM	50	36.5 µM		
194000	Thromboxane Synthetase		100 µM	66	38.6 µM		
203400	Adrenergic α ₁₀		10 µM	92	1.36 µM	0.667 µM	0.981
204010	Adrenergic β ₁		10 µM	56	6.18 µM	3.57 µM	0.561
204410	Adrenergic, Norepinephrine Transporter		10 µM	52			
219800	Dopamine D ₃		10 µM	78	0.814 µM	0.046 µM	0.465
220100	Dopamine D ₄		10 µM	69	3.39 µM	1.36 µM	0.656
220320	Dopamine Transporter		10 µM	64	5.33 µM	4.23 µM	0.975
252000	Monoamine Transporter		10 µM	84	1.44 µM	1.32 µM	0.979
252600	Muscarinic M ₁		10 µM	90	2.45 µM	0.59 µM	2.32
252900	Muscarinic M ₄		10 µM	70	6.31 µM	0.881 µM	1.82

^a A standard error of the mean is presented where results are based on multiple, independent determinations.

PRIMARY							
CAT. #	BIOCHEMICAL ASSAY	SPECIES	CONC.	% INH.	IC ₅₀ *	K _i	n _H
254000	Muscarinic, Non-Selective		10 μM	73	4.4 μM	1.45 μM	1.19
260410	Opiate μ		10 μM	63			
271210	Serotonin 5-HT _{1B}		10 μM	59	6.16 μM	3.77 μM	0.718
271700	Serotonin 5-HT _{2B}		10 μM	59	3.75 μM	2.39 μM	0.583
278200	Sigma α ₂		10 μM	59	6.83 μM	4.21 μM	0.489
278300	Sigma, Non-Selective		10 μM	73	3.66 μM	3.55 μM	1.08
279500	Sodium Channel, Site 2		10 μM	71	3.35 μM	3 μM	0.472
255600	Tachykinin NK ₁		10 μM	63	3.62 μM	1.21 μM	0.906

ABOVE PRIMARY TESTS IN RANK ORDER OF POTENCY

PRIMARY							
CAT. #	ENZYME ASSAY	SPECIES	CONC.	% INH.	IC ₅₀ *	K _i	n _H
176000	Protein Kinase, Ick (p56 ^{lck}) Tyrosine Kinase		10 μM	52	7.87 μM		
156000	Phosphodiesterase PDE5		100 μM	71	17.5 μM		
168000	Protein Kinase, Ca ²⁺ /Calmodulin-Dep. PK II		100 μM	57	19.9 μM		
177000	Protein Kinase PKA, Non-Selective		100 μM	50	36.5 μM		
194000	Thromboxane Synthetase		100 μM	66	38.6 μM		
156100	Phosphodiesterase PDE6		100 μM	65	45.9 μM		
106000	Acyl CoA-Cholesterol Acyltransferase, Intestine		100 μM	67	49.3 μM		
132000	Leukotriene C ₄ Synthetase		100 μM	52	97.6 μM		

PRIMARY							
CAT. #	RADIOLIGAND ASSAY	SPECIES	CONC.	% INH.	IC ₅₀ *	K _i	n _H
219800	Dopamine D ₂		10 μM	78	0.814 μM	0.046 μM	0.465
252600	Muscarinic M ₁		10 μM	90	2.45 μM	0.59 μM	2.32
203400	Adrenergic α _{1D}		10 μM	92	1.36 μM	0.667 μM	0.981
252900	Muscarinic M ₄		10 μM	70	6.31 μM	0.881 μM	1.82
255600	Tachykinin NK ₁		10 μM	63	3.62 μM	1.21 μM	0.906

*A standard error of the mean is presented where results are based on multiple, independent determinations.

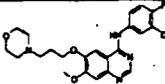
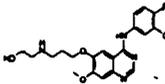
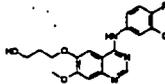
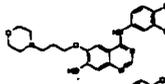
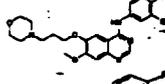
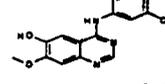
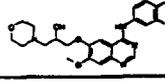
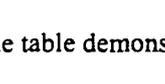
ABOVE PRIMARY TESTS IN RANK ORDER OF POTENCY							
PRIMARY							
CAT. #	RADIOLIGAND ASSAY	SPECIES	CONC.	% INH.	IC ₅₀ ^a	K _i	n _H
252000	Monoamine Transporter		10 μM	84	1.44 μM	1.32 μM	0.979
220100	Dopamine D ₂		10 μM	69	3.39 μM	1.36 μM	0.656
254000	Muscarinic, Non-Selective		10 μM	73	4.4 μM	1.45 μM	1.19
271700	Serotonin 5-HT _{2B}		10 μM	59	3.75 μM	2.39 μM	0.583
279500	Sodium Channel, Site 2		10 μM	71	3.35 μM	3 μM	0.472
278300	Sigma, Non-Selective		10 μM	73	3.66 μM	3.55 μM	1.08
204010	Adrenergic β ₁		10 μM	56	6.18 μM	3.57 μM	0.561
271210	Serotonin 5-HT _{1A}		10 μM	59	6.16 μM	3.77 μM	0.718
278200	Sigma σ ₂		10 μM	59	6.83 μM	4.21 μM	0.489
220320	Dopamine Transporter		10 μM	64	5.33 μM	4.23 μM	0.975

In patients given 250 mg of IRESSA per day the steady state trough concentration of the drug is about 0.5 μM. Peak concentrations are 1 μM or greater. Thus, clinical concentrations rise well with in the range of k_i for many of these pharmacological sites.

Reviewed in summary only:

The sponsor submitted the following table in their integrated pharmacology and toxicology summary. They did not provide a reference for this study and I have been unable to find the study in the sponsor's submissions.

Table 2.9 ZD1839 metabolite pharmacology

	MW	M No.	<i>In vitro</i> kinase assay				Cell assay	
			EGFR	erbB2	KDR	FTKR	EGF stim	Basal
	447	ZD1839	0.027**	>1.2	>1.2	>33	0.054*	8.8*
		M305956	0.033*					
	421	M537194	0.011	>1.2	>1.2	>11	0.375	5.51
	378	M527301	0.037	>1.2	>0.4	>33	0.123	12.2
	433	M523595	0.036	>3.7	>33	>33	0.73	15.1
	445	M387783	0.199	>3.7	>1.2	>33	0.788	19.91
	320	M295820	<0.005	>11	>1.2	>33	0.231	23
	463	M301361	0.035	>3.7	>1.2	>33	0.161	10.0

The table demonstrates that at least five of the major metabolites are active.

Safety Pharmacology Summary

Clinically relevant concentrations of ZD1839 range from 0.5 to 1.0 μM or greater. At concentrations only a few fold greater, 2.5 and 10 μM , ZD1839 caused significant dose dependant prolongation of the Purkinje action potential *in vitro*. This represents a delay in Purkinje repolarization of as much as 10 ms at the lower dose. This effect was not reversible after a 30-minute washout period and did not appear to have reached a maximum at the 10- μM concentration.

ZD1839 caused half-maximal inhibition of a voltage-dependent potassium channel, the slow potassium rectifier known as IKs, at a concentration of about 1 μM *in vitro*. Cardiomyocytes express this channel in high copy number. Inhibition of this channel causes delayed repolarization, QTc prolongation, and in severe cases, arrhythmias leading to Torsade-du-pointes. At a concentration of 10 μM , inhibition was almost 100%.

In vivo in dogs, at a dose of 50 mg/kg, ZD1839 caused hypotension; the diastolic pressure decreased about 18% at three hours post dosing and recovering slowly over the next 21 hours. Systolic pressure followed a similar pattern but the changes were not as severe. The pressure drop caused a transient compensatory tachycardia (about 20% increase) followed by prolonged bradycardia. This toxicity is probably dose dependant. The combined data did not show a statistically significant change in T-wave morphology at either 5 or 50 mg/kg. But, at 2 hours post-dose, 1 out of 6 dogs at 5 mg/kg and 2 out of 6 dogs at 50 mg/kg showed greater than a 10% QTc prolongation in comparison to baseline values. Nevertheless, pharmacokinetic data from dogs given doses of 50 mg/kg of ZD1839 suggest that these effects occur at plasma concentrations significantly greater than those encountered clinically.

Thus, ZD1839 has measurable effects on heart rate and blood pressure at doses less than one order of magnitude above the clinical dose. This toxicity is probably related to direct interference with the slow potassium channel (IKr), to interference with cellular metabolism or interference with expression.

ZD1839 causes significant inhibition of numerous pharmacologically important receptor sites. The mechanism of this inhibition has yet to be studied but is possibly related to kinase activity, direct interaction with tyrosine sites or direct interaction with phosphate binding sites. Clinical concentrations of ZD1839 rise well within the range of k_i for many of these pharmacological sites.

The following table shows but a few of these interactions.

ABOVE PRIMARY TESTS IN RANK ORDER OF POTENCY							
PRIMARY							
CAT. #	RADIOLIGAND ASSAY	SPECIES	CONC.	% INH.	IC ₅₀ ^a	K _i	α_H
252000	Monoamine Transporter		10 μM	84	1.44 μM	1.32 μM	0.979
220100	Dopamine D ₂		10 μM	69	3.39 μM	1.36 μM	0.656
254000	Muscarinic, Non-Selective		10 μM	73	4.4 μM	1.45 μM	1.19
271700	Serotonin 5-HT _{2B}		10 μM	59	3.75 μM	2.39 μM	0.583
279500	Sodium Channel, Site 2		10 μM	71	3.35 μM	3 μM	0.472
278300	Sigma, Non-Selective		10 μM	73	3.66 μM	3.55 μM	1.08
204010	Adrenergic β_1		10 μM	56	6.18 μM	3.57 μM	0.561
271210	Serotonin 5-HT _{1A}		10 μM	59	6.16 μM	3.77 μM	0.718
278200	Sigma σ_2		10 μM	59	6.83 μM	4.21 μM	0.489
220320	Dopamine Transporter		10 μM	64	5.33 μM	4.23 μM	0.975

Pharmacokinetics and Toxicokinetics:

- 1) The disposition of ¹⁴C-ZD1839 in the Rat.
Study number ZD1839 KMR007

Animals	Six male and six female Alpk:APfSD rats Aged between 41 and 54 days Weighing between 164 and 210 g,
Drug	[¹⁴ C]-ZD1839 (batch 1R1), specific activity of 44.8 μCi/mg (salt) radiochemical purity in excess of 98 % The compound was labeled at the two most distal carbons of the propoxy group. Vehicle 0.5% hydroxypropyl methylcellulose (HPMC) in 0.1% Polysorbate 80
Dose	5.0 mg/kg (83 μCi/kg) PO 3.4 mg/kg (62 μCi/kg) IV
Schedule	Single dose
Route	IV, three rats per sex PO, three rats per sex
Tissues	Urine, and faeces were collected daily for five days from each animal and stored frozen. An aqueous cage wash was made at each collection time and stored refrigerated. Expired CO ₂ was collected in 25% ethanolamine in isopropoxyethanol for 24 hours after oral dosing. Following the five day collection period the animals were killed by inhalation of halothane (FLUOTHANE, Zeneca Limited) and the carcasses retained for analysis.
Analysis	liquid scintillation counting (LSC),

Investigators at ZENECA Pharmaceuticals, Cheshire, England did this study in 1996. Mr. E. A. Partridge signed the GLP statement.

Results:

APPEARS THIS WAY
ON ORIGINAL

Table 1. D1839-KMR#07. Recovery of radioactivity in excreta following administration of single oral and intravenous doses of [¹⁴C]-ZD1839 to rats

Time after dose (hours)	Intravenous		Oral	
	Male	Female	Male	Female
Urine				
0-24	4.07 ± 0.23	2.92 ± 0.28	3.12 ± 0.75	1.95 ± 0.37
24-48	0.47 ± 0.05	0.50 ± 0.06	0.52 ± 0.19	0.36 ± 0.11
48-120	0.33 ± 0.02	0.31 ± 0.03	0.33 ± 0.15	0.19 ± 0.06
Total	4.87 ± 0.26	3.73 ± 0.34	3.97 ± 1.08	2.50 ± 0.52
Faeces				
0-24	69.27 ± 4.46	68.49 ± 6.17	61.77 ± 13.99	77.84 ± 4.51
24-48	18.79 ± 3.70	24.50 ± 5.68	20.58 ± 6.71	19.73 ± 4.34
48-72	3.99 ± 1.06	3.02 ± 0.33	8.12 ± 5.87	3.37 ± 0.68
72-96	1.34 ± 0.35	1.51 ± 0.43	1.86 ± 1.05	0.50 ± 0.09
96-120	0.80 ± 0.13	0.82 ± 0.20	0.61 ± 0.19	0.68 ± 0.33
Total	94.19 ± 1.11	98.34 ± 1.38	92.93 ± 1.45	102.13 ± 2.06
Cage wash	0.73 ± 0.14	0.55 ± 0.04	1.86 ± 1.11	0.54 ± 0.21
Expired air	NC	NC	1.05 ± 0.25	0.48 ± 0.10
Carcass	5.10 ± 0.95	4.92 ± 0.75	2.51 ± 0.55	2.87 ± 0.88
Total recovery	104.90 ± 1.90	107.54 ± 1.78	102.32 ± 3.30	108.53 ± 2.87

Each value, representing the mean ± SE obtained from three animals, is expressed as a percentage of the dose administered

NC = Not collected

The table above shows that males eliminated slightly more total radioactivity in the urine than females in the first 24 hours after either oral or IV dosing. No other differences between the sexes were clearly distinguishable. Measurements of fecal elimination showed considerable variability. Irrespective of route, less than 5% of the total radioactivity was recovered in the urine; 93% or more was recovered in the feces. After an IV dose, less than 1% was recovered in expired air. Nevertheless, the result suggests that there is some metabolism of the propoxy chain, leading to the release of a small amount of the radiolabel as CO₂.

The majority of the IV dose was recovered in the feces indicating that most of the compound is eliminated in the bile. The recoveries of radioactivity in carcass were 3% and 5% respectively after oral and intravenous dosing.

The investigators did not examine the urine chromatographically because it contained so little radioactivity. Methanol extraction recovered an average of 77% of the 0-24 hour fecal radioactivity. After extraction, the chromatographic profiles from all the rats were similar independent of sex and dose route. The major radiolabelled component extracted from feces co-chromatographed with [¹⁴C]-ZD1839, suggesting that much of the drug is eliminated unchanged. In male rats, this component accounted for

56% and 66% of the extracted radioactivity respectively after intravenous and oral administration. The proportion of ZD1839 excreted unchanged in female rats was approximately 8% higher than in males. A second component, apparently less polar than ZD1839 accounted for between 8% and 15% of total fecal radioactivity. The remainder of the radioactivity was associated with at least six minor components, none of which accounted for more than 8% of the radioactivity.

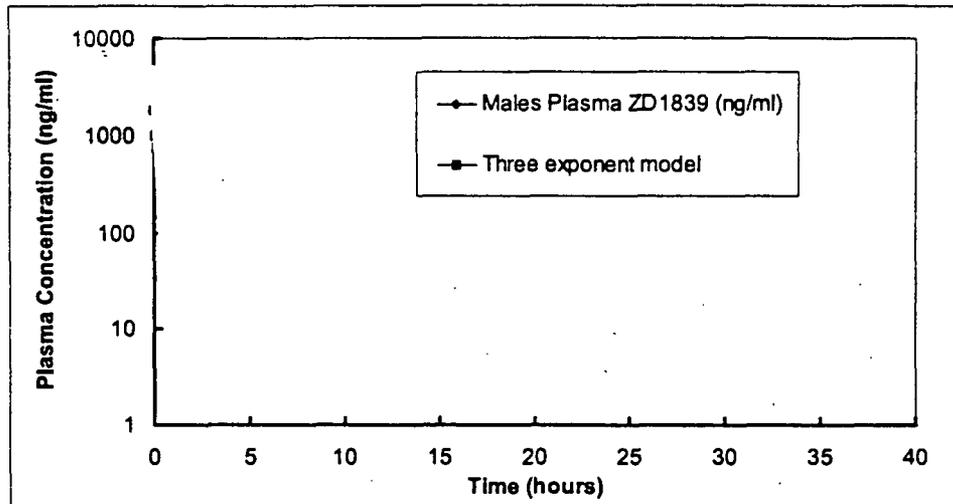
2) The distribution of radioactivity in the blood after oral and intravenous administration of ^{14}C -ZD1839 to rats. Study Number D1839 KKR008.

Animals	One hundred and seventeen Alpk:APfSD rats of each sex, aged between 41 and 62 days weighing between 165 and 293 g,
Drug	^{14}C -ZD1839 (batch 1R1), specific activity of 44.8 mCi/mg (salt) radiochemical purity in excess of 99 % diluted with ZD1839 (ADM 35414H96) to give material (batch 1R1B) with a specific activity of 21.3 mCi/mg (salt), a radiochemical purity in excess of 99 %.
Vehicle	0.5% hydroxypropyl methylcellulose (HPMC) in 0.1% Polysorbate 80 for oral dosing 10% aqueous hydroxypropyl β -cyclodextrin
Dose	5.05 mg/kg (base) actual PO dose, 116 $\mu\text{Ci}/\text{kg}$, Group 1 11.55 mg/kg (base) actual PO dose, 266 $\mu\text{Ci}/\text{kg}$, Group 2 5.00 mg/kg (base) actual IV dose, 115 $\mu\text{Ci}/\text{kg}$, Group 3
N	36 per dose group per sex for PO doses, 45 per sex for IV doses
Route	PO and IV
Schedule	Single dose
Sampling	Blood samples were taken from groups of three animals of each sex at the following times: 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24, 36 and 48 hours after oral dosing and 2, 5, 10, 20 and 40 minutes, 1, 2, 3, 4, 5, 7, 9, 12, 24 and 36 hours after intravenous dosing under anesthesia
Analysis	LSC, —

Investigators at ZENECA Pharmaceuticals, Cheshire, England did this study in 1996. Mr. E. A. Partridge signed the GLP statement.

Results:

After an IV dose of ^{14}C -ZD1839 at 5 mg/kg, the terminal half-lives of the parent compound were 3.2 and 5.3-hours for male and female rats respectively by the investigators calculations using a log-linear estimation. I analyzed the data for male rats using both two and three exponential models. The three-exponential model gave a better fit to the data and suggested that the log-linear calculation underestimated the terminal half-life by about 20% ($t_{1/2\gamma} \sim 6.5$ hours). The following graph shows the data and a curve for the three-exponential model.



Male rats cleared the parent from plasma more rapidly than females (42.0 mL/min/kg vs. 23.6 mL/min/kg respectively). Distribution of ZD1839 was similar in both sexes (9.2 and 9.8 L/kg respectively) and much greater than total body water. The fit of the three-exponential model suggests that the initial short half-life (~ 2 minutes) is due to distribution in the blood. The second half-life (~1.2 hours) is due to distribution to the periphery reflected in the large volume of distribution. This distribution possibly involves non-specific binding. The following table shows that in all measurable instances the AUC in females was higher than in males by about a factor of two. This is consistent with the more rapid clearance by males.

	C _{max} (ng/ml)	T _{max} (hr)	AUC ₀₋₆ (ng*hr/ml)	AUC _{0-infinity} (ng*hr/ml)	t _{1/2} (hours)	Clearance (ml/min/kg)	V _d (litres/kg)	Bioavailability (%)
5 mg/kg IV								
Male				1990	3.2	42	9.2	
Female				3540	5.3	23.6	9.8	
5 mg/kg PO								
Male	127	6	620	NC	NC			NC
Female	230	4	1130	1780	4.1			49.8
12.5 mg/kg PO								
Male	381	5	1940	3520	4.7			76.6
Female	590	3	3550	7160	5.7			87.6

The lack of sufficient information to calculate an AUC at infinity for the 5 mg/kg orally dosed males precluded the calculation of the bioavailability. In female rats, it was 49.8 %. The table shows that the bioavailability appears to increase with increasing dose in males and females. The concentrations of radioactivity were uniformly higher in blood than in plasma (ratio approximately 1:0.8), suggesting the possibility of binding on or within RBCs. At all times after dosing the concentrations of total radioactivity in the plasma were greater than the concentrations of ZD1839 in the plasma, indicating the presence of circulating metabolites.

- 3) The disposition of ¹⁴C-ZD1839 in male bile duct cannulated rat. Study Number D1839 KMR022

Animals	Six male Alpk:APfSD rats The rats were surgically fitted with biliary cannulae three days prior to dosing. weighing between 254 and 297 g aged 58 days
Drug	[¹⁴ C]-ZD1839 (batch 1R1), specific activity of 44.8 mCi/mg (salt) Radiochemical purity in excess of 99% diluted with ZD1839 (ADM 37551D96).
Vehicle	0.5% hydroxypropyl methylcellulose (HPMC) in 0.1% Polysorbate 80 concentration of the suspension was 0.81 mg/g.
Dose	3.9 mg/kg incorporating 99 mCi/kg
Route	PO
Schedule	Single dose
Tissues	Bile was collected 0-3, 3-6, 6-12, 12-24 and 24-48 hours after dosing. Urine samples were collected at 0-6, 6-12, 12-24 and 24-48 hours after dosing. Feces and aqueous cage-wash were collected daily.
Analysis	liquid scintillation counting (LSC), One sample containing a high concentration of radioactivity, and with a reasonable volume of bile remaining (rat 4, 12 hour sample) was incubated overnight at 37.C with β-glucuronidase, in the presence and absence of the inhibitor Saccharolactone (D-saccharic 1,4-lactone).

Investigators at ZENECA Pharmaceuticals, Cheshire, England did this study in 1998. Mr. E. A. Partridge signed the GLP statement.

Results:

The following table shows the incremental and cumulative recovery of radioactivity in the various excreta.

Table 1 D1839 KMR022. Recovery of radioactivity in excreta following administration of a single oral dose of [¹⁴C]-ZD1839 to biliary cannulated male rats

Time after dose (hours)	Bile	Faeces	Urine	Cagewash	Carcass	Total
0-3	2.68 ± 0.43					
3-6	6.39 ± 0.93					
0-6	9.08 ± 1.00		0.71 ± 0.28			
6-12	10.88 ± 1.53		2.69 ± 0.69			
12-24	6.48 ± 1.37		1.83 ± 0.36			
0-24	24.63 ± 3.91	40.35 ± 3.95	5.24 ± 0.86	0.35 ± 0.06		
24-48	3.43 ± 0.63	11.83 ± 2.43	0.97 ± 0.27	0.11 ± 0.05		
0-48	28.05 ± 4.14	52.17 ± 3.07	6.20 ± 1.07	0.45 ± 0.09	10.28 ± 1.10	97.17 ± 1.42

The table shows that a mean of 97% of the radioactivity was recovered over two days. The recovery in bile was 28%, feces 52%, 6% in urine and less than 1% in cage-wash. Ten percent of the dose remained in the carcass at the end of 48 hours. The rate of elimination was slow. Only 70% of the dose was recovered within 24 hours and a further 15% 24-48 hours after dosing. The radioactivity

recovered in feces probably represents the unabsorbed fraction of the dose. The absorption of [^{14}C]-ZD1839, based on biliary, urinary and carcass recoveries was at least 44%. Again the investigators did not examine urine chromatographically because they recovered less than 7% of the dose via this route.

Methanol extracted 56% of the total radioactivity from pooled fecal homogenates (0 to 48 hours).

— the major component co-chromatographed with parent compound and accounted for 23% of the extracted radioactivity. Approximately 40% of the material remained at or close to the origin. Two further metabolites were detected, each representing less than 17% of the extracted radioactivity. All components were apparently more polar than [^{14}C]-ZD1839.

— of the fecal extracts resolved at least ten radiolabelled components. Three significant areas of radioactivity were apparent in all of the extracts. The largest of these accounted for 45% of the radioactivity and co-chromatographed with ZD1839. The other two significant components accounted for 19 and 12% of the extracted radioactivity respectively, the former eluting before and the latter after ZD1839. The minor components accounted for less than 6% each of the extracted radioactivity.

— of bile showed that 10% of the radioactivity (equivalent to 3 % of the dose) co-chromatographed with parent compound. There were three other significant metabolites. Two of these were very close to the origin and accounted for 58% of the extract radioactivity; the third accounted for 23% of the radioactivity. The remainder of the radioactivity was associated with two minor components. All observed components were apparently more polar than ZD1839.

— of pooled bile 0 to 48 hours resolved at least ten radiolabelled components. Parent compound accounted for approximately 8% of the bile radioactivity. There was only one peak common to all samples, this accounted for 6% of the radioactivity. The largest peak eluted after ZD1839 and accounted for 41% of the radioactivity in five of the samples. Three other significant peaks eluted before ZD1839 and individually accounted for between 8 and 14% of the radioactivity. The remainder of the radioactivity was associated with several minor components the largest accounting for 6% of the sample radioactivity.

Enzyme hydrolysis of bile was inconclusive. Only one major change occurred in all samples; the major biliary metabolite that accounted for 33% of the radioactivity in the control sample virtually disappeared. Its disappearance gave rise to a major metabolite which was less polar than ZD1839 and that accounted for approximately 50% of the sample radioactivity.

The results show that rats absorb only about half of an oral dose of ZD1839, but the absorbed drug is extensively metabolized to more polar compounds in the liver.

4) The transplacental transfer of ^{14}C -ZD1839 in the rat.
Study Number D1839 KMR016.

Animal	Four pregnant Alpk:ApfSD rats
Drug	^{14}C -ZD1839 (batch 1R1) specific activity of 44.8 mCi/mg (salt) radiochemical purity in excess of 99% diluted with ZD1839 (ADM37551D96). Diluted drug specific activity of 20.22 mCi/mg free base.
Formulation	suspended in 0.5% hydroxypropyl methylcellulose (HPMC) in 0.1% Polysorbate 80
Concentration	1 mg/ml nominal actual concentration 1.02 mg/g.
Dose	5.0 mg/kg incorporating 101 μCi /kg.
Necropsy	The rats were killed by inhalation of halothane at 4 hours
Samples	whole fetuses (oxidized) and maternal blood separated to plasma
Analysis	liquid scintillation

Investigators at ZENECA Pharmaceuticals, Macclesfield, England did this GLP study. They dosed animals on day 16 of pregnancy. The mean plasma and fetal tissue concentrations of radioactivity were 291 ± 8 and 112 ± 4 ng equiv/g respectively. Thus, ZD1839 does cross the rat placenta. The mean ratio of fetal to plasma radioactivity was 0.39.

- 5) The transplacental transfer of ^{14}C -ZD1839 in the rabbit.
Study Number D1839 KMB017.

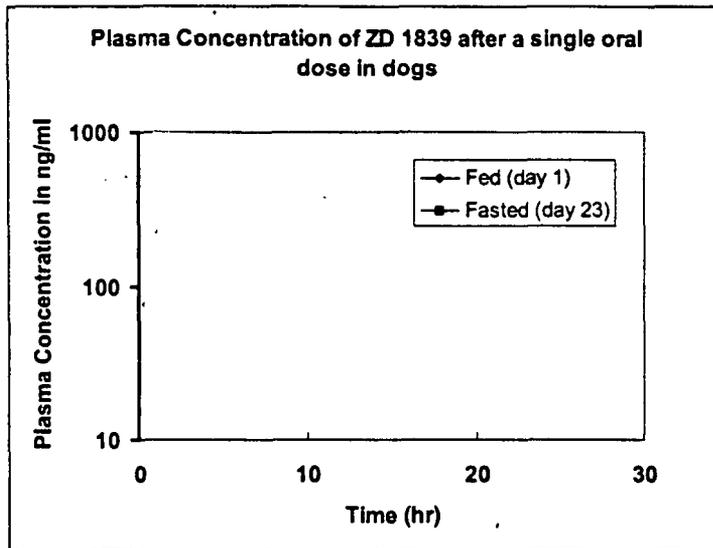
Animal	Four pregnant New Zealand White (Hsd Poc:NZW) rabbits,
Drug	^{14}C -ZD1839 (batch 1R1) specific activity of 44.8 mCi/mg (salt) radiochemical purity in excess of 99% diluted with ZD1839 (ADM 37551D96) purity of 99.2% diluted material specific activity of 20.43 mCi/mg free base.
Formulation	suspended in 0.5% hydroxypropyl methylcellulose (HPMC) in 0.1% Polysorbate 80
Concentration	1 mg/ml nominal, actual concentration 1.02 mg/g.
Dose	5.3 mg/kg, incorporating 108 mCi/kg.
Necropsy	The rabbits were killed by injection of 'Euthatal' at 4 hours
Samples	whole fetuses (oxidized) and maternal blood separated to plasma
Analysis	liquid scintillation

Investigators at ZENECA Pharmaceuticals, Macclesfield, England did this GLP study. They dosed animals on day 19 of pregnancy. The mean plasma and fetal tissue concentrations of radioactivity were 199 ± 29 and 40.4 ± 5.2 ng-equiv/g respectively. Thus, ZD1839 crosses the rabbit placenta. The mean ratio of fetal to plasma radioactivity was 0.21.

- 6) Evaluation of the effect of food on the pharmacokinetics of Seneca SD 1839 following a single (100 mg) oral tablet dose to the dog.
Study Number D1839 KPD025.

Animal	Male beagle dogs
Drug	ZD1839 ((batch ADM37432C96)
Dose	100 mg
Formulation	Brown film coated tablet (clinical formulation) within a gelatin capsule
Route	PO

In this GLP study, investigators at Zeneca Pharmaceuticals, Macclesfield, England gave ZD1839 as a single 100-mg oral tablet to six male dogs one-hour after feeding. About three weeks later they gave a second single oral 100 mg tablet to the same six dogs when the feeding regime had been altered so that the dogs were dosed after an approximately 18 hour fast. Whole blood was taken just before dosing and at intervals up to 72 hours post dose. The blood was separated to obtain plasma, stored at 20 C and analyzed for ZD1839 concentration by . — This analysis showed that this dose caused significant systemic exposure ZD1839 in all dogs in both the fed and fasted state. The following graph shows that exposure was similar for both the fed and fasted states.



At most time points, particularly early ones, the plasma concentrations were higher in the fed animals, but the differences were small. The average maximum concentrations were 1190 ± 89.2 and 919 ± 244 ng/ml for fed and fasted dogs respectively. The following table shows that the calculated pharmacokinetic parameters were not significantly different between the fed and fasted state.

	AUC ₀₋₂₄ (ng*hr/ml)	AUC _{0-∞} (ng*hr/ml)	t _{1/2} (hours)	C _{max} (ng/ml)	t _{max} (hours)
Fed	7295	7436	5	1190	2 - 8
sd	541	565	0.4	89	
Fasted	7930	8380	6.6	919	2 - 8
sd	1400	1380	1.2	244	

Thus feeding one hour before Iressa administration has little effect on the pharmacokinetics of the drug in dogs.

7) The Binding of ¹⁴C- ZD1839 to Purified Plasma Proteins. Study Number D1839 KPJ046

Proteins Human serum albumin (HSA) (Lot 30K7607 catalogue number A-1653) and human α-1-acid glycoprotein (α-1-AGP) (lot 128H7606 catalogue number G9885)

- Solutions
- 1) Human α-1-acid glycoprotein 0.4 mg/ml
 - 2) Human α-1-acid glycoprotein 0.8 mg/ml
 - 3) Human α-1-acid glycoprotein 3.2 mg/ml
 - 4) Human α-1-acid glycoprotein 0.4 mg/ml + Human serum albumin 40 mg/ml
 - 5) Human α-1-acid glycoprotein 0.8 mg/ml + Human serum albumin 40 mg/ml
 - 6) Human α-1-acid glycoprotein 3.2 mg/ml + Human serum albumin 40 mg/ml
 - 7) Human serum albumin 40 mg/ml

Solvent phosphate buffered saline

Drug ¹⁴C ZD1839 (batch 1R4) specific activity of 111 mCi/mg (hydrochloride) radiochemical purity in excess of 98%

Drug Conc. Nominal 0.05, 0.2, 2, 5, 8 mg/ml (free base) in the protein solutions
 Method One ml aliquots of each spiked protein solution were dialysed at 37 C against an equal volume of isotonic 0.067 M phosphate buffer, pH 7.4 using a dialysis system over night.
 Analysis liquid scintillation counting (LSC)

Investigators at ZENECA Pharmaceuticals, Macclesfield, England did this GLP study.

The investigators determined the percentage binding of ZD1839 to α -1-AGP at three concentrations of the protein. The following table shows that at the lower concentrations of 0.4 and 0.8 mg/ml the percentage binding declined with increasing ZD1839 concentration suggesting that binding to the purified protein is saturable.

Table 1 D1839 KPJ046. Binding of ZD1839 at various concentrations to purified proteins

Nominal ZD1839 concentration (μ g/ml)	% Binding						HSA
	0.4 mg/ml α -1-AGP	0.4 mg/ml α -1-AGP + HSA	0.8 mg/ml α -1-AGP	0.8 mg/ml α -1-AGP + HSA	3.2 mg/ml α -1-AGP	3.2 mg/ml α -1-AGP + HSA	
0.05	52.6	88.1	82.0	87.1	95.8	91.1	87.9
0.2	46.7	86.5	78.2	88.5	96.4	91.7	87.8
2	49.0	85.9	74.2	86.4	95.8	89.9	89.8
5	NR	87.0	69.8	87.4	95.8	91.7	86.6
8	31.4	86.4	63.8	85.5	94.7	89.7	87.7
Mean \pm SD	NC	86.8 \pm 0.82	NC	87.0 \pm 1.13	95.7 \pm 0.62	90.8 \pm 0.95	88.0 \pm 1.13

Concentration of human serum albumin (HSA) was 40 mg/ml

NC Not calculated

NR No result

Values show the mean for two cells

Concentration of human serum albumin (HSA) was 40 mg/ml

At the higher concentration of 3.2 mg/ml there was no change in binding to the purified protein with increasing concentration of ZD1839 because the concentration did not reach saturation.

The investigators then determined the percentage binding of ZD1839 to α -1-AGP at these three concentrations in the presence of HSA (40 mg/ml). At the two lower concentrations of the purified protein binding remained relatively constant, between 86 and 88%, irrespective of the ZD1839 concentration and was statistically indistinguishable from the percentage binding to HSA alone.

At 3.2 mg/ml, the binding to α -1-AGP with HSA was in between, and statistically different from the values for α -1-AGP or HSA alone. In the presence of HSA a four fold increase in α -1-AGP concentration caused an increase in binding from 87 to 91% which corresponds to a reduction of approximately 30% in free ZD1839 (from 13 to 9%). These results suggest that in cancer patients with elevated α -1-AGP concentrations, where concentrations of up to 3 mg/ml have been reported (Kremmer et al, 1988), plasma concentrations of free ZD1839 may be less than therapeutically optimal.

8) The Disposition of ¹⁴C-AstraZeneca ZD1839 in the male dog following oral and intravenous administration.

Number: DBIT1117

AstraZeneca Reference Number: D1839 KMD041

Animal Three male Beagle dogs, 6 and 9 months old, 10.5 to 11.4 kg
 Drug ¹⁴C-ZD1839 (Batch 1R3; specific activity 121.8 μ Ci/mg, radiochemical purity >99%)
 Non-radiolabelled ZD1839 (Batch C253/4, ADM No 60517H99, chemical purity 99.1%)

Dose Each dog received a oral doses ¹⁴C-ZD1839 at 5 and 25 mg/kg (800-1200 μCi) and an IV dose 5 mg/kg (1200-1300 μCi), with 4 weeks between each of the three doses.

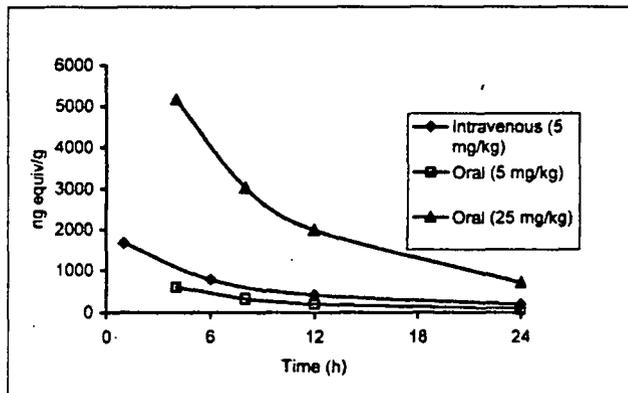
Formulation Oral hydroxypropyl methylcellulose (0.5%, w/v) in aqueous Polysorbate 80 (0.1%, w/v) IV aqueous hydroxypropyl-β -cyclodextrin (10%, w/v) pH reduced to ca 5.0 by HCl

Analysis Radioactivity in aliquots of urine, cage wash, plasma, and dose material was determined by liquid scintillation counting. Aliquots of fecal samples (homogenized in water) were oxidised before liquid scintillation counting.

analysis

Investigators at _____ did this GLP study. This is a study of the metabolites excreted in urine or feces in the dog.

The following chart shows the mean plasma concentrations of total radioactivity following single oral (5 and 25 mg/kg) and intravenous (5 mg/kg) doses of ¹⁴C-ZD1839 to male dogs in ng*equiv/g.



The investigators did not calculate pharmacokinetic parameters from this data and did not estimate oral bioavailability. It looks to be about 10%.

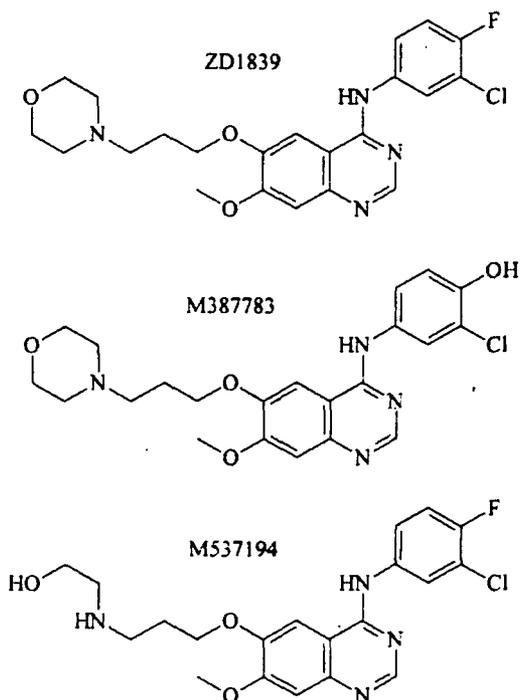
The following table shows the recovery of radioactivity at different time intervals. I have omitted some time intervals that had recovery amounts less than 2%.

Time interval	Intravenous 5 mg/kg	Oral 5 mg/kg	Oral 25 mg/kg
Urine			
0 to 24 hours	1.8 ± 0.2	1.2 ± 0.1	2.4 ± 0.5
Total (to 168 hours)	2.6 ± 0.4	1.8 ± 0.3	3.4 ± 0.7
Cage wash			
Total (to 168 hours)	0.7 ± 0.1	1.6 ± 0.7	0.9 ± 0.0
Feces			
0 to 24 hours	53.2 ± 9.6	67.1 ± 4.9	50.8 ± 8.5
24-48 hours	23.6 ± 9.4	14.9 ± 4.8	29.0 ± 7.5
48-72 hours	6.1 ± 3.2	2.4 ± 0.4	3.2 ± 0.3
Total (to 168 hours)	85.6 ± 0.2	86.1 ± 2.6	84.8 ± 2.1
Total recovery			
Total (to 168 hours)	88.9 ± 0.4	89.5 ± 2.2	89.1 ± 1.6

The table shows that most, greater than 85%, of the radioactivity was recovered in feces within 48 hours irrespective of route. Urinary excretion of radioactivity was minimal and was essentially complete after 24 hours.

The recovery of radioactivity from the pooled samples following extraction was 88-114% for feces, and 54-91% for plasma. In the plasma samples the percent recovered by extraction decreased with time. The _____ profiles produced by _____ analysis of plasma and fecal extracts and of urine were very similar irrespective of route. The following three

structures were identified and used as reference standards. The first is the parent compound (retention 45.73 min). In the second a fluorine is replaced by a hydroxy group (M387783, retention 30.89 min) and the last (M537194, retention 38.76 min) is a de-ethyl derivative.



In plasma the parent compound predominated and decreased with time similar to total radioactivity. A poorly resolved complex eluted before the parent and contained two components identified as the de-ethyl reference standard, M537194, and desmethyl ZD1839. These components increased through the early time points before decreasing. The investigators tentatively identified two other minor plasma components as desmethyl M537194 and the M387783 reference standard.

The following table shows the percentages of recovered metabolites in urine and feces. I have included only those chromatographic peaks that were identified and present in quantities greater than 1 % of the dose. The values for urine are totals for collection between 0 and 24 hours and those for feces are totals for collection between 0 and 72 hours.

Retention time	Proposed Identity	5 mg/kg Oral, Table 7			25 mg/kg Oral, Table 11			5 mg/kg IV, Table 15		
		Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total
29.9	M387783	0.015	3.83	3.84	0.015	7.94	7.96	0.01	5.24	5.25
37.08	dihydroxy ZD1839	0.13	2.58	2.71	0.123	5.98	6.1	0.11	2.72	2.83
37.81	Carboxypropyl ZD1839		5.14	5.14		5.9	5.9		5.17	5.17
39.23	Desmethyl ZD1839		15.2	15.2		10	10		11.6	11.6
39.66	M537194	0.929	17.4	18.3	0.834	11.6	12.5	0.61	13.6	14.2
43.96	Hydroxy ZD1839	1.58	20.1	21.7	2.76	33.4	36.1	2.16	22.9	25.1
44.94	ZD1839*		14.6	14.6		3.91	3.91		14.5	14.5
	Total **	3.43	86.1	89.5	4.17	84.9	89	3.17	85.4	88.6

- * See text for explanation of a discrepancy.
- * *Total includes all peaks including those minor components not shown in this table.

I constructed this table from the sponsor's data tables #7, 11, and 15. In table 11, the authors identify the peak eluting at 44.94 minutes as the hydroxy derivative and the one eluting at 43.96 minutes as the parent compound. This is not only inconsistent with the other two tables but is unreasonable in a system since the hydroxy should elute before the less polar parent. I must assume this is a typographical error; the text offers no explanation. In the table above, I have identified the values at 44.94 as the parent and those at 43.96 as the hydroxy derivative among the values for the 25 mg/kg oral dose (numbers in bold). Thus, they are reversed from the author's original identification but are consistent with the other two tables.

The study summary states "Urinary profiles from all phases also comprised of two major components found at the same retention time and masses as seen in the plasma profiles confirming the presence of unchanged ZD1839, however the earlier peak was found to contain the reference standard M537194 and a hydroxylated ZD1839 metabolite. Again two other minor metabolites were also tentatively identified as desmethyl M537194 and a dihydroxylated form of ZD1839." These statements are inconsistent with the tabular results.

The report of this study is poorly organized and contains conflicting statements so I will let the tabular results stand on their own. The experiment does show that dogs extensively metabolize ZD1839 and they excrete most of the radioactivity in the feces. The presence of hydroxy, desmethyl, and N-deethyl derivatives and major excretion in the feces implicates cytochromes P450 as the major agents of metabolism.

- 9) The characterization of metabolites in the plasma and feces from healthy male (human) volunteers and plasma from rats following single oral administration of ¹⁴C-ZD1839. study number DBIT1148 AstraZeneca Reference Number D1839 KMN047

Species	Rats (NOS) and healthy male human volunteers
Drug	¹⁴ C-ZD1839 batch IR3
Dose	Specific activity 130.5 μC/mg for rats and 3.31 μC/mg for humans
Route	40 mg/kg in rats (240 mg/m ²), 50 mg for humans (about 28 mg/m ²)
Samples	PO
	rat plasma taken at hour six post dosing pooled from rats 1 through 6
	Rat plasma taken at hour 24 post dosing pooled from rats 7 through 12
	Human plasma taken at hours 5 and 24 post dosing pooled from six individuals
	Human feces collected over 120 hours post dosing pooled from six individuals.
Analysis	and liquid scintillation counting.

Investigators at _____ did this study for AstraZeneca. Mr. W. J. Herron signed the GPL statement.

The total recovery of radioactivity after extraction from pooled rat plasma was 70 percent for the 6-hour sample and only 30 percent for the 24 hour sample. The extraction was a protein precipitation method using acetonitrile. The poor recovery at 24 hours suggests irreversible protein binding of some components. Because of this unexpectedly poor recovery, the investigators analyzed unextracted plasma by _____. The recovery: _____ after this analysis was only 39%, demonstrating considerable holdup _____. The recovery of radioactivity from human plasma samples was 71 to 81 percent and that from feces was 73 percent, consistent with that from pooled rat plasma.

I have combined the results from the investigators tables 2, 3 and 4 to construct the following table. The table presents the percentage of total recovered radioactivity in a sample found to elute with a specific retention time. The partial matching of retention times is mine but is consistent with the author's text. The proposed identities for the peaks are from the author's analysis of the — analysis in comparison with radiolabeled standards (see above). Most of their assignments were for human feces. The dose in humans was sufficiently low as to cause analytical problems; the amounts eluted from some of the peaks were too low to allow accurate . — analysis.

Retention Time Minutes	Rat Plasma			Human Plasma			Human Feces		Proposed Identity*
	6 hr samples	24 hr extracted	24 hr unextracted	Retention Time Minutes	5 hr samples	24 hr	Retention Time Minutes	Retention Time Minutes	
4.61	4.07	7.48	19.2	3.75	1.24	6.56			
							24.29	11.6	
							26.21	3.88	
							27.37	1.29	
							30.75	6.9	
31.77	1.38	4.98	3.97	32.25	6.91	6.84	31.62	3.45	
							34.8	12.1	M387783 aldehyde
							36.01	7.76	
							37.47	0.43	
38.21	0.81			39	7.97	11.7	38.23	12.1	Carboxypropyl ZD1839
39.22	0.54	1					39.34	0.43	
40.13	4.07	13.4	13.2	42.25	13.5	22.8			M537194 and hydroxy ZD1839 Desmethyl ZD1839 and desmethoxy morpholino ring opened ZD1839
							40.7	26.3	
				46	2.45				
44.18	82.7	65.2	58.3	46.75	56.6	27.1	44.54	12.1	ZD1839
45.23	0.81	1.99					45.55	1.29	
46.31	2.17	0.5					46.62	0.43	
49.63	3.52	5.47	5.3	49	4.36	14.3			
				56	7.02	10.9			
	100	100	100		100	100		100	

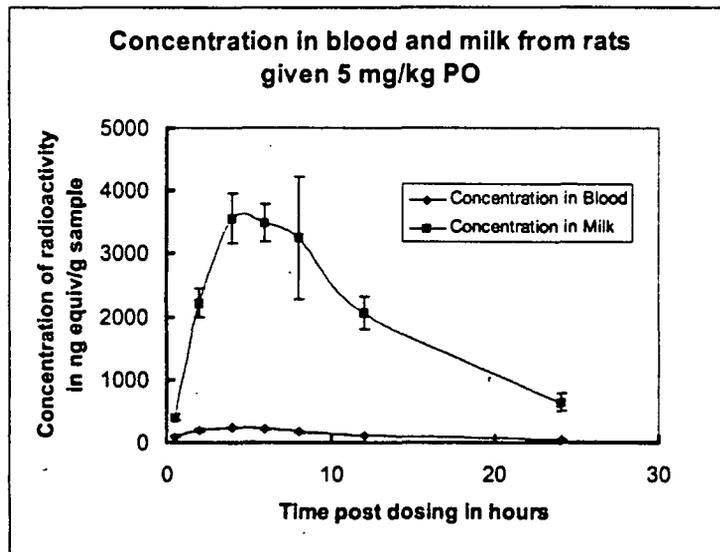
The experiment demonstrates significant qualitative similarities between rat and human plasma though humans appear to metabolize ZD1839 more extensively than rats as demonstrated by the amount of parent compound in plasma at 5 and 24 hours. Not enough peaks were identified to accurately compare the metabolism across species. As one might expect the number of peaks in human feces is significantly greater than in plasma, implying significant first pass metabolism. The absence of any significant identifiable conjugates is intriguing.

- 10) The excretion of drug-related material in the milk following oral administration of ¹⁴C-ZD1839 (5 mg/kg) to lactating rats
Study Number D1839 KMR051

Animal - Eight pregnant Alpk rats 325 to 390 g
Drug ¹⁴C-ZD1839, Batch 2R1
specific activity 115 µC/mg, radiochemical purity greater than 98%
Unlabeled ZD1839, batch C253/1 P1
Final specific activity 39.25 grams
Dose 5 mg/kg given 13 days *post-partum*
Route PO
Formulation 10% w/v hydroxypropyl-β-cyclodextrin, pH 7, 0.92 mg/ml
PK times 0.5, 2, 4, 6, 8, 12, 24 three animals per time point except 24 hours where there were 6.
Samples blood and milk (5 to 10 minutes after an IP dose of oxytocin)
Analysis against reference standards.

Investigators at AstraZeneca UK Ltd, Cheshire, UK did this GLP study.

The following graph demonstrates that radioactivity from the ZD1839 dose concentrates in rat milk.



The peak concentration in both blood and milk occurred at about 4 hours. The maximum ratio of the concentration in milk to that in blood occurred at about eight hours post-dosing and had a value of about 20. The authors estimated that the pups were exposed to a maximum of 18% of the maternal dose over 24 hours using worst case analysis. The major component in milk (greater than 90%) was the parent compound. Two minor components were the metabolite M527301 (morpholinyl group replaced by an hydroxy group) and desmethyl-ZD1839.

- 11) AstraZeneca ZD1839: Investigation of Plasma Metabolites Following Oral Administration of ^{14}C - AstraZeneca ZD1839 at 40 mg/kg to Male Rats. Study Number D1839 KMR036. EDR submission 21399 RRZ 003, November 30, 2001, Module4/pk/krm036.pdf.

Investigators at ZENECA Pharmaceuticals, Cheshire, England did this study in 2001. Mr. E. A. Partridge signed the GLP statement. The investigators gave a dose of 38.6 mg/kg (231.6 mg/m²) to 12 male rats. They harvested plasma samples at 6 and 24 hours, using six rats at each time point. The study yielded few useful results because the procedure required 5 fractions to achieve about 94% recovery of total radioactivity. The elution profiles in these five fractions were considerably different between 6 and 24 hours and indicated significant metabolism; the more polar fractions contained a greater amount of radioactivity at 24 hours. Nevertheless, the parent compound predominated in all but the most polar extract at 24 hours. The investigators did not identify other metabolites.

- 12) AstraZeneca ZD1839 : The Disposition of AstraZeneca ^{14}C -ZD1839 following a Single Intravenous Dose to Male and Female Rats. Study Number D1839 KKR044, EDR submission 21399 RRZ 003, November 30, 2001, Module4/pk/KKR044.pdf.

Animals nine male and nine female Alpk:APfSD rats, aged between 43 and 47 days weighing between 166 and 190 g,
 Drug ¹⁴C-ZD1839 (batch 2R1), specific activity of 115 µCi/mg radiochemical purity in excess of 98 % diluted with ZD1839 (batch not specified)
 Vehicle 10% aqueous hydroxypropyl β-cyclodextrin
 Dose 19.9 mg/kg actual PO dose, 21.8 µCi/kg,
 Route IV
 Schedule Single dose
 Sampling 1, 6, 12 hours (N=1/sex), 24 hours (N=2/sex), 7 days (N=4/sex) urine, feces and tissues
 Analysis LSC

Investigators at ZENECA Pharmaceuticals, Cheshire, England did this study in 2001. J. A Clarkson-Jones signed the GLP statement.

The study determined the following pharmacokinetic parameters:

Parameter	Male	Female	Mean
AUC ₀₋₆ (µg.h/ml)	NC	14.2	NC
AUC ₀₋₂₄ (µg.h/ml)	5.68	13.4	9.56
t _{1/2} (hours)	NC	6.16	NC

Each value was derived from the composite profiles obtained using 1 animal at each time point other than 24 hours where 2 animals were used
 NC Not calculated

Contrary to what the authors say in the results section and summary, the data indicates that most all the radioactivity was associated with the plasma fraction of the blood; little was associated with the cellular portions of the blood. The following table shows the amount of radioactivity recovered in the various tissues 24 hours after dosing. As one would expect, most of the radioactivity is associated with the liver, lungs, and kidney. The drug appears to concentrate in the female adrenal gland to a greater degree than in the male. The value in the heart in rat 18 appears unusual. That it is identical to the value for the spleen suggests a typographical error. The high tissue-to-blood concentration ratios are consistent with the large volume of distribution observed in other pharmacokinetic studies.

Table 3 D1839 KKR044. Concentrations of radioactivity in the tissues and ratios of tissue to blood radioactivity concentrations at 24 hours after a single intravenous dose (20 mg/kg) of [¹⁴C]- ZD1839 to rats

Tissue	Male rats		Female rats		Male rats		Female rats	
	Rat 12	Rat 13	Rat 17	Rat 18	Tissue : blood	Tissue : blood	Tissue : blood	Tissue : blood
	Tissue radioactivity (µg equiv/g)	Tissue : blood	Tissue radioactivity (µg equiv/g)	Tissue : blood	Tissue radioactivity (µg equiv/g)	Tissue : blood	Tissue radioactivity (µg equiv/g)	Tissue : blood
Adrenal glands	8.19	45.64	4.65	149.93	10.57	86.63	19.06	89.48
Heart	1.17	16.72	1.81	58.44	0.53	4.37	21.02	98.66
Kidney	7.01	100.18	6.18	199.44	6.70	54.94	11.21	52.61
Liver	28.78	411.15	26.03	839.62	22.96	188.16	43.41	203.80
Lung	8.11	115.83	7.75	250.07	10.92	89.52	15.50	72.78
Spleen	5.80	82.91	3.96	127.89	8.17	66.96	21.02	98.66

The following data shows that male rats excrete more radioactivity into the feces in the first 12 hours than do females. Though this difference does not reach statistical significance because of the low sample numbers, it is consistent with the lower exposures and shorter half-lives observed consistently in male rats. Excretion into the urine is complete for the most part in the first 12 hours.

Table 4 D1839 KKR044. Recovery of radioactivity in excreta following administration of a single 20 mg/kg intravenous dose of [¹⁴C]-ZD1839 to rats

Time after dose (hours)	Male	Female
Urine		
0-12	3.11 ± 0.37	2.28 ± 0.19
12-24	0.91 ± 0.16	0.65 ± 0.04
24-48	0.27 ± 0.04	0.26 ± 0.05
48-168	0.20 ± 0.02	0.18 ± 0.04
Total	4.50 ± 0.42	3.38 ± 0.19
Faeces		
0-12	44.60 ± 7.25	27.20 ± 4.91
12-24	23.39 ± 0.60	32.42 ± 8.95
24-48	21.72 ± 4.87	21.58 ± 4.53
48-168	9.01 ± 0.89	7.76 ± 0.79
Total	98.71 ± 3.17	88.96 ± 4.57
Cagewash	0.64 ± 0.10	1.14 ± 0.34
Expired air	ND	ND
Carcass	2.82 ± 0.38	3.55 ± 0.60
Total recovery	106 ± 3.03	97.02 ± 4.44

Each value, representing the mean ± SE obtained from four animals, is expressed as a percentage of the dose administered
 ND Not detected

The following table shows that much more of the radioactivity in the urine of male rats (0-12 hours) bound at the origin than did that of females. This strongly suggests that males metabolize more ZD1839 to significantly more polar metabolites shortly after injection than do females. In females more of the parent compound remains unmetabolized.

Table 5 D1839 KKR044. Profiles of metabolites in urine obtained 12 hours following administration of a single intravenous dose of [¹⁴C]- ZD1839 to rats

R _f	Percentage of urine sample radioactivity	
	Male	Female
0.01-0.03 (origin)	35.9 ± 5.3	16.7 ± 2.09
0.090-0.100	4.73 ± 0.30	5.65 ± 1.41
0.160-0.170	4.73 ± 0.55	4.08 ± 0.35
0.333-0.53 (M537194)	28.75 ± 1.35	26.25 ± 1.57
0.583-0.613 (ZD1839)	23.33 ± 3.37	40.15 ± 2.11

Values show the mean ± SE obtained from four animals; these represent the proportion of radioactivity associated with each component
 R_f indicates the approximate relative mobility of each component

of fecal extracts show that little of the radioactivity excreted into the feces is highly polar as reflected by the small percentage bound at the origin. Again, male rats appear to metabolize the parent more extensively than females.

Table 6 D1839 KKR044. — profiles of metabolites in faeces obtained following administration of a single intravenous dose of [¹⁴C]-ZD1839 to rats

R _f	Percentage of faeces sample radioactivity	
	Male	Female
0.024-0.041 (origin)	5.9 ± 0.56	4.78 ± 0.39
0.108-0.228	5.73 ± 0.97	4.93 ± 1.05
0.436-0.476 (M537194)	16.78 ± 1.06	9.30 ± 1.48
0.520-0.588	11.50 ± 0.58	7.25 ± 0.95
0.696-0.740 (ZD1839)	45.10 ± 4.79	59.88 ± 2.83

Values show the mean ± SE obtained from four animals; these represent the proportion of radioactivity associated with each component
 R_f indicates the approximate relative mobility of each component

The levels of radioactivity in organ tissues at 24 hours were much greater than the concentrations found plasma at that time. — of tissue extracts indicated that most of the radioactivity was attributable to parent compound. The metabolite M537194 was bound in all tissues sampled. M523595 was found in all tissues sampled except the heart.

- 13) AstraZeneca ZD1839 : The Characterisation of Metabolites in the Plasma and Faeces from Healthy Male Volunteers and Plasma from Rats Following Single Oral Administration of ¹⁴C- AstraZeneca ZD1839. — Study Number: DBIT1148 AstraZeneca Reference Number: D1839 KMN047. EDR submission 21399 RRZ 003, November 30, 2001, Module4/pk/KMN047.pdf.

In this GLP study investigators at — reanalyzed the plasma and feces samples from the rats dosed in study KMR036 (see above) using — and scintillation counting (30 second collection samples —) to obtain more useful results. They compared these results to those obtained from samples from human volunteers (study number IL003). The following table defines the parameters of the study.

Study Number	D1839 KMR036	D1839 IL0003
Species	Rat	Human
Dose level	40 mg/kg	50 mg
Route	Oral	Oral
Sp Act	130.5 µCi/mg	3.31 µCi/mg
Pooled plasma samples	6 hour (pooled from rats 1-6) 24 hour (pooled from rats 7-12)	5 & 24 hour (pooled from volunteers 1-6)
Pooled faeces samples	-	0-120 hour (pooled from volunteers 1-6)

The investigators extracted the pooled plasma samples by acetonitrile precipitation. The recovery of total radioactivity was unacceptably low for the 24 hour rat samples, so they also prepared these samples by direct dilution. They extracted fecal samples with tetrahydrofuran.

The following table shows the results for rat plasma. The percentages add to about 100% so I assume that they are calculated on the basis of applied radioactivity and not the total determined by scintillation counting from the unextracted sample. This makes it difficult to discern the real problem with the extraction procedure for the 24-hour sample. Nevertheless, the data suggests a small shift to more polar metabolites in the unextracted sample.

Table 2 DBIT1148 (AstraZeneca Reference Number D1839 KMN047) Radioactivity profiles of rat plasma following a single oral administration of [¹⁴C]-ZD1839

Results expressed as percentage of radiochemical profile

Peak Number	Time (mins)	6 hr	24 hr (extracted)	24 hr (unextracted)	m/z (M+H) ⁺	Proposed Identity
1	4.61	4.07	7.46	19.2		
2	31.77	1.36	4.98	3.97		
3	38.21	0.81				
4	39.22	0.54	1.00			
5	40.13	4.07	13.4	13.2	423/465	M537194 Hydroxy ZD1839
6	44.18	82.7	65.2	58.3	449	ZD1839
7	45.23	0.81	1.99		451	
8	46.31	2.17	0.50			
9	49.63	3.52	5.47	5.30	463	

m/z values given in this table indicate an increase of 2 Da due to the very high specific activity of the [¹⁴C]-ZD1839 material

The following table shows the metabolites found in human plasma.

Table 3 DBIT1148 (AstraZeneca Reference Number D1839 KMN047) Radioactivity profiles of human plasma following a single oral administration of [¹⁴C]-ZD1839

Results expressed as percentage of radiochemical profile

Peak Number	Mean Retention Time (mins)	5 hour	24 hour	m/z (M+H) ⁺	Proposed Identity
1	3.75	1.24	6.56		
2	32.25	6.91	6.64		
3	39.00	7.97	11.7		
4	42.25	13.5	22.8		
5	46.00	2.45			
6	46.75	56.6	27.1	447	ZD1839
7	49.00	4.36	14.3		
8	56.00	7.02	10.9		

The table demonstrates that humans metabolize ZD1839 more extensively than rats particularly at the 24-hour time point. The presence of metabolites significantly less polar than the parent is unusual but these are probably the aromatic rings after more polar functional groups have been cleaved.

The following table shows the percentage of metabolites found in human feces.

Table 4 DBIT1148 (AstraZeneca Reference Number D1839 KMN047) Radioactivity profiles of human faeces following a single oral administration of [¹⁴C]-ZD1839

Results expressed of percentage of radiochemical profile

Peak Number	Time (mins)	% Area	m/z (M+H) ⁺	Proposed Identity
1	24.29	11.6		
2	26.21	3.88		
3	27.37	1.29		
4	30.75	6.90		
5	31.62	3.45		
6	34.80	12.1	459	M387783 aldehyde
7	36.01	7.76		
8	37.47	0.43		
9	38.23	12.1	392	Carboxypropyl ZD1839
10	39.34	0.43		
11	40.70	26.3	433/435	Desmethyl ZD1839/Desmethoxy morpholino ring-opened ZD1839
12	44.54	12.1	447	ZD1839
13	45.55	1.29		
14	46.62	0.43		

The total amount of parent converted to metabolites is about the same as in plasma but there are considerably more peaks. The study suffers due to the lack of more comprehensive identification of the metabolites. The rat appears to be a relatively good model for human metabolism in that a significant amount of parent is excreted unchanged and most of the metabolites appear to be side-chain cleavage products.

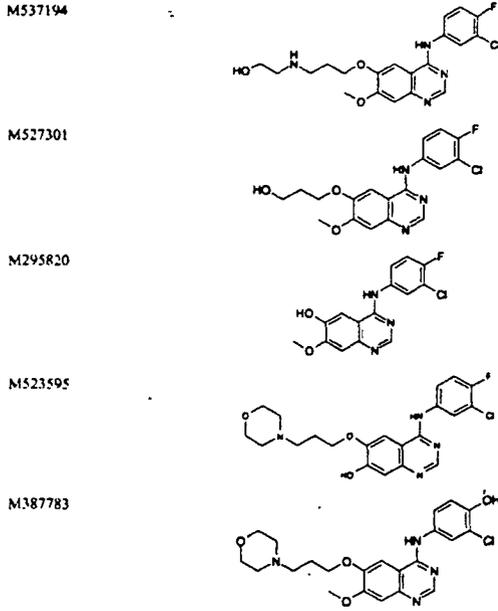
14) Zeneca ZD1839 : The Pharmacokinetics of ZD1839 and its Metabolites in Male Dogs Following Oral and Intravenous Dosing at 5 mg/ kg. Study Number D1839 KPD050. EDR submission 21399 RRZ 003, November 30, 2001, Module4/pk/KMN050.pdf.

Animal three male Beagle dogs, 15 to 17 months old, between 12.1 and 12.3 kg
 Drug Oral dose – ZD1839 (reference number ADM60517H99 and Batch Number C253/ 4)
 IV dose – ZD1839 (ADM60281B99)
 Dose 5 mg/kg
 Schedule one oral dose followed three weeks later with a single IV dose.
 Formulation Oral dose – suspension in 0.5% w/ v hydroxypropyl methylcellulose (HPMC) solution containing 0.1% w/ v aqueous polysorbate 80
 IV dose – 4.5% w/ v glucose solution (pH 4)
 Concentration 2 mg/ml,
 Sampling , Oral dose – immediately before dosing and 15 and 30 minutes, 1, 2, 3, 4, 6, 8, 12, 24, 32, 48, 72, 96 and 120 hours after dosing
 IV dosing – immediately before dosing and at 5, 10, 15 and 30 minutes, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, and 120 hours after dosing
 Analysis

Investigators at AstraZeneca UK Limited, Macclesfield Cheshire UK did this GLP study.

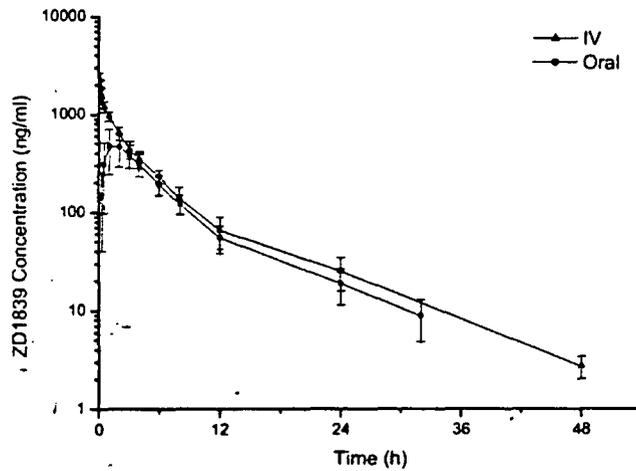
Standards

Figure 2 D1839 KPD050. Analytical reference standard structures



The following graph shows the pharmacokinetic profiles for the parent compound in plasma after an oral and an IV dose. The profiles are remarkably similar, attesting to the apparent quality of the data.

Figure 3 D1839 KPD050. Mean plasma concentrations of ZD1839 in male dogs following single oral and intravenous doses of ZD1839 at 5 mg/kg



The following table shows the pharmacokinetic parameters obtained from the data graphed above using WinNonLin and log-linear regression (non-compartmental analysis).

Table 2 D1839 KPD050. Pharmacokinetic parameters for ZD1839 derived following administration of ZD1839 to male dogs

Parameter	Dose route	
	Oral	Intravenous
Dose mg/kg	5	5
C ₀ (ng/ml)	-	2805 ± 288
C _{max} (ng/ml)	510 ± 206	2280 ± 359
t _{max} (hours)	1 to 3	0.083
AUC ₀₋₂₄ (ng.h/ml)	3138 ± 1027	5031 ± 984
AUC _{0-∞} (ng.h/ml)	3380 ± 1135	5768*
t _{1/2} (hours)	6.97 ± 0.46	7.77*
Absolute bioavailability (%)	63.9*	-
Clearance (ml/min/kg)	-	16.1*
Volume of distribution (l/kg)	-	6.33*

Each value shows the mean ± SE obtained from three animals
 * Mean of results from two animals

The bioavailability of ZD1839 in dogs is similar to that in humans, but the half-life is considerably shorter and the volume of distribution is considerably less. Exposure at a comparable dose in humans is about the same based on AUC.

- 15) 13-week dose range finding study in mice with administration by gavage. — study # 455544, Sponsors reference THM/1227, IND [redacted] submission 624 page 1.

In the toxicokinetics portion of this study (reviewed below in the toxicology section), the 125 mg/kg/day dose (327 mg/m²) resulted in an AUC_{0-∞} of 36.6 and 49.9 µg*h/ml in males and females, respectively.

- 16) Six Month oral dosing study of ZD1839 in rats. TPR2576, Serial 014.

The following table shows the pharmacokinetic parameters derived from this study (reviewed below in the toxicology section). There is evidence that the ability to clear ZD1839 decreases significantly with time and half-life is not constant with dose.

Parameter	Day 1		Week 26	
	Group III 5 mg/kg	Group II 1 mg/kg	Group III 5 mg/kg	Group IV 15 mg/kg
Half-life (h)	NC	NC	9	20.1
AUC ₀₋₈ (ng.h/ml)	430	145	1480	4280
AUC ₀₋₂₄ (ng.h/ml)	NC	NC	3370	11400
C _{max} (ng/ml)	70.7	20.4	222	640
t _{max} (h)	4	8	4	8

NC – not calculated due to insufficient data.

In the toxicokinetic portion of this study, the 15 mg/kg dose, or 90 mg/m², resulted in an AUC of 9.1 and 13.5 µg*h/ml in males and females, respectively. AUC appears to increase with repeated dosing.

17) One-month oral toxicity study in dogs. Study Number TAD/876.

For the details of this study see the toxicology section below.

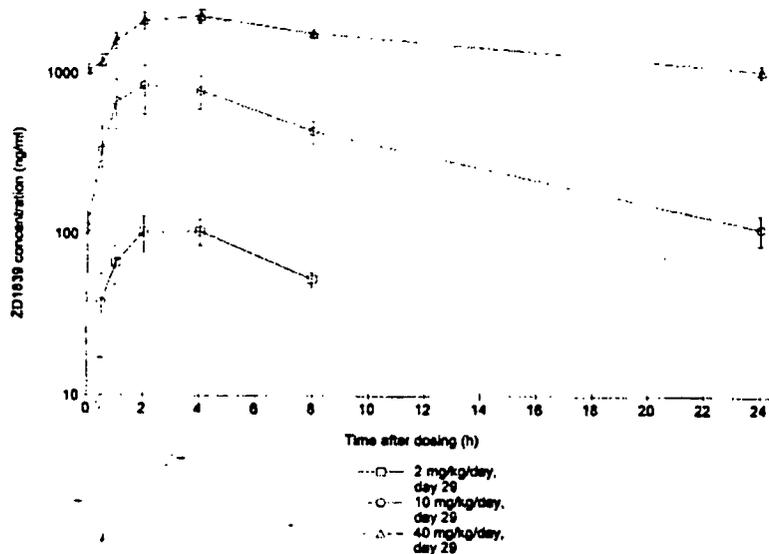
Table 3 Summary of pharmacokinetic parameters

Parameter	Day 1		Day 29	
	2 mg/kg/day	2 mg/kg/day	10 mg/kg/day	40 mg/kg/day
Half-life (h)	NC	NC	5.8	NC
AUC ₀₋₈ (ng.h/ml)	352	652	5300	15800
AUC ₀₋₂₄ (ng.h/ml)	NC	NC	9700	38600
C _{max} (ng/ml)	71	114	938	2360
t _{max} (h)	2.3	3.0	3.0	3.7

NC Not calculated

The following graph shows the pharmacokinetic profiles on day 29

Figure 2 Study number TAD/876. Mean ± standard error ZD1839 plasma concentration in male and female dogs in the 2, 10 and 40 mg/kg/day dose groups on day 29



The sample size is small and there is significant variability. The number of time points is insufficient and the pharmacokinetic sampling was not carried out long enough. Nevertheless, the data suggests some accumulation with repeated dosing. Sampling of the high or mid dose group on days 1 and 29 would have been more informative than using the low dose group. Nevertheless, the difference in exposure between day 1 and 29 at the low dose was statistically significant. The investigators arguments against accumulation are unconvincing. AUC increases linearly through this dose region but there is

some suggestion that absent unacceptable toxicity it would probably be limited by absorption at higher doses. The elimination half-life probably has two components but the data is inadequate to characterize them.

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Pharmacokinetics and Toxicokinetics Summary:

The following table summarizes the major findings of pharmacokinetics studies in mice, rats and dogs.

Study	Species	Route	Dose	Time	T _{max}		C _{max}		AUC		t _{1/2} ^b	
					hr		µg/ml		µg*hr/ml		hr	
			mg/m ² /day		male or combined	female	male or combined	female	male or combined	female	male or combined	female
TAR/2492	Rat	PO	60	d1	5	6	0.2	0.2	0.97	1.85		
male and female		PO	300	d1	2	3	1.5	1.7	12.7	17.6		
		PO	750	d1	2	6	4.6	4.9	30.9	41.3		
		PO	60	d14	3	3	0.4	0.4	2.1	3		
		PO	300	d14	6	5	2.1	1.9	14.1	17.4		
		PO	750	d14	5	2	3.4	3.5	23.5			
TAR/2570	Rat	PO	12	d1	4	4	0.02	0.03				
male and female		PO	12	d28	4	0.5	0.05	0.06		0.35		6.8
		PO	60	d28	2	2	0.54	0.58	2.55	3.22	6.8	5
		PO	240	d28	8	1	1.46	1.69	22.8	27.3	7.1	6.7
TPR/2576	Rat	PO	30	d1	4		0.071					
male and female		PO	6	w26	8		0.02					
		PO	30	w26	4		0.22		3.37			
		PO	90	w26	8		0.64		11.4			
TAD/876	Dogs	PO	40	d1	2.3		0.071					
male		PO	40	d29	3		0.114					
		PO	200	d29	3		0.938		9.7		5.8	
		PO	800	d29	3.7		2.36		38.6			
TAD/870	Dog	PO	100	d1	1	2	2.6	1.6	19	16	7.1	6.7
male and female		PO	1,000	d1	4	4	8.7	11.8	102	142	4.8	7.6
		PO	1,500	d1	1	1	12.8	16	149	177	9.9	8.8
		PO	100	d14	2	4	2	1.1	20	15	7.2	6.7
		PO	1,000	d14	6	8	3.5	5.1	63	93		NC
		PO	1,500	d14	8		9.6		192			NC
TAD/876	Dog	PO	40	d1	2.3		0.07		0.35			
male		PO	40	d28	3		0.14		0.65			
		PO	200	d28	3		0.94		5.3		5.8	
		PO	800	d28	3.7		2.36		15.8			
KKR008	Dog	IV	40	d1					1.99	3.54	3.2	5.3
male and female		PO	40	d1	6	4	0.127	0.23	0.62	1.78		4.1
		PO	250	d1	5	3	0.381	0.59	3.52	7.16	4.7	5.7
KPD025	Dog	PO Fed	250	d1	2 to 8		1.19		7.44		5	
male		PO Fasted	250	d1	2 to 8		0.919		8.38		6.6	
KMN050	Dog	PO	40	d1	1 to 3		0.51		3.38		6.97	
male		IV	40	d1	0.083		2.28		5.77		7.77	

^a AUC0-8 for studies TAR/2570 and TAD/876, AUC0-12 for study TAR/2492, AUC0-24 for study TAD/870

^b AUC0-12 cannot be determined due to the lack of sampling points;

The following table summarizes the major findings of the most relevant pharmacokinetic studies in humans.

Study	Species	Route	Dose mg/m ² / day	Time	T _{max} hr		C _{max} µg/ml		AUC µg*hr/ml		t _{1/2β} hr	
					male or combined	female	male or combined	female	male or combined	female	male or combined	female
(1839IL/0001 male	Human		6.3	d1	5.5		0.0028		0.086 ^g		14.8 ^g	
			15.6	d1	5		0.0079 ^f		0.126 ^f		12.9 ^f	
			31.2	d1	5.5		0.013		0.261 ^g		15.6 ^g	
			46.9	d1	5		0.026		0.424		12.1	
(1839IL/0010) Healthy Volunteers	Human	male	62.5 ^h	d1	5		0.044 ^h		0.558 ^h		12.7 ^h	
			62.5 ^h	d3	6		0.068 ^h		1.078 ^h		31.5 ^h	
1839IL/0033 Healthy Volunteers Male	Human		31	d1	5		0.015		0.57		31	
			63	d1	3		0.049		1.1		23	
			156	d1	5		0.098		3.3		32	
			313	d1	3		0.21		6.2		28	
1839IL/0005 Cancer Patients male & female	Human		31	d1	3		0.044		0.59		34	
			63	d1	5		0.046		0.57			
			94	d1	3		0.14		2			
			141	d1	5		0.15		1.9			
			188	d1	4		0.24		3.5			
			250	d1	5		0.4		4.6			
			328	d1	3		0.47		5.7			
438	d1	3		0.83		11						

Assuming the body surface area = 1.6 m²; ^g n = 1, ^f n = 2, ^g n = 3, ^h n = 5

For analysis and comparison of this information, see the overall summary below.

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Multi-Dose Toxicology:

- 1) 13-week dose range finding study in mice with administration by gavage. —
study # 455544, Sponsors reference THM/1227, IND [redacted] submission 624 page 1.

Animals male and female CD-1 mice
Drug AD1839, batch ADM60517H99, purity 99.6%
Doses controls, 50, 125, 175 mg/kg/day (0, 150, 375, 525 mg/m²/day)
Vehicle 0.5% w/v hydroxypropylmethyl cellulose (HPMC):0.1% w/v Tween 80.
Route PO gavage
Schedule daily
Duration 13 weeks
N 10 per dose group per sex
20 per dose group per sex in a satellite group dosed for pharmacokinetics
(reported separately below)
Histopathology Limited to only the eye, kidney, liver (with gall bladder), lung and skin
Necropsy week 13

— completed this study for AstraZeneca in March of 2001. This is a draft report of a GLP study; it remains to be signed.

Results:

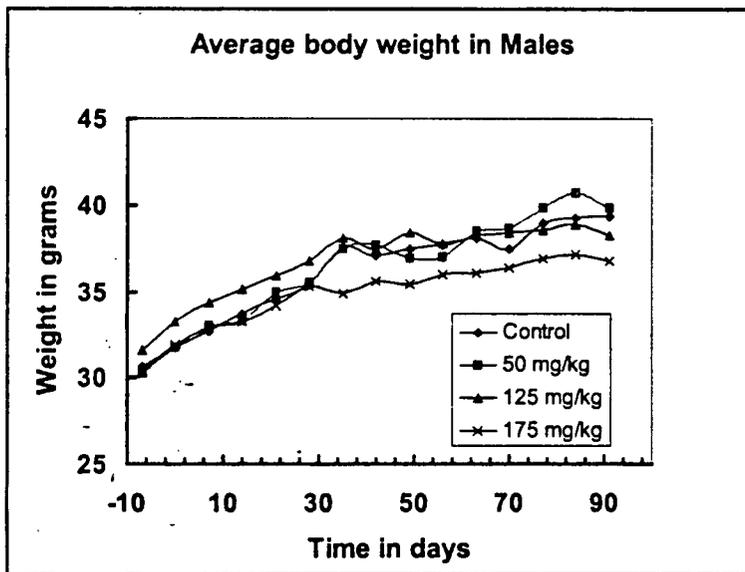
Mortality: Only one animal died in the main study group, a low dose animal suffered injury from the dosing procedure.
In the satellite group four mid-dose animals and 1 high dose animal died or were killed moribund, three of the five were considered treatment related deaths (sex not reported).

Clinical Signs/Symptoms, Gross and Histopathology:

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Observation		Control	Low-Dose	Mid-Dose	High-Dose
Clinical Signs	Daily		partially closed eyes (3/10 M) with swollen or encrusted eyelids	Dose related alopecia less severe in males, partially closed eyes (8/10 M, 8/10 F) with swollen or encrusted eyelids	Dose related alopecia less severe in males, partially closed eyes (9/10 M, 8/10 F) with swollen or encrusted eyelids
Body Weight	Daily reported weekly	See graph below			
Food Cons.	Weekly		NC	NC	NC
Water Cons	Weekly		NC	NC	NC
Ophthalmoscopy	Pre-trial and week 13			Animals with closed eyes were not examined. Others showed no pathology	Animals with closed eyes were not examined. Others showed no pathology
Abs Spleen Weight				52% increase M	120% increase M 50% increase F dose related
Abs Liver Weight				17% increase M	31% increase M Dose related
Necropsy				Pallor, swollen lymph nodes, pale foci or prominent liver lobes	Pallor, swollen lymph nodes, pale foci or prominent liver lobes
Histopathology Lung			Macrophage infiltration without reaction	Macrophage infiltration without reaction, greater than low dose	Macrophage infiltration without reaction, about the same as the Mid dose
Histopathology Liver			Macrophage infiltration	Macrophage infiltration	Macrophage infiltration, dose dependant
Histopathology Skin			Some animals with Chronic active folliculitis	Chronic active folliculitis, associated with alopecia	Chronic active folliculitis, associated with alopecia
Histopathology Eye			NC	NC	NC

NC = No Change relative to control



The body weight chart for males above shows that the mice in the high dose group weighed less than controls from day-thirty onward. The result for the mid-dose group is less clear but this is because randomization inadvertently resulted in a higher starting weight for these animals. The following chart shows average body weight gain for males and females at the end of the study. By this measure both mid

and high dose animals gained significantly less weight than controls (**p < 0.01). Females did not show a decrease in weight gain.

Net Body Weight Gain

	Male			Female		
	N	mean	SD	N	mean	SD
Control	10	7.8	2.7	10	4.7	2.4
50 mg/kg	9	8.0	2.6	10	4.6	3
125 mg/kg	10	5.2**	1.8	10	4	3.4
175 mg/kg	10	4.9**	1.2	10	5	1.2

Histopathology:

The macrophages that infiltrated the lung tissue were primarily in the alveolar spaces but some were seen in the bronchioles and interstitium. "They were large cells with abundant brown pigment stippled with cytoplasm with relatively small nuclei. Generally there was no interstitial reaction to the presence of these cells." This effect appears to be dose related with a possible plateau in the severity and incidence of the response at the mid- and high doses.

The macrophage infiltration in the liver was similar to that in the lung but more obviously dose dependant. Infiltration was more intense in the centrilobular regions. But, in the liver this infiltration was accompanied by inflammatory cell infiltration "mainly consisting of lymphocytes and other mononuclear inflammatory cells. Centrilobular hepatocytes were enlarged by the presence of pigment in all groups receiving ZD1839." This pigmentation was similar to that seen in the lungs. The cytoplasm was paler than usual but was not accompanied by vacuolation. The changes were consistent with the observation that the liver was pale on gross inspection. In one animal studied more closely, the pigment reacted with Masson-Rontana and long Ziehl-Neelsen stains. "This pattern of staining reaction is consistent with old (highly polymerized) lipofuscin," a result that suggests sustained oxidative stress.

In the skin, chronic or chronic-active folliculitis was present in most animals in the mid and high dose groups and was seen in some low dose animals. This folliculitis was consistent with alopecia seen grossly. Some treated animals showed signs of low-grade dermal inflammatory cell infiltration. There was evidence of dose related *epithelial hyperplasia*.

In the eye, keratitis, corneal hyperplasia and retinal atrophy were seen in some treated animals, but the incidence was too low to establish a dose relationship. Note that the protocol for this study called for the examination of only five tissues, yet significant histopathological damage was observed.

In an earlier 13-week dose range-finding study (THM960), a daily dose of 250 mg/kg caused unacceptable mortality. The 250-mg/kg dose group was terminated at 9 weeks. This result, coupled with the decrease in weight gain seen in the present study, establishes 125 to 175 mg/kg/day as an upper limit for long term dosing in mice. The sponsor proposes 125 mg/kg as the high dose for the mouse carcinogenicity study. This dose is half the dose that caused mortality in the dose range finding study. It caused a decrease in body weight gain, and distinct dose related histopathology in this 13-week study.

See the "pharmacokinetics and toxicokinetics" section above for the toxicokinetic information.

2) Six Month oral dosing study of ZD1839 in rats. TPR2576, Serial 014.

Animal Rat (Alpk:ArfSD (Wistar derived))
Drug ZD1839, analytical reference number ADM35887E97.
Doses 0, 1, 5 or 25 (reduced to 15 in week 9) mg/kg/d
(0, 6, 30 or 150/90 mg/m²/d)
Group I, II, III and IV respectively
Dose volume 0.5 mL per 100 g body weight
Route PO gavage
Schedule Daily
Vehicle 0.5% w/v hydroxypropylmethyl cellulose (HPMC):0.1% w/v Tween 80.
Duration seven days per week for 6 months
N 20 per dose group per sex
plus 10 per sex at control & top dose for recovery (12 weeks post-dosing)
PK Blood samples (approximately 2 ml) were collected from three male and three female rats in Group III at 0.5, 1, 2, 4, 8 and 24 hours after dosing on day 1, at 0.5, 1, 2, 4, 8 and 24 hours after dosing from Groups II and III during week 26 and at 0.5, 1, 2, 4, 8, 24, 28, 32 and 48 hours after dosing from Group IV during week 26. Additional samples were taken from the Group II males at 0.5, 1, 2, 4 and 24 hours on day 180 because they were erroneously dosed at 5 mg/kg on day 175 (reviewed above).
Histopathology See table
Necropsy after 26 weeks dosing for 20 rats per sex per dose in all group
12 weeks after end of dosing for 10 rats per sex in control and high dose groups

Investigators at the Safety of Medicines Department of Zeneca Pharmaceuticals completed this study in November of 1998. The study director, Mr. C. D. Berent, signed the GLP statement.

Mortality:

Neither male nor female rats tolerated the 25-mg/kg dose so the investigators reduced it to 15-mg/kg/d in week 9. They had to kill one male rat humanly a week before the dose reduction and another in week 11 due to poor health despite the dose reduction. Two other deaths were incidental.

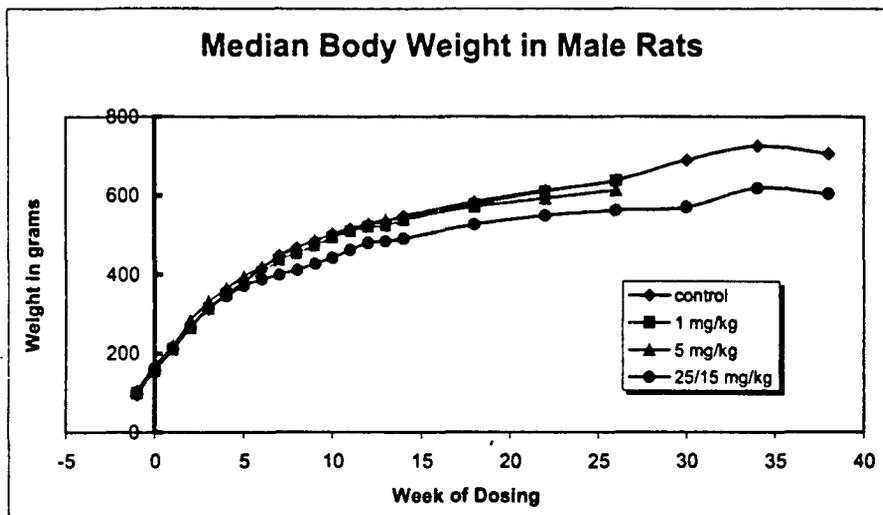
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Clinical Signs or Symptoms, Gross and Histopathology:

Parameter		1 mg/kg	5 mg/kg	25/15 mg/kg
Clinical signs	Twice daily	Similar to mid dose but lesser	Similar to high dose but lesser	coat abnormalities, skin lesions, tail lesions, curly whiskers and urine staining.
Body Wt.	Weekly to 14 wks then monthly			Males 10 to 17% < control from week 7 on Females 0 to 10% < controls from week six on
Food Cons.	Weekly to 14 wks then monthly			Males increased to 12% > control Females increased to 14%
Ophthalmology	Pre-study, week 15, 26, 30, 34, 38			keratitis (granular appearance of the cornea) in some females, reversible
Hematology	Wk 25 and 38			
White Count			Females 50% > control	Males increased 60% Females increased 160% Predominantly neutrophils, NCR
Red Cells				Females 5% < control, reticulocytes increased
Clinical Chem.	Wk 25 and 38			
Albumin			Slightly < control F	10 to 15% < control
ALT		-20% > control in males & females	-2X in males and females	-2.3X > control in males -2X in females, reversible
AST		-10% > control in females	-40% > control males -1.8X in females	-2.5X in males -1.7X in females, reversible
ALP			-2X increased in females	-50% > control in males, -2.5X > control in females, reversible
Total Bilirubin			33% > controls in F	2X > control in females
K ⁺				8% > control M, 16% F
Cholesterol			<10% increase males	25 to 35% < control in males
Triglycerides		10% < control M	-20% < control males	-30% < control in males
glucose			7% < control females	15% < control males, 20% females
Urine volume	Wk 25 and 38			2X > control in females with concomitant decreased SG, creatinine, & electrolytes
Rel. Kidney Wt		5.3% > control	8.8% > control males	24.5% > control males, NCR smaller changes in females
Rel. Liver Wt				11.4% > control in males 25.6% > control in females NCR
Rel. Ovary Wt		7% < control	10% < control	10% < control
Rel. Spleen Wt				32% > control males NCR 28% > control females reversible
Relative Heart Wt				14% > control in males 5% > control in females
Histopathology				
Eyes				mild corneal epithelial atrophy, reversible
Liver			Same as high dose but less severe	mild hepatocellular necrosis & eosinophilic sinusoidal macrophage infiltration seen in almost all males & females, not reversible
Kidney				papillary necrosis and papillary microlithiasis in females, not reversible
Spleen				mild/moderate diffuse extramedullary haematopoiesis, not reversible
Lymph node		lymphoid hyperplasia	lymphoid hyperplasia	lymphoid hyperplasia
Skin		folliculitis & scab formation	folliculitis & scab formation	Minor incidence of folliculitis and scab Formation, both sexes dose dependent

In this chart "reversible" means the changes were not seen at the end of the recovery period, "not reversible" or NCR (not completely reversible) means that some or all of the changes were still present at the end of the recovery period.

The following chart shows the mean body weight for males. Body weight decreased somewhat less in females than in males.



The high dose caused skin and tail lesions, subdued behavior and reduced body weight gain (about 10% in males after 26 weeks). White cell counts increased and red cell parameters decreased slightly. Plasma albumin decreased by as much as 20% in both sexes but total protein was the same as controls. Serum ALP, ALT, AST and total bilirubin all increased suggesting liver damage. Urine volume increased in females with a concomitant decrease in electrolytes, specific gravity and creatinine. Only red cell parameters and female urine volume had returned to normal at the end of the 12-week recovery period.

Gross Pathology and Histopathology:

Kidney, liver, heart and spleen weights increased and ovary weight decreased significantly. Microscopically, high-dose females showed signs of reversible corneal epithelial atrophy and kidney damage (papillary necrosis and papillary microlithiasis with some associated hemorrhage). In the liver, there was a drug-related increase in the incidence of hepatocellular necrosis and eosinophilic sinusoidal macrophage infiltration in both sexes. The number of corpora lutea in the ovaries diminished in high dose females. In the skin, there was drug related folliculitis, micro-abscesses and scab formation in both sexes. All these changes appeared reversible but were not completely absent at the end of the recovery period. The 5 mg/kg/day dose caused similar but less severe toxicity at the same sites. Body weight reduction was about 4% in males at 26 weeks. The 1 mg/kg/day dose caused minor skin and tail lesions and a slight (5%) increase in kidney weight. This dose was not a NOEL. Rats are less tolerant of ZD1839 than are mice when their response to drug doses is compared on a mg/m² basis.

The sponsor proposed a high dose for the 2-year carcinogenicity study of 12.5 mg/kg/day (75.0 mg/m²/day). This is half the dose that caused unacceptable morbidity or mortality by week nine in the six-month oral toxicity study in rats. This dose should produce a significant decrease in body weight gain at the end of the study and it is likely to cause significant pathology in the liver and kidney.

See the "pharmacokinetics and toxicokinetics" section above for the toxicokinetic information.

3) One month oral toxicity study in dogs. Study Number TAD/876.

Animal male and female beagle dogs (36 to 47 weeks old)
Drug ZD1839, analytical reference number ADM36757E96.
Doses 0, 2, 10 or 40 mg/kg/d
(0, 40, 200, or 800 mg/m²/d)
Dose Groups I, II, III, or IV
Dose volume 0.1 ml/100 g body weight
Route PO gavage
Schedule Daily
Vehicle 0.5% w/v hydroxypropylmethyl cellulose (HPMC):0.1% w/v polysorbate 80.
Duration seven days per week for one month
N Groups I and IV – 6 per sex (3 per dose group were killed after dosing and three after 4 weeks recovery)
Groups II and III – 3 per sex
PK In Group II at 0.5, 1, 2, 4, 8, 24 hours after the first dose
In all groups at 0.5, 1, 2, 4, 8, 24 hours after the "penultimate" dose
Histopathology See table
Necropsy 4 weeks (after dosing) or 8 weeks (recovery animals)

Investigators at the Safety of Medicines Department of Zeneca Pharmaceuticals completed this study in January 1997. The study director, Mr. A. W. Russel, signed the GLP statement.

Mortality: On day 18 of dosing, one high dose male was trembling, subdued, had an ocular discharge bilaterally and changes in skin tone. The animal was killed and necropsied. This was probably a dose related death.

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Clinical Signs or Symptoms, Gross and Histopathology:

Parameter	Schedule	Group I	Group II	Group III	Group IV
Clinical signs	Twice daily	No Symptoms	No Symptoms	No Symptoms	Loose feces (12/12), abnormal feces. Animals became cold and thin toward end of study (3/5 m, 2/6 f). 5/12 had reddened, partially closed eyes or a discharge
Body Wt.	Weekly	NC	NC	NC	Animals lost between 4 and 18% of day 1 body wt. More serious in male. All animals gained weight in recovery
Food Cons.	Daily				Consistent with weight loss, animals offered food supplementation still lost weight.
Ophthalmology	Pre-study, wks 2, 4, and 8				See table below
EKG	Pre-study, days 2-4, days 24-26 and day 52			Small changes consistent with those seen in high dose suggestive of a dose effect.	Lengthened PR in 2 of 12 large variability. Some QT prolongation and decreased heart rate and pressure. 2 nd degree heart block 1 male.
Temperature	Pre-study, days 2-4 & days 24-26	NC	NC	NC	NC
Hematology	Pre-study, days 29 or 30, day 53				
White Count	Day 29 relative to control			Increased 10 to 15% Neutrophils and lymphocytes	Increased ~25% in males and 10% in females reversible
Platelets	Day 29 relative to control			Increased ~25%	Increased > 25% did not reach significance because of variability
Red Cell Parameters	Day 29 relative to control			Decreased 10 to 14% reversible	Decreased 12 to 14% reversible, worse in females
Clinical Chem.	Pre-study, days 2-4 & days 24-26				
Albumin				~20% decrease	~45% decrease
Total Pi				~15% decrease	>25% decrease
Albumin/Globulin				Decreased ~10%	Decreased ~40%
ALT				Decreased 10 to 30%	Decreased 20 to 60% NCR
ALP				Decreased ~10%	Decreased ~50%
Total Bilirubin				Some decrease	Decreased 50 to 100%
Cl-				Increased ~5%	Increased ~10% reversible
Total Calcium			Decreased 5 to 8%	Decreased 10 to 12%	Decreased ~20% reversible
Cholesterol				Increased ~10%	Increased 20 to 30%, NCR
Urinalysis	Pre-study, days 2-4 & days 24-26			Minor changes of questionable significance, decreased Na ⁺	Minor changes of questionable significance, Decreased Na ⁺
Relative organ Wts			NC	No significant changes	No significant changes
Gross Pathology			NC	No significant changes	No significant changes
Histopathology					
Eyes					Corneal epithelial atrophy or ulceration 2M 2F. Epidermal micro-abscess of the eyelid 2M and 2F.
Kidney					Papillary necrosis 1 M
Spleen					Vacuolization 1 male
Thymus					2 male 1 female
Lymph node					Vacuolization 3 male 1 female

In this chart "reversible" means the changes were not seen at the end of the recovery period, "not reversible" or NCR means that some or all of the changes were still present at the end of the recovery period.

The study investigators compiled the following table of ocular signs and symptoms.

Table 5 Study number TAD/876. Ophthalmology - Incidence of observations in the 40 mg/kg/day group

Timepoint	Number of animals (number of eyes) affected				
	Pre-study	Week 2	Week 3*	Week 4	Week 8 (withdrawal)
MALES					
Number of animals (number of eyes) examined	6 (12)	6 (12)	1 (2)	5 (10)	3 (6)
OBSERVATION					
Area of diffuse corneal translucency	0 (0)	0 (0)	0 (0)	2 (3)	2 (4)
Rough appearance to whole cornea	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
Retention of Rose Bengal stain by cornea	0 (0)	NA	1 (1)	3 (5)	0 (0)
Speckled appearance to tear film or surface of cornea	0 (0)	3 (6)	1 (2)	5 (8)	0 (0)
Tear film moves slowly on blinking	0 (0)	2 (4)	1 (2)	2 (4)	0 (0)
Conjunctival reddening	0 (0)	2 (4)	1 (2)	1 (2)	0 (0)
FEMALES					
Number of animals (number of eyes) examined	6 (12)	6 (12)	0 (0)	6 (12)	3 (6)
OBSERVATION					
Area of diffuse corneal translucency	0 (0)	0 (0)	NA	4 (7)	2 (3)
Rough appearance to whole cornea	0 (0)	0 (0)	NA	0 (0)	0 (0)
Retention of Rose Bengal stain by cornea	0 (0)	NA	NA	4 (7)	0 (0)
Speckled appearance to tear film or surface of cornea	0 (0)	1 (2)	NA	1 (2)	0 (0)
Tear film moves slowly on blinking	0 (0)	0 (0)	NA	0 (0)	0 (0)
Conjunctival reddening	0 (0)	1 (1)	NA	1 (2)	0 (0)

NA Not applicable

* Unschedule examination of M834

Most of these changes were reversed after four weeks recovery but some corneal changes remained, though diminished, in 5 of 6 animals. These changes manifest mostly as corneal translucency.

The administration of 40 mg/kg/day of ZD1839 caused significant toxicity and caused the early death of one male dog. The other dogs in the high dose group lost weight during the course of dosing but recovered from most clinical signs and symptoms within thirty days after dosing. Dosing for a period of longer than a month at 40 mg/kg/day would probably cause unacceptable mortality.

At 40 mg/kg/d, ZD1839 caused an increase in PR interval in 2 of 12 with a very large variability in one male and one female that lasted over 2 hours. It caused some QT prolongation, decreased heart rate and increases in both systolic and diastolic pressure in some animals. It caused transient 2nd degree heart block 1 male. All these cardiac changes indicate significant interference with conduction across the atrio-ventricular node. The experiment provides too little information to fully characterize this toxicity. The authors postulate that EGF stimulates adenylate cyclase activity in the heart and causes the accumulation of cyclic AMP by means of the same G protein as isoproterenol. Inhibition of this EGF signal could account for the conduction failure. Nevertheless, in light of the fact that ZD1839 inhibits numerous other pharmacological sites and the evidence that it blocks the slow sodium channel, I suspect the mechanism for this toxicity may have little to do with EGF.

The investigators considered the decreases in albumin, ALP, and ALT a consequence of reduced food intake and weight loss. This seems a plausible explanation in the absence of any microscopic pathology in the liver or other major organs. And with frank liver damage, the values for ALT should increase. Nevertheless, liver damage is seen in other species. An alternative explanation would be decreased metabolic capacity, possibly secondary to ZD1839 competition at an undefined ATP site.

The changes in red cell parameters do not correlate with changes in the marrow. Thus, the origin of this toxicity remains unknown. Large concentrations of EGF are found in eye tissues and tears, thus, the ocular toxicity is possibly a direct result of the pharmacology of ZD1839. Likewise, the microscopic changes seen in the kidney of one dog are probably directly related to ZD1839 pharmacology as large concentrations of EGF are found in the kidney and the toxicity is seen in other species. The authors attribute the changes in the thymus and lymphoid tissue to stress. The changes in white cell parameters are unexplained, but are seen in other species. The authors consider 10 mg/kg/day an NOAEL; I do not. There were many changes in the low and mid dose group that were too small to easily quantify but that were in the same direction as the changes seen in the high dose group. Most of the results above strongly suggested a dose effect.

See the "pharmacokinetics and toxicokinetics" section above for the toxicokinetic information.

3) Six month oral toxicity study in dogs. Study Number TAD/877.

Animal	male and female beagle dogs (8 to 11 months old)
Drug	ZD1839, analytical reference number ADM35887E97.
Doses	0, 1, 5 or 25 (reduced to 15 on day 11) mg/kg/d (0, 20, 100, or 500 reduced to 300 mg/m ² /d)
	Dose Groups I, II, III, or IV
Route	PO gavage
Schedule	Daily
Formulation	Brown film coated tablets. Gelatin capsules were filled weekly with the appropriate number of tablets.
Duration	6 months
N	Groups I and IV – 7 per sex (4 per dose group were killed after dosing and three after 12 weeks recovery) Groups II and III – 4 per sex
PK	In Group II and IV at 0.5, 1, 2, 4, 8, 24 hours after the first dose (day 1) on 4 dogs/sex In Groups II, III, and IV at 0.5, 1, 2, 4, 8, 24 hours after the dose on day 182 on 4 dogs/sex. Additional samples were taken at 28, 32 and 48 hours post-last dose from 3 male and female withdrawal animals in group IV.
Histopathology	See table
Necropsy	Six months (after dosing) or after 12 weeks recovery (recovery animals)

Investigators at the Safety of Medicines Department of Zeneca Pharmaceuticals completed this study in July 1998. The study director, Mr. A. W. Russel, signed the GLP statement.

Mortality: Group IV female #547 lost 1.4 kg body weight by day 10. This dog was thin and had loose feces and so was killed and examined. Group III male #587 was extremely subdued, hunched, trembling and shaking, had red gums and red colored abnormal feces on day 120. The animal was killed and examined.

Clinical Signs or Symptoms, Gross and Histopathology:

Parameter	Schedule	Group I	Group II	Group III	Group IV
Clinical signs	Twice daily		Abnormal gums (red in appearance) 3/8, loose feces 1/M	Abnormal gums (red in appearance) 4/8; subdued, trembling, shaking, hunched, thin & discharge from eye 1 M; loose feces 4/4 M, 1/4 F.	Hair loss & rough/coarse coat 13/13. Abnormal gums (red in appearance) 12/13, loose feces 13/13; discharge from eye 6/7 M, 6/6 F. More severe in males.
Body Wt.	Weekly		NC	NC	< control 8% M, 7% F, NCR.
Food Cons.	Daily				Consistent with wt loss.
Ophthalmology	pre-study & wks 5, 13 & 26 during TX & wks 4, 9, & 12 of withdrawal			Corneal lesions 3/4 M, NCR	Corneal lesions 13/13, NCR
EKG	pre-study & weeks 13 & 25 during TX and wk 30 of withdrawal				P-R prolongation 1 F
Hematology	pre-study & wks 13 & 26 during TX, wks 30 & 38 withdrawal.				
White Count					16 & 70% increase wks 13 & 26
Clinical Chem.	pre-study & wks 13 & 26 during TX, wks 30 & 38 withdrawal.				
Albumin				Decreased 10% M	decrease 20 to 25% M & F
Albumin/Globulin				Decreased 10%	Decreased 30%
ALT					Decreased 17% and 34% M
Total Calcium					Decreased 10% M & F
Creatinine					Decreased to 20 % M & F
Urinalysis	Pre-study, days 2-4 & days 24-26		NC	NC	NC
Organ Wts					
Brain absolute					14.8% decreases in M
Relative liver Wt					Increased 14.7% in M & 10.8% in F
Rel thyroid Wt					Increased 33% in F
Relative lung Wt				Increased 14.4% M	Increased 18.4% M
Gross Pathology				Did not establish cause of death in moribund M	Did not establish cause of death in moribund F
Histopathology				Did not establish cause of death in moribund M	minimal renal papillary necrosis in moribund F
Eyes lids				sebaceous adenitis of the tarsal gland	sebaceous adenitis of the tarsal gland, NCR. Granulomata and micro-abscesses involving hair follicles
Eyes				Corneal epithelial atrophy 2/3 M	corneal epithelial atrophy & limbal inflammatory cell infiltration
Liver			pigment deposits	pigment deposits	pigment deposits, severity increased with dose intensity
Ovaries				multiple, bilateral, large, partly luteinised follicular cysts 1/4	multiple, bilateral, large, partly luteinised follicular cysts 1/6
Skin					degeneration & reduction in number of hair shafts

In this chart, changes were reversible or recovered unless otherwise noted, "NCR" means that some or all of the changes were still present at the end of the recovery period. Changes are for male and female unless otherwise noted.

The corneal lesions consisted of an horizontal area of corneal translucency occurring bilaterally in the ventro-lateral quadrant, accompanied in many cases by conjunctival reddening. They first appeared in week 5 as faint lesions in Group IV dogs. In six animals dosed at 15 mg/kg/day, the

translucencies had progressed by week 26 into corneal opacities, 2 of which retained rose bengal in part of the lesion. No lesion retained fluorescein. The opacities were not reversible but translucencies not associated with opacity and conjunctival reddening were.

Increased variability and prolongation of P-R interval (first degree atrio-ventricular heart block), and several incidents of isolated P waves (second degree AV heart block) were observed in one Group IV female during week 25. First-degree AV heart block was also seen in this animal during the repeated recordings during week 26, again at pre-dose only.

Iressa caused an increase of between 16 and 70% in total white cell count in both sexes dosed at 15 mg/kg/day at weeks 13 and 26. In males, these increases were attributable to a rise in both neutrophils and lymphocytes, while, in the females, it appeared to be associated only with an increase in neutrophil count. White cell counts returned to normal by the fourth week of recovery.

Plasma albumin concentration was decreased 20 to 25% in high dose males and females at weeks 13 and 26. Plasma albumin concentration was 10% lower than controls or pre-dose values in mid-dose males in week 13, but was recovering toward normal at the end of dosing. Decreases in total plasma protein were discernable in high dose dogs in week 13 but did not reach significance. The change in albumin-globulin ratios was a consequence of the decrease in albumin. The reductions of approximately 10% seen in plasma total calcium for both sexes dosed at 15 mg/kg/day at weeks 13 and 26 of the study are directly related to the reduction in plasma albumin. These changes were reversible. Male and female high dose dogs showed a reduction of 10 to 20% in plasma creatinine at weeks 13 and 26. High dose males showed a reduction of 17% and 34% in plasma ALT activity weeks 13 and 26 respectively. All these changes are consistent with a reduction in general liver function, but the microscopic changes in the liver were minimal.

See the "pharmacokinetics and toxicokinetics" section above for the toxicokinetic information.

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Toxicology Summary:

The following table summarizes the results of single dose toxicology studies.

Summary of the Single Dose Toxicology Studies						
Species (Study No.)	Route	N/sex/dose	Critical Dose(s)	mg/kg	mg/m ²	Significant findings
Mouse (TLM/958)	Gavage	5	MTD NOAEL	> 2,000 ≤ 2,000	> 6,000 ≤ 6,000	6,000 mg/m ² : no deaths occurred and no clinical signs observed for up to 13 days. No Histopathology was done.
Rat (TLR/2571)	Gavage	5	Lethal	2,000	12,000	12,000 mg/m ² : 4/10 deaths (3F, 1M). clinical signs occurred by d5: hunched posture (10/10), loss of skin tone (10/10); piloerection (10/10), subdued behavior (6/10), trembling/shaking (1/10) and urinary staining (4/10). Histopathology showed discoloration of adrenal glands and adrenal medullary vacuolation; myocarditis; necrosis and ulceration in GI tract; mucosal atrophy or necrosis or both in the trachea; multifocal hemorrhage in lung; multifocal hepatocyte necrosis; microabscess formation on the skin; splenic atrophy.

The following table summarizes the results from multi-dose toxicity studies in mice, rats and dogs.

Summary of the Multiple Dose Toxicology Studies						
Species (Study No.)	Route & Duration	N/sex/dose	Critical Doses	mg/kg/d	mg/m ² /d	Significant findings
Rat (TAR/2492)	Gavage 14-days	5	LD ₁₀ ^a	50	300	750 mg/m ² /d: 3/5 females died; stains around muzzle (10/10), red exudate around eyes (9/10), hunched posture (10/10), loss of skin tone (9/10), subdued behavior (4/5 F); ↓ body weight. (28% for M and 35% for F). ↓ Food intake (29% for M and 69% for F) by d15. ↑ WBC, NEUT, PLT; ↑ blood urea, ALP (F), ALT, TG and AST. Dry and rough corneal surface. Enlarged adrenals; ↑ organ weight for adrenals; thymus atrophy; abnormal microscopic findings in the adrenals, small intestine, kidneys, liver, lungs, lymph nodes, ovaries, skin, spleen and thymus.
			NOAEL	10	60	300 mg/m ² /d: no deaths. Stains around muzzle (5/5 F), red exudate around eyes (7/10), loss of skin tone (1/5 F), subdued behavior (1/5 F). Minor decrease in body weight gain (6%) and food intake (12-19%) by the end of the dosing. ↑ WBC and NEUT. Dry and rough corneal surfaces in female rats. Abnormal microscopic findings in small intestine, kidney (F only), liver, lymph nodes and skin.
			NOAEL			10 mg/m ² /d:
Rat (TGR/2616) ^a Seg. I	Gavage 21-days	10	LD ₁₀	< 40	< 240	240 mg/m ² /d: 1/10 was killed on D9 and 9/10 were killed on d11 primarily because of 6.5% wt. loss (less tolerance for wt loss than in other studies). Clinical signs: urine staining, loose feces, hunched posture, piloerection, discharge from eyes, and partially closed eyes. ↓ food intake (21%).
			LD ₁₀	> 20	> 120	120 mg/m ² /d: No deaths occurred. 1.5% wt loss in first week. Scabs around the mouth and nose. Loose feces.
			NOAEL			60 mg/m ² /d: No deaths. Same as 120 but less severe. 12 mg/m ² /d:
Rat (TAR/2570)	Gavage 1-month	10-15	LD ₁₀	> 40	240	240 mg/m ² /d: no death; clinical signs: eyes partially closed, slight inflammation and encrustation of the ankles and toe joints; red exudate, and scab on mouth; ↓ body weight (12-19%) on d29; ↓ body weight by 16% for F on d57; ↓ food intake (7-25%) on d22; ↑ WBC (126-142%) and NEUT (400-546%) on d30; ↓ Alb (16%) and A/G (22-24%) on d30; ↑ ALT (32-46%) and AST (29-55%); granular appearance of the cornea (10/60); discolored papilla in kidney (2/10 F); enlarged lymph nodes (17/20); discoloration of the skin (8/20); scab in skin (5/20); enlarged spleen (4/20); ↑ relative organ weight:

			NOAEL	10	60	adrenals (19% in M); kidney (10-13%); thymus (13-16%); liver (15% in F); ↓ relative organ weight: ovary (21%), uterus (24%) & prostate (12%); histopathology: corneal epithelial atrophy, papillary necrosis or inflammation of kidney; focal folliculitis; scab formation on skin; abscesses; extramedullary hematopoieses and reactive lymphoid hyperplasia in lymph nodes 60 mg/m²/d: 12 mg/m²/d:
Dog (TAD/876)	Gavage 14-days	1	Lethal	75	1,500	1,500 mg/m²/d: 1 female died on d11. Loose feces, emesis, reddened eyes, reddened inside of the mouth, thin, eyes partially closed. ↓ body weight by 22% for female on d10 and by 13% for male d3. ↓ food intake by 59-61% during wk1 and 66% (male) during wk2. ↓ RBC and HB (8-19%); ↑ WBC and NEUT (16-30%); ↓ Alb (25-41%) and ALP (19-36%); ↑ ALT (30-70% for M and 183-155% in F) and blood urea (32-83%). Diffuse staining of the cornea surface, corneal epithelial atrophy; germinal center vacuolation in lymph nodes and spleen; thymus atrophy, and multifocal epidermal microabscesses. 1,000 mg/m²/d: emesis, loose feces, reddened eyes and reddened inside of the mouth; ↓ body weight (10% for males on d3). ↓ food intake by 43-60% during wk1; ↓ HB (9-10%) on d14. ↓ Alb (13-41%); ↓ ALP (29-39%); ↓ ALT (25-51%); ophthalmology abnormalities; corneal epithelial atrophy, germinal center vacuolation in lymph nodes & spleen; diffuse thymus atrophy, & multifocal skin microabscesses.
			NOAEL	10	200	200 mg/m²/d:
Dog (TAD/876)	Gavage 1-month	3	Lethal	40	800	800 mg/m²/d: one death (1/3 F); clinical signs during the study: loose feces, thin, reddened mouth or eyes, discharge from eyes, partially closed eyes; emesis, eyelids swollen, sore in mouth, cold, subdued, trembling and loss of skin tone; clinical signs during recovery period: loose feces, thin, reddened mouth or eyes, discharge from eyes, corneal damage, partially closed eyes and sore in mouth; ↓ in cumulative body weight gain (0.9-2.0 kg) on d31; ↓ in food intake (40-100%) from wk1-5; lengthened PR intervals in EKG at wk4; ↓ RBC (8-11%) and HB (7-13%) on d30; ↑ WBC (6-27%) and NEUT (30% M); ↓ Alb (40-47%), A/G (39-48%), ALT (18-58%) and ALP (44-46%) on d30; ↑ TCHOL (20-36%) and TRG (10-58%) on d30; ↑ urine NAG (185-539%), ↓ urine K+/creatinine (42-77%), and ↓ urine Na+/creatinine (79-92%) on d30 200 mg/m²/d: no death or clinical signs, ↓ Alb (20-23%), A/G (16-19%), ALT (17-301%); ↓ urine Na+/creatinine (87-96%) on d30 40 mg/m²/d: Not a no effect level. Slight changes consistent with those described above suggestive of a dose effect.
			HNSTD	10	200	
Mouse THM/1227	Gavage daily for 13 weeks	10	HNSTD	175	525	525 mg/m²/d: Clinical signs: Dose related alopecia less severe in males, partially closed eyes (9/10 M, 8/10 F) with swollen or encrusted eyelids. Decreased body wt gain (37% M) Absolute spleen wt increased (120% M, 50% F). Absolute liver wt increased (31% M). Histopathology: lungs - Macrophage infiltration without reaction, about the same as the Mid dose. Liver - Macrophage infiltration, dose dependant. Skin - Chronic active folliculitis, associated with alopecia. 375 mg/m²/d: Clinical signs: Dose related alopecia less severe in males, partially closed eyes (8/10 M, 8/10 F) with swollen or encrusted eyelids. Decreased body wt gain (33% M, 15% F). Absolute spleen wt increased (52M). Absolute liver wt increased (17M). Histopathology: lungs Macrophage infiltration without reaction. Liver - Macrophage infiltration. Skin - Chronic active folliculitis, associated with alopecia. All

						greater than low dose.
						150 mg/m²/d: Clinical signs: partially closed eyes (3/10 M) with swollen or encrusted eyelids. Histopathology: lungs Macrophage infiltration without reaction. Liver - Macrophage infiltration. Skin - Some animals with chronic active folliculitis, associated with alopecia.
Rat TPR2576	Gavage daily for six months	20	Lethal	25 reduced to 15	150 reduced to 90	150/90 mg/m²/d: Clinical signs: coat abnormalities, skin lesions, tail lesions, curly whiskers and urine staining. Body wt. Males 10 to 17% < control from week 7 on, Females 0 to 10% < controls from week six on. Eyes - keratitis (granular appearance of the cornea) in some females, reversible. WBC - Males increased 60%, Females increased 160%, Predominantly neutrophils, not completely reversible. Alb 10 to 15% < controls. ALT -2.3X > control in M, -2X in F, reversible. AST -2.5X in M, -1.7X in F, reversible. ALP -50% > control in M, -2.5X > control F, reversible. Billi 2X > controls in F. K+ 8% > control in M, 16% in F. Cholesterol 25 to 35% < control in M. Urine Vol. 2X > controls in females with concomitant decreased SG, creatinine, and electrolytes. Rel Wt of kidney liver and spleen 10 to 32% increased. Rel Wt of heart increased 5 to 15% more in males. Rel. Wt of Ovaries decreased 10%. Histopathology: eyes - mild corneal epithelial atrophy, reversible. Liver - mild hepatocellular necrosis & eosinophilic sinusoidal macrophage infiltration seen in almost all males & females, not completely reversible. Kidney - papillary necrosis and papillary microolithiasis in females, not completely reversible. Lymph nodes - lymphoid hyperplasia. Skin - Minor incidence of folliculitis and scab formation, both sexes dose dependent
			HNSTD	5	30	30 mg/m²/d and 6 mg/m²/d: All the toxicities in these groups were similar to those in the high dose group but lesser in severity. 6 mg/m²/d: not a NOAEL but all the toxicities were near the limit of detection.
Dog TPD877	Gavage for six months	7 control and HD 4 low and mid dose	Lethal Dose reduction day 11 Lethal NOAEL	25 15 5 1	500 300 100 20	300 mg/m²/d: One female lost 1.4 kg body weight by day 10. This dog was thin and had loose feces and so was killed and examined on day 11. The dose was reduced to 300 mg/m ² /d after day 11. Toxicities were similar to those seen at the high dose in the one month studies but considerably less severe. 100 mg/m²/d: One male was extremely subdued, hunched, trembling and shaking, had red gums and red colored abnormal feces on day 120. The animal was killed and examined. 20 mg/m²/d:

^a this is a reproductive toxicity study conducted on female rats;

^b approximate LD₁₀ based on MTD for the female, this 14 study did not have a recovery observation period.

For analysis of these findings see the overall summary below.

Genetic Toxicology:

- 1) Zeneca ZD1839 + 4 Isomer : Bacterial Mutation assay in *S. typhimurium* and *E. coli*. Study Number YV6075. AZ reference number TMV/1008. Submitted to IND [redacted] submission number 526, page 1.

[redacted] did this GLP study for AstraZeneca to qualify an impurity that forms during the synthesis of ZD1839, that is [redacted]. The nominal amount of the [redacted] was [redacted]%; the assayed amount actually used in the experiments was [redacted] w/w. This is a standard Ames assay with *S. typhimurium* test strains TA1535, TA1537, TA98 and TA100 and *E. coli* strains WP2P and WP2P uvrA. The protocol incorporated all appropriate positive controls. To test for activation the chemical mixture was tested with and without S9 (phenobarbital and β -naphthoflavone induced) in the top agar. The vehicle control was DMSO.

In an initial dose range finding experiment with strain TA100, the chemicals precipitated at doses of 1000 μ g/plate, the bacterial lawn was absent at higher concentrations (to 5000 μ g/plate). The subsequent definitive tests were done using doses of 5 to 1000 μ g/plate, with and without S9, three plates per dose. The number of revertants declined in some tests at 200 or 500 μ g/plate or both because of toxicity. The number of revertants did not increase in any of the tests at any dose with or without S9. All positive controls caused the anticipated increase in revertants. Thus, ZD1839 + [redacted] of its [redacted] does not cause mutations in the standard Ames test strains.

- 2) Zeneca ZD1839 + 4 Isomer : In vitro cytogenetic study using cultured human lymphocytes study TYX123. Submitted to IND [redacted] submission number 526, page 33.

[redacted] did this GLP study for AstraZeneca to qualify an impurity that forms during the synthesis of ZD1839, that is [redacted]. The nominal amount of the [redacted] [redacted]%; the assayed amount actually used in the experiments was [redacted] w/w. This is a standard *in vitro* assay using cultured human peripheral lymphocytes with and without S9 (induction not specified). The positive control was cyclophosphamide with S9, and mitomycin C without. The vehicle control was DMSO. In the presence of S9, the cultures were incubated with the test compound for three hours; without S9 some were incubated for three hours and a second set was incubated for twenty hours. At the end of the exposure period the cells were exposed to colcemid and evaluated microscopically for mitotic index and chromosomal aberrations.

In an initial dose range finding study, concentrations of 24 and 32 μ g/ml caused marked decreases in the mitotic index (60 and 29 percent of vehicle control respectively in the absence of S9). In the definitive assay the investigators used concentrations of 8, 12, and 24 μ g/ml in the absence of S9 and 6, 8, and 12 μ g/ml in the presence of S9. The high dose was lowered to 16 μ g/ml in the second assay.

In the first assay cells from two male donors were used; cells from each donor were analyzed separately. In this assay there were no toxicologically or statistically significant increases in the number of cells with structural chromosomal aberrations under with or without S9 activation.

In a second confirmatory assay, cells from one male and one female donor were used. In this experiment, cells treated for 20 hours in the absence of S9 showed the following percent abnormal cells (excluding gaps); 0.0, 1.5, 0.5, and 2.5% for concentrations of 0, 8, 12, and 16 μ g/ml respectively. If gaps are included the results change only in the vehicle control to 0.5%. The result at the highest dose rose to statistical significance ($p = 0.05$, one-sided Fishers exact test). Nevertheless, there is no dose response and the values fall well within the laboratories historical solvent control values. The investigators conclude that this result is not toxicologically relevant and that ZD1839 plus [redacted] not clastogenic to cultured human peripheral lymphocytes. I agree.

Summary:

Both of these studies were valid and appropriately conducted. They showed that the — of ZD1839, a contaminant formed during synthesis, is not mutagenic in the Ames test or clastogenic to cultured human peripheral lymphocytes.

Dr. Zheng reviewed the remainder of the genotoxicity studies. See below.

Labeling recommendations:

Comments on the lack of mutagenicity of the — are not necessary in the label.

Carcinogenicity:

The sponsor has yet to complete any carcinogenicity studies but plans to do so. They brought their proposed protocols and doses before the ExecCAC in March of 2002 and received concurrence. The minutes of the ExecCAC are appended below.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Dr Zheng reviewed several of the pertinent reproductive and developmental toxicity studies in his earlier reviews of IND [redacted] I have included a summary of his results in the overall summary.

I will review two definitive Segment II studies in rats and rabbits in a subsequent supplement. I have included information from these studies in the summary below.

DETAILED CONCLUSIONS AND RECOMMENDATIONS:

The following summary includes information reviewed by Dr. Hua Zheng.

Pharmacology

EGF is a 53 amino acid protein that stimulates the growth of a variety of epithelial cells, including epidermal cells and gastric epithelium. Epidermal growth factor receptor (EGFR) was the first receptor protein recognized to be a tyrosine-specific protein kinase (see G. Carpenter, *Annu. Rev Biochem* 56:881-914, 1987 for a review). Since that time the number of receptor molecules identified as tyrosine-specific protein kinases has grown to include receptors for platelet-derived growth factor (PDGF), fibroblast growth factors (FGFs), hepatocyte growth factor (HGF), insulin, insulin like growth factor-1 (IGF-1), nerve growth factor (NGF), vascular endothelial growth factor (VEGF) and macrophage colony stimulating growth factor (M-CSF). The names of these transmembrane receptor tyrosine kinases usually derive from the tissue from which they were first isolated. Nevertheless, the name should not imply that those tissues are the only ones where the various receptors are found. The mechanisms of action for these receptor tyrosine-specific kinases are for the most part similar. The following discussion will use EGFR as the paradigm to illustrate the mechanism of action of this family of receptor proteins.

EGF binds to the extracellular domain of EGFR, a large (about 1200 kD) single-pass transmembrane receptor protein. When two receptor protein in close proximity bind EGF (or other

ligand for the other receptors), they form a dimer on the intracellular side. The two components of the dimer phosphorylate each other at multiple sites in a process called autophosphorylation. A complex menagerie of intracellular signaling proteins then binds to the various phosphorylated receptor sites, which in turn phosphorylate the signaling proteins. Many of these signaling proteins are members of the Ras family. They initiate a cascade of signals within the cellular cytoskeleton and the nucleus that regulates the process of cell division. Inhibition of this signal process at many different points in the cascade can prevent cell division. The other receptor tyrosine kinases control cell division or other cell functions similarly. Cross signaling among the downstream signaling proteins is considerable.

Many human tumors overexpress EGFR and other receptor tyrosine-specific kinases. This overexpression often correlates with a poor clinical prognosis. Transfection of cells with genes that produce dominant negative mutants of EGFR in high copy number blocks the EGF signal and prevents cell division. Blockade of EGFR expression with antisense nucleotides also prevents cell division. This blockade manifests as growth retardation in cultured cells. Experiments have shown that blocking the binding of EGFR receptor to prevent EGF binding with antibodies specific to EGFR can cause similar growth retardation.

AstraZeneca developed ZD1839 by testing the effectiveness of various synthetic 4-anilinoquinazolines for activity against KB oral carcinoma cells *in vitro* and as tumor implants (A Baker *et al. Bioorganic & Medicinal Chemistry Letters* 11 (2001) 1911 – 1914). This molecule shares structural characteristics with other tyrosine kinase inhibitors. ZD1839 probably inhibits tyrosine kinases by disrupting the dimerization of the intracellular domains. It binds competitively at the ATP site.

Despite claims to the contrary in both the NDA submission and in the literature, the data does not support the concept that ZD1839 binds specifically at the EGF Receptor. In HUVEC cells, the IC_{50} for inhibition of EGF stimulated growth differed from that of inhibition of VEGF, and FGF by less than an order of magnitude (0.03 to 0.1 μM versus 1 to 3 μM respectively). In NIH 3T3 cells the IC_{50} for inhibition of PDGF stimulated growth by ZD1839 was 0.04 μM , that for IGF was 0.047 μM and that for EGF was 0.22 μM . The following table provides a few of the many examples of the variability of ZD1839 inhibition.

Cells	Growth factor/Stimulation	ZD1839 IC_{50} (μM)
HUVEC ^b	EGF	0.03-0.1
HUVEC ^b	FGF	1-3
HUVEC ^b	VFGF	1-3
NIH 3T3 (morphology)	H-ras	> 10
NIH 3T3 (proliferation)	H-ras	~5
NIH 3T3	PDGF	0.04
NIH 3T3	FGF	3.4
NIH 3T3	EGF	0.22
NIH 3T3	IGF	0.047
NIH 3T3	Lysophosphatidic acid (LAP)	0.05

These results suggest that ZD1839 inhibits phosphorylation at many sites on proteins of the intracellular transmembrane tyrosine-specific protein kinase family. Maximum plasma concentrations resulting from clinically relevant doses are 0.5 to 1 μM or more, well within the range of many of these IC_{50} values. Cellular susceptibility to ZD1839 inhibition possibly correlates with the expression of downstream signal proteins or other factors more closely than it does with the specific tyrosine protein kinase.

Safety Pharmacology

At physiological concentrations ZD1839 caused dose dependant prolongation of the Purkinje action potential *in vitro* of as much as 10 ms at 2.5 μM . This effect was not reversible after a 30-minute washout period. This inhibition is probably related to inhibition of the slow potassium rectifier, I_{Ks} . Concentrations of about 1 μM *in vitro* cause half-maximal inhibition. Inhibition of this channel can cause delayed repolarization, QT_c prolongation, and in severe cases, arrhythmias leading to Torsade-du-

pointes. At a concentration of 10 μM , inhibition was almost 100%. *In vivo* in dogs, at a dose of 1000 mg/m^2 , ZD1839 caused systolic and diastolic hypotension (about 18% below controls). The effect was maximal at about 3 hours post dosing, a time that corresponds to T_{max} in the dog. It persists for over 21 hours, or about four half-lives, suggesting tight binding. The concentrations that cause this effect are about 6 to 10 times greater than those seen in humans at the clinical dose based on C_{max} . The pressure drop caused a transient compensatory tachycardia followed by prolonged bradycardia. Dosing with ZD1839 caused mild QTc prolongation (>10%) at both doses in some dogs. Thus ZD1839 has measurable effects on heart rate and blood pressure at doses less than one order of magnitude above the clinical dose. This toxicity is possibly related to direct interference with the slow potassium channel (IKr), to interference with cellular metabolism, or other secondary pharmacology or a combination of these. It may also result from interference with a signal mediated by angiotensin II through its interaction with EGFR (S. Kagiya *et al. Circulation* 2002 Aug 20;106(8):909-12). This effect is possibly the source of the increased heart weights seen in rats after prolonged dosing. Nevertheless, single doses of 600 mg/m^2 did not cause changes in heart rate or blood pressure in spontaneously hypertensive rats. And *in vitro* ZD1839 at 10 μM had no agonist or antagonist activity in the guinea-pig right or left atria. In normal rats, single doses of 300 and 3000 mg/m^2 caused mild dose dependant hypotension consistent with the results in the dog.

ZD1839 causes significant inhibition of numerous pharmacologically important receptor sites. The mechanisms behind these inhibitions have yet to be studied but they possibly include decreased kinase activity, direct interaction with tyrosine sites or direct interaction with ATP or phosphate binding sites. The following table shows but a few of these interactions. Note that adrenergic sites, serotonin sites, and dopamine sites are affected.

ABOVE PRIMARY TESTS IN RANK ORDER OF POTENCY							
PRIMARY							
CAT. #	RADIOLIGAND ASSAY	SPECIES	CONC.	% INH.	IC_{50}°	K_i	ρ_{H}
252000	Monoamine Transporter		10 μM	84	1.44 μM	1.32 μM	0.979
220100	Dopamine D_{2L}		10 μM	69	3.39 μM	1.36 μM	0.656
254000	Muscarinic, Non-Selective		10 μM	73	4.4 μM	1.45 μM	1.19
271700	Serotonin 5-HT _{2B}		10 μM	59	3.75 μM	2.39 μM	0.583
279500	Sodium Channel Site 2		10 μM	71	3.35 μM	3 μM	0.472
278300	Sigma, Non-Selective		10 μM	73	3.66 μM	3.55 μM	1.08
204010	Adrenergic β_1		10 μM	56	6.18 μM	3.57 μM	0.561
271210	Serotonin 5-HT _{1B}		10 μM	59	6.16 μM	3.77 μM	0.718
278200	Sigma σ_2		10 μM	59	6.83 μM	4.21 μM	0.489
220320	Dopamine Transporter		10 μM	64	5.33 μM	4.23 μM	0.975

In vitro ZD1839 at 10 μM had no agonist or antagonist activity guinea-pig ileum. Single PO doses of ZD1839 at 100 mg/kg did not effect gastrointestinal motility in the mouse. These results suggest a lack of cholinergic interaction. Single PO doses of ZD1839 at 100 mg/kg caused no stimulant or depressant activity, or any effects on a range of other behavioral parameters in the mouse. It caused no bronchoconstrictor or bronchodilator activity in the anesthetized guinea-pig, and had no effect on the delayed hypersensitivity response to Freund's adjuvant in the mouse.

Rats given ZD1839 at 100 mg/kg PO showed significant decreases in the excretion of water, sodium, chloride and urine osmolality. PK Carmines *et al. (Hypertension* 2001 Feb;37(2 Part 2):569-73) have shown that angiotensin II signaling in renal afferent and efferent arteriolar vascular smooth muscle is either mediated or modulated by tyrosine kinase activity, including that of the epidermal growth factor receptor tyrosine kinase. The 100 mg/kg dose also caused statistically significant inhibition (16%) of acute inflammatory response to carrageenin.

In studies of effects on the respiratory system, doses of 300 and 3000 mg/m² caused a mild dose related decreases peak inspiratory flow, peak expiratory flow, tidal volume and minute volume in rats. Numerous papers have demonstrated various roles for tyrosine kinase signaling in pulmonary function, usually involving VEGF or HGF. Inhibition of signaling at these sites could account for the mild pulmonary toxicity. It could also contribute to pneumonitis sometimes observed clinically.

Single doses of 300 and 3000 mg/m² also caused slight reduction in motoractivity (CNS toxicity) in mice and rats. The clinical significance of this toxicity is unknown.

Pharmacokinetics and Toxicokinetics

The absorption of ZD1839 after oral dosing is relatively slow. T_{max} ranged from 2 to 8 hours, in rats, 1 to 6 hours in dogs and 3 to 6 hours in humans. In rats, bioavailability was greater at 12.5 mg/kg (66% to 88% in females) than at 5-mg/kg, (39% to 50% in females). ZD1839 is extensively metabolized in the liver, so this result suggests saturation of a metabolic pathway during first pass. The possibility of a dose dependant increase in absorption is more remote. In rat study TAR/2492, AUC increased hyperbolically between the low and mid dose suggesting a first pass effect. This effect was not seen in dogs, but absorption in the dog showed greater variability. This variability decreased when dogs were dosed after a meal, but there was no obvious feeding effect on AUC or C_{max}. In humans, oral bioavailability was about 60% in fasted volunteers and in cancer patients. AUC and C_{max} were linear (r² > 0.98 in Trial 33). But in humans given 250 mg after a high fat meal, AUC increased by 33% and C_{max} increased by 29%.

Rats and dogs eliminate less than 7% of a total radioactive single dose in the urine. Ninety percent or more of an oral dose is recovered in the feces in both species. Results are similar for humans. Studies in cannulated rats suggest little enterohepatic recirculation. Only small amounts (<10%) of parent drug were found in the bile of cannulated rats suggesting that elimination in rats is dependant on metabolism. As one would expect with excretion dependant on metabolism, excretion is slow. The amount of total radioactivity recovered at 24 hours was about 70%, 40% and 50% in rat, rabbit and dog respectively. Recovery of a single dose was complete in rats and rabbits after five days but was incomplete in dogs and humans after seven to ten days. This slow elimination manifests in relatively long half-lives. The following table provides a comparison of major pharmacokinetic parameters in rats, dogs and humans.

	Study	Clearance ml/min/kg	V _{ss} L/kg	t _{1/2} hr
Male Rat	KKR008	42 to 25	9 to 10	3 to 14
Female Rat	KPR055	24 to 16	8 to 10	5 to 8
Dog	KPD050	11 to 16	2 to 6	3 to 8
Human	IL/0035	12	28	47

Human data is normalized for a 50-kg person.

The table shows that rats clear the drug almost at the rate of hepatic blood flow. The values are two to four-fold higher than those for humans and dogs. This is inconsistent with the observation that rats are more sensitive to ZD1839 toxicity and suggests the influence of an active metabolite. At least five active metabolites have been identified in human plasma. The very long half-life in humans is reflected in the more than three fold greater volume of distribution. The unusually large volumes of distribution are consistent with the large tissue-to-blood concentration ratios seen in distribution studies in the rat. ZD1839 rapidly partitions into the highly metabolic tissues.

In cancer patients given repeated daily doses, concentrations of parent drug reach steady state in seven to ten days. These concentrations are two to six-fold higher than those achieved by a single dose. C_{max} in humans reaches μM concentrations after 14 days of dosing (trial 005) and AUC reaches 16

$\mu\text{M}\cdot\text{hr}$ at a dose of 300 mg/day. Binding of ZD1839 at the intracellular active site is competitive so one would expect AUC to drive the process. This coupled with the long half-life suggests that inhibition should approach 100% *in vivo* at the clinical doses in any system where the effective k_i was 0.2 μM or less. Repeat dosing causes similar increases in C_{max} and AUC in rats and dogs. In the rat, both AUC and C_{max} were frequently higher in females. This is consistent with the observation that females seemed more sensitive to ZD1839 toxicity (below). This sex difference likely has little clinical relevance.

ZD1839 binds extensively to plasma proteins, 86 to 94% in all species studied including humans. ZD1839 binds both human serum albumin (HSA) and human α -1-acid glycoprotein (AGP) and binding was saturable. High AGP concentrations in cancer patients may cause a decrease in the free concentration of ZD1839.

Quantitative whole body autoradiography of rats demonstrated that ZD1839 was widely distributed. Concentrations of radioactivity were highest in liver, kidney and gastrointestinal tract. This is consistent with the target organ toxicity seen in toxicology studies (below). High concentrations were seen in lachrymal, salivary and adrenal glands. ZD1839 did not appear cross the blood-brain barrier in high concentrations. ZD1839 appeared to accumulate in tissues containing melanin such as the eye and skin. This suggests the possibility of racial differences in dermal toxicity.

ZD1839 is metabolized extensively by rats, humans and dogs. *In vivo* studies using human hepatocytes demonstrated that cytochrome P450 3A4 was responsible for almost all of this metabolism in humans. Rifampicin induces cytochrome P450 3A4 expression. Dosing with this compound prior to ZD1839 challenge reduced C_{max} by more than 60% and reduced AUC by 85% in humans (Trial 30). Itraconazole inhibits cytochrome P450 3A4. Co-administration of this drug with ZD1839 caused an increase in C_{max} of 54% and an increase in AUC of almost 100% in humans (Trial 51). Inhibition of cytochrome P450 2D6 by metoprolol caused an increase in AUC of about 25% in humans (Trial 38). *In vitro*, ZD1839 did not significantly inhibit cytochrome P450 activity so the possibility of it causing large increases in the concentrations of other drugs may be limited. Nevertheless, a significant and potentially dangerous prolongation of clotting times has been seen clinically in several patients taking concomitant warfarin. ZD1839 should be used cautiously with any drug that interacts with cytochrome P450 3A4.

All the major metabolites found in human samples *in vivo* or *in vitro* were formed by rat and dog hepatocytes. Some studies of *in vivo* metabolism, particularly early ones, in rats and dogs suffered from a lack of sensitivity, so the comparative data is somewhat incomplete. Human metabolism by cytochrome P450 3A4 usually corresponds to metabolism by 3A isoenzymes in the dog and 2B isoenzymes in the rat. Studies in rats and dogs suggest that ZD1839 does not significantly induce cytochrome P450 activity. The following illustration shows the major metabolites of ZD1839 in rats, dogs and humans.

APPEARS THIS WAY
ON ORIGINAL