

ZD1839 is lipophilic as are many of its metabolites. Extraction and chromatographic techniques frequently provided inadequate separation of metabolites especially in fecal extracts. In rat plasma ZD1839 was the main component. Other components included M537194 and an hydroxylated metabolite of ZD1839. In dog plasma, ZD1839, M523595 and M537194 predominated. In human plasma parent compound and M523595 are the major components. In rat feces, ZD1839 and M537194 predominated. In dog feces, ZD1839, M537194, M523595, M387783, and the hydroxylated, dihydroxylated and carboxypropyl derivatives were isolated and identified. Human fecal extracts contained ZD1839, M387783, a proposed carboxypropyl metabolite and an unknown metabolite. M523595 (O-desmethyl ZD1839) was at relatively high concentrations (0.027 μM). This metabolite is active (IC_{50} at the tyrosine kinase site of 0.025) and the plasma concentrations are similar to the IC_{50} . This compound possibly contributes significantly to ZD1839 inhibition. Work on characterizing the metabolites in rats, dogs and humans is ongoing.

Acute toxicity

A single oral dose of 6 g/m^2 caused no deaths and no clinical signs in mice. All mice gained weight normally after dosing.

A dose of 12 g/m² killed three of five female rats and one of five male rats on days 3 and 6 after dosing respectively. Clinical signs seen in survivors began on day five and included hunched posture, loss of skin tone, piloerection, subdued behavior, trembling and shaking and urinary staining. These signs resolved by day 15. The rats lost weight through day eight then recovered. At necropsy, animals showed discoloration of the adrenal glands and the GI tract. Microscopic changes included adrenal medullary vacuolation, myocarditis, necrosis or ulceration in duodenum, necrosis with ulceration in cecum and stomach, mucosal atrophy or necrosis of the trachea, periportal hepatocyte vacuolation in the liver, renal tubular dilatation, splenic atrophy, and cutaneous microabscesses.

The site of these toxicities and the time of onset point to multi-organ failure as the cause of death in rats. Due to the complex pharmacology of ZD1839 it would be difficult to isolate any specific cause. Nevertheless, most of the tissues involved – with the notable exception of the cardiac toxicity seen in the dog (above) – are proliferative, consistent with the primary pharmacology of the drug, that is inhibition of tyrosine kinase.

Repeat Dose Toxicities

Irrespective of schedule, dogs appear to be somewhat more tolerant of ZD1839 than rats. For example, 200 mg/m²/day given for one month caused only minor changes in liver function tests in dogs, whereas 240 mg/m²/day given for one month cause considerable toxicity in the rat. Likewise, dogs tolerated 300 mg/m²/day for six months with relatively mild toxicities whereas 90 mg/m²/day caused significant changes clinically and microscopically in rats. Mice are also more tolerant than rats, surviving 525 mg/m²/day for 13 weeks. Humans show clinically significant toxicity at about 300 mg/m²/day much like the dog. In all tested animal species, the difference between a chronic dose that caused relatively little toxicity and one that was lethal was less than twofold. This is consistent with clinical results; patients tolerated a dose of 500 mg/day reasonably well but they did not tolerate doses of 750 and 1000 mg/day. I consider the toxic dose response curve relatively steep though there is insufficient information to accurately characterize such a curve. The species differences in the severity of toxicities are probably due to metabolism and absorption as are the sex differences; female rats appear more sensitive than males. For all these reasons, dogs are probably the best models for toxicity testing if not the most sensitive.

Rodents given toxic doses daily for two weeks or longer loose weight. This weight loss correlates with dose. Dogs also loose weight but again at relatively higher doses. Weight loss is probably related to diminished appetite. Damage to the intestine was apparent microscopically only at relatively high doses in rats. Nevertheless, submicroscopic damage in the GI tract, a site of highly proliferative tissue, could account for the diminished appetite. Chronically dosed moribund animals frequently have loose feces or diarrhea consistent with damage to the intestines. This last toxicity correlates with the observation that EGFR signaling appears to be involved in normal intestinal repair post surgical resection in rats.

Consistent with the inhibition of EGFR, ZD1839 causes alopecia, chronic active folliculitis, scabbing and tail lesions in rodents. The tail lesions possibly result when ZD1839 inhibits the proliferation necessary to repair normal photo-damage. In human patients, skin rash and other skin damage, along with diarrhea and asthenia, can be dose limiting.

During the phase I development of ZD1839, investigators monitored patients for signs of ocular toxicity. This was because studies in rats and dogs demonstrated microscopic signs of corneal atrophy after chronic dosing. Other signs of ocular toxicity in test animals included exudation around the eye, encrusting, reddened eyes, swollen eyelids and closed eyes. These toxicities are consistent with retardation of high cellular turnover in tissues of and around the eye, but they were seen only at relatively high doses, usually above 300 mg/m²/day. Despite the fact that sporadic ocular toxicities were seen in some of the early trials, ZD1839 has not appeared to cause significant ocular toxicities in patients at the 250 or 500 mg doses in large well conducted trials. Nevertheless, a few patients have suffered irritation

from aberrant eyelashes. Lashes have grown in the wrong direction down into the eye. When these lashes were removed the irritation resolved.

Macrophage infiltration in the liver (centrilobular) and lung (bronchioles and interstitium) associated with pigmented inclusions is consistent with inflammation and cell death in these tissues. The papillary necrosis occasionally seen in the kidneys probably is also associated with the interference with angiotensin II signaling noted above. Macrophage infiltration and a generalized immune stimulation is consistent with increased weight seen in these organs at high doses in some studies seen in the liver, lungs, spleen, thymus and lymph nodes. Dogs and rats both show signs of liver damage but again in dogs this toxicity is only seen in doses higher than those tolerated by rodents on a mg/m² basis. In rats, microscopic liver damage was usually associated with two to three fold increases in ALT and AST and decreases albumin, as one would expect with direct liver damage. But, in dogs, microscopic damage to the liver was less apparent and was associated with only slight decreases (10 to 20 %) in ALT and albumin. I suspect that in rats liver damage is associated with necrosis due to direct toxicity but in dogs it is associated with apoptosis or a derangement of normal hepatocyte replacement. Again these species differences are probably due to differences in metabolism.

Repeat dosing causes mild (to about 50% above controls) dose dependant increase in white count in both dogs and rodents. It also causes a dose dependant increase in platelets. Again, this is possibly a generalized inflammatory response, that is a response to increased cell death. Nevertheless, IL-10 mediates the containment and eventual termination of inflammatory responses. This mediation is through the initiation of cellular signal cascades through the Jak/stat tyrosine kinase system (see the review by KW Moore *et al. Annu Rev Immunol* 2001;19:683-765). Thus, it is possible that ZD1839 directly interferes with this or some other tyrosine kinase mediated cytokine signal in white cells. A decrease in red cell parameters in dogs does not correlate with marrow changes and is not seen in rodents. I suspect it is related to sub-microscopic changes in the hematopoietic feedback system.

The increase in the absolute and relative weight of the heart in rats is somewhat disturbing. It did not appear to be related to macrophage infiltration or some other inflammatory response. It may be due to cardiac hypertrophy associated with the unusual and extensive secondary pharmacology caused by ZD1839 described above. It is also almost certainly related to the cardiac toxicity caused by large single doses.

My impression is that at sub-lethal doses given for greater than 14 days, many of the toxicities caused by ZD1839 are associated with its primary pharmacology, that is inhibition of tyrosine kinases and competition at ATP sites. Higher doses appear to cause other more profound toxicities that lead quickly to death. Again, I suspect that this steep dose response may be related to secondary pharmacologies, but I have insufficient information to confirm this.

Genotoxicity

ZD1839 was non-mutagenic in the Ames assay in four *S. typhimurium* strains and two *E. Coli* strains in the presence or absence of S9-mix. ZD1839 at single oral doses of 1,200, 4,020 and 12,000 mg/m², did not increase the incidence of the micronucleated polychromatic erythrocytes in live rats 24 or 48 hr post-dose. ZD1839 was not genotoxic in an *in vitro* mouse lymphoma assay. Carcinogenicity studies have not been done.

Reproductive and Developmental Toxicology

In a pilot teratology study in rats (TTR/2950), ZD1839 caused no developmental or maternal toxicity at the highest dose tested (120 mg/m²/day). In an initial pilot teratology study in rabbits (TRB/725) doses greater than or equal to 1180 mg/m²/d cause unacceptable maternal mortality. In a

second teratology study in rabbits (TBR/696), ZD1839 at 2950 mg/m²/day caused 100% maternal mortality. A dose of 590 mg/m²/day was not embryo-toxic or teratogenic in rabbits.

At doses that were toxic to the dams (60, 120 and 240 mg/m²/day), ZD1839 had no effect on female fertility and embryonic survival when female rats were treated from 2 weeks before pairing through day 7 of gestation (Segment I). The high dose caused 100% mortality (1 dead 9 moribund) by day 11. ZD1839 did not impair sperm function in male rats. Nevertheless, there was an increased incidence of irregular estrous cycles in rats given 120-mg/m²/day dose. This dose also caused significant embryo-fetal toxicity manifest as a decrease in corpora lutea (about 10% less than controls), uterine implants (about 25% less) and live-embryos per litter (25% less). Thus, ZD1839 is an embryo-fetal toxin at doses approximately equivalent to the proposed clinical dose on a mg/m² basis.

In a definitive segment II study, doses of 20 or 75 mg/kg/day caused dose dependent maternal toxicity manifest as scabs around the mouth and decreases in body weight and food consumption. The 75-mg/kg/day dose caused unacceptable toxicity in 15 of 24 dams. Severe villous atrophy of the duodenum appeared to be dose limiting. In surviving animals, there was no evidence of adverse effects on embryonic or fetal survival or development. There was a dose dependent decrease in fetal body weight at doses of 20 mg/kg/day and above (about 25% in the high dose group). The low dose, 5 mg/kg/day appeared to be a no effect level for both dams and offspring.

In a definitive segment II study, rats were given doses of 0, 1, 5 or 30 mg/kg/day. The low, mid and high dose caused decreases in maternal body weight gain of 12%, 13% and 63% respectively relative to control during the course of dosing. There were no dose related adverse effects on embryonic or fetal survival or development at any of the doses.

General Toxicology Issues:

ZD1839 is metabolized almost exclusively by cytochrome P450 3A4. Its concentration increases significantly in the presence of inhibitors of this enzyme. It has significant potential for drug-drug interactions involving this metabolism. An interaction with warfarin has been seen in clinical studies.

ZD1839 has numerous secondary pharmacologies with receptor binding constants that approach physiologically significant concentrations. The significance of these pharmacologies remains to be defined. I consider the toxic dose response curve of ZD1839 to be relatively steep. It is likely that toxicities seen at doses above 500 mg/d are related to some of these secondary pharmacologies.

ZD1839 appears to have the potential to inhibit numerous tyrosine kinase sites. The possibility of drug-drug interactions with drugs that act at these sites has not been characterized.

The indication proposed in this NDA is for patients with a life-threatening condition. No further pharmacology or toxicology studies are needed to support this indication.

Recommendations:

There are no pharmacology and toxicology issues to prevent the approval of IRESSA for the indication proposed in this NDA.

W. David McGuinn, Jr., Ph. D. D.A.B.T

Appendices**Appendix I, Dr. Zheng's first review****Division of Oncology Drug Products, HFD-150****REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA****Safety Review**IND No. Type IND Serial No(s). 000Date(s) of Submission IND dated: 11/17/97
Received by CDER: 11/18/97

Information to be Conveyed to Sponsor: Yes (X), No ()

Reviewer: Hua Zheng, Ph.D.

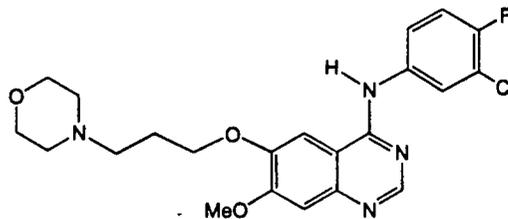
Date Review Completed: Dec. 16, 1997

Sponsor: Zeneca Pharmaceuticals
Wilmington, DE 19850-5437

Drug Name: ZENECA ZD1839

Chemical Name: 4-(3-Chloro-4-fluorophenylamino)-7-methoxy-6-(3-(4-morpholinyl)propoxy)
quinazoline

CAS Number: None

StructureMolecular Formula: $C_{22}H_{24}ClFN_4O_3$

Molecular Weight: 446.91

Related INDs/NDAs/DMFs: IND

Class: Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor

Indication: Solid malignant tumors

Clinical Formulation: The finished product is presented as a range of round, biconvex, brown film coated tables containing 5 mg, 25 mg and 100 mg of ZD1839.

Qualitative and quantitative composition of drug product

Ingredient	5 mg (mg/tablet)	25 mg (mg/tablet)	100 mg (mg/tablet)	Function
Core				
ZD1839	5.0	25.0	100.0	Active ingredient
Lactose				
Microcrystalline cellulose				
Croscarmellose sodium				
polyvidone				
Sodium lauryl sulphate				
Magnesium stearate				
Core tablet weight				
Coating				
Methylhydroxypropylcellulose				
Polyethylene glycol, PEG 300				
Red iron oxide E172				
Yellow iron oxide E172				
Titanium dioxide				
Coated tablet weight	229.6	224.6	204.6	

Route of Administration: Oral

Proposed Clinical Protocol: This is a multi-center, phase I safety and pharmacokinetic clinical trial of orally administered ZD1839 with multiple rising dose and sequential block escalation in patients with solid malignant tumors known to commonly overexpress EGFR, which are refractory to other treatments.

Objective: To assess

- a) the tolerability and toxicity,
- b) multiple dose PK,
- c) antitumor activity of ZD1829 in patients with advanced cancer, and
- d) effect on biological surrogates of antitumor efficacy (Ki67, c-fos mRNA and EGFR) in tumor biopsies

Starting Dose:

- a) Single dose: 100 mg (63 mg/m²)
- b) Multiple dose: 100 mg/day (63 mg/m²/day)

Escalation: Multiple dose: 250, 500, 750 and 1,000 mg/day (156, 313, 469 and 625 mg/m²/day)

Frequency and duration:

- a) Single dose: one day treatment followed by 6 days washout
- b) Multiple dose: daily x 14, followed by 14 days off treatment

Age of patient population: ≥ 18 years

Previous Review(s), Date(s), and Reviewer(s): None

Studies reviewed for IND:**I. Pharmacology**

1. Pharmacology Relevant to Cancer (Vol. 1, p265)

II. Safety Pharmacology

2. General pharmacology (Vol. 1, p273)

III. Toxicology*Single dose Studies*

3. TLM/958 Acute toxicity (limit) study in mice: oral administration (Vol. 2, p1)
4. TLR/2571 Acute toxicity (limit) study in rats: oral administration (Vol. 2, p47)

Multi-dose Studies

5. TAR/2492 Rat pilot toxicity study (Vol. 2, p112)
6. TAD/870 Dog pilot toxicity study (Vol. 2, p167)
7. TAR/2570 One month oral toxicity studies in rats (Vol. 3, p1)
8. TAD/876 One month oral toxicity study in dogs (Vol. 4, 1)

IV. Toxicokinetics/ADME The PK studies were integrated into the above toxicity studies

9. KMR/010 The tissue distribution of total radioactivity in the rat following oral administration of [¹⁴C]-ZD1839 (Quantitative whole body autoradiography) (Vol. 5, p45)
10. KMR/007 The disposition of [¹⁴C]-zeneca ZD1839 in the rat (Vol. 5, p92)
11. BIT/00401 The effects of ZD1839 on the hepatic microsomal mixed function oxidase enzymes of the male rat (Vol. 5, p111)
12. KKR/008 The distribution of radioactivity in the blood after oral and intravenous administration of [¹⁴C]-zeneca ZD1839 to rats (Vol. 5, p132)
13. KKD/009 The disposition of [¹⁴C]-zeneca ZD1839 in male dogs (Vol. 5, p164)
14. KPJ/013 The binding of [¹⁴C]-ZD1839 to plasma proteins (Vol. 5, p191)
15. KMN/012 Metabolism of zeneca ZD1839 in rat, dog and human hepatocytes (Vol. 5, p208)

V. Special Toxicology*Reproductive Toxicity*

16. TGR/2616 Single female fertility study in rats: oral administration (Vol. 4, p307)

Genetic Toxicity

17. TMV/668 An evaluation of mutagenic potential using *S. typhimurium* and *E. coli* (Vol. 4, p332)
18. TQR/2573 Micronucleus test in the rat: oral administration (Vol. 4, p369)
19. TYX/78- *In vitro* cytogenetic study using cultured human lymphocytes (Vol. 5, p1)

VI. Previous human experience

- Clinical experience (Vol. 1, p101)

Studies not reviewed for safety review**III. Toxicology**

- TLM/958 Acute toxicity (limit) study in mice: intravenous administration (Vol. 2, p20)
- TLR/2571 Acute toxicity (limit) study in rats: intravenous administration (Vol. 2, p87)

IV. Toxicokinetics and ADME

- KPV/006 Validation of

method

- for the determination of zeneca ZD1839 concentrations in rats and dog plasma (Vol. 5, p234)
- KPV/014 Validation of _____
method for the determination of zeneca ZD1839 concentrations in human plasma (Vol. 5, p274)
- KML/002 The synthesis of N-(3-Chloro-4-fluorophenyl)-7-methoxy-6-(3-morpholino-[2,3-¹⁴C]propoxy)quinazolin-4-amine hydrochloride (Vol. 5, p311)

Note that portions of this review were excerpted directly from the sponsor's submission.

Overall Summary and Evaluation

Introduction: The EGFR tyrosine kinase is activated by binding of a variety of ligands to the external domain which causes EGFR itself and a number of cellular substrates to become phosphorylated on tyrosine domains. Overexpression of EGFR has been shown in a significant percentage of human tumors, and has been correlated in many cases with poor prognostic features. Evidence suggests that blockade of the EGFR pathway, either by the dominant negative mutant or the antisense approach, reduces the proliferation and invasive properties of a human colon tumor cell line. Antibodies that block the EGF binding site of EGFR inhibit tumor cell proliferation in cell cultures and produce complete regressions of some human tumor xenografts. Mice with mutant EGFR representing a model of partial inhibition of EGFR *in vivo* are healthy and fertile, but have hair, skin and eye abnormalities. Recent studies have shown that EGFR can protect tumor cells from apoptosis and anti-EGFR antibody induce both G1 arrest and apoptosis. ZD1839 is being developed as an orally active anti-tumor agent for the treatment of a broad range of major human solid tumor types.

Enzyme assay showed that ZD1839 markedly inhibited the autophosphorylation of EGF-stimulated EGFR in multiple tumor cells in culture. The selectivity of ZD1839 in growth inhibition was demonstrated in a panel of cells grown in medium with different growth factors. In a series of experiments with NIH 3T3 mouse fibroblasts, ZD1839 was shown to inhibit proliferation induced by multiple growth factors. Statically significant responses to ZD1839 have been demonstrated in 8/16 xenograft models (listed in the following table). Regression and continued suppression of tumor xenograft growth was also demonstrated in A431 human squamous vulval carcinoma xenografts in nude mice given 200 mg/kg/day > 100 days and the inhibition of tumor growth sustained for up to 4 months. Antitumor activity of ZD1839 (50 mg/kg/day) was also seen in the AO strain rat with transplantable rat uterine tumors, T169 and 3010, with tumor growth inhibition of 83% and 56%, respectively. However, the time point at which the tumor inhibition for this study was measured was not specified in the summary report.

Safety pharmacology studies showed that *in vitro* ZD 1839 at 10 μ M had no agonist or antagonist activity in, 1) the guinea-pig right or left atria, 2) the guinea-pig ileum. Rats given ZD1839 at 100 mg/kg po had significant decreases in the excretion of water, sodium, chloride and urine osmolality, and a weak, statistically significant inhibition (16%) of acute inflammatory response to carrageenin. However, ZD1839 at 100 mg/kg po had 1) no significant effect on the blood-pressure or heart-rate in the spontaneous hypertensive rat; 2) not effect on gastrointestinal motility in the mouse; 3) no stimulant or depressant activity, or any effects on a range of other behavioral parameters in the mouse; 4) no bronchoconstrictor or bronchodilator activity in the anesthetized guinea-pig; and 5) no effect on the delayed hypersensitivity response to Freund's adjuvant in the mouse.

The target organs of ZD1839 in preclinical toxicity studies were identified to be the skin, adrenal glands, heart, GI tract, liver, kidney, spleen and trachea. Single dose studies revealed a lethal oral dose of

12,000 mg/m² in the rat while ZD 1839 at 6,000 mg/m² in the mouse was not frankly toxic.

Multiple dose studies confirmed that the target organs for ZD1839 toxicity are similar to those observed in the acute toxicity studies; end organ toxicity was also observed on cornea of the eyes and thymus in both rat and dogs. The approximate LD₁₀ of 14-day multiple oral dose in rats was 300 mg/m²/day in one study but was < 240 mg/m²/day in pregnant rats. The HNSTD of 30-day repeat oral dose in rats was 240 mg/m²/day. Gender difference in sensitivity was seen in rodents but not obvious in dogs; female rats were generally more sensitive than the males to ZD1839 toxicity. The HNSTD for dogs were 1,000 mg/m²/day × 14 or 200 mg/m²/day × 30.

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 death occurred and no clinical signs observed for up to 13 days. Histopathology examination was not performed.
Rat (TLR/2571)	Gavage	5	Lethal	2,000	12,000	12,000 mg/m ² /day: 4/10 deaths (3 ♀ and 1 ♂). 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 revealed discoloration of adrenal glands and adrenal medullary vacuolation; myocarditis; necrosis and ulceration in GI tract; mucosal atrophy and/or necrosis in the trachea; multifocal hemorrhage in lung; multifocal hepatocyte necrosis; microabscess formation on the skin; splenic atrophy; etc.

Summary of the Multiple Dose Toxicology Studies						
Species (Study No.)	Route & Duration	N/sex/dose	Critical Doses	mg/kg /day	mg/m ² /day	Significant findings
Rat (TAR/2492)	Gavage 14-days	5	LD ₁₀ ^b NOAEL	50 10	300 60	750 mg/m ² /day: 3/5 ♀ 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 ♀); ↓ b.w. (28% for ♂ and 35% for ♀), ↓ food intake (29% for ♂ and 69% for ♀) by D15; ↑ WBC, NEUT, PLT; ↑ blood urea, ALP (♀), ALT, TG and AST; dry and rough corneal surface; enlarged adrenals; ↑ organ weight for adrenals; thymus atrophy; abnormal histopathologic findings in the adrenals, small intestine, kidneys, liver, lungs, LN, ovaries, skin, spleen and thymus. 300 mg/m ² /day: no deaths, stains around muzzle (5/5 ♀), red exudate around eyes (7/10), loss of skin tone (1/5 ♀), subdued behavior (1/5 ♀); minor decrease in b.w. gain (6%) and food intake (12-19%) by the end of the dosing; ↑ WBC and NEUT, dry and rough corneal surfaces in ♀ rats; abnormal histopathology findings in small intestine, kidneys (♀ only), liver, LN and skin.
Rat (TGR/2616) ^a Seg. I	Gavage 21-days	10 ♀	LD ₁₀	< 40	< 240	240 mg/m ² /day: 1/10 was euthanized on D9 and 9/10 were euthanized on D11; clinical signs: urine staining, loose feces, hunched posture, piloerection, discharge from eyes, and partially closed eyes; ↓ food intake (21%)
Rat (TAR/2570)	Gavage 1-month	10-15	LD ₁₀ ^b	40	240	240 mg/m ² /day: no death occurred; clinical signs: deposit on nose, eyes partially closed, slight inflammation and encrustation of the ankles and toe joints; red exudate, and scab on mouth; ↓ b.w. (12-19%) on D29; ↓ b.w. by 16% for ♀ 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 ♀); enlarged LN (17/20); discoloration of the skin (8/20); scab in skin (5/20); enlarged spleen (4/20); ↑ relative organ weight: adrenals (19% in ♂); kidney (10-13%); thymus (13-16%); liver (15% in ♀); ↓ 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 LN.
Dog (TAD/870)	Gavage 14-days	1	Lethal NHSTD	75	1,500	1,500 mg/m ² /day: 1 ♀ died on D1. Loose feces, emesis, reddened eyes, reddened inside of the mouth, thin, eyes partially closed; ↓ b.w. by 22% for ♀ on D10 and by 13% for the ♂ on D3; ↓ food intake by 59-61% during wk1 and 66% (♂) during wk2; ↓RBC and HB (8-19%); ↑ WBC and NEUT (16-30%); ↓ ALB (25-41%) and ALP (19-36%); ↑ ALT (30-70% for ♂ and 183-155% in ♀) and blood urea (32-83%); diffuse staining of the cornea surface, corneal epithelial atrophy; germinal center vacuolation in LN and spleen; thymus atrophy, and multifocal epidermal microabscesses. 1,000 mg/m ² /day: emesis, loose feces, reddened eyes and reddened inside of the mouth; ↓ b.w. (10%) for ♂ 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 LN & spleen; diffuse thymus atrophy, & multifocal skin microabscesses.
				50	1,000	
Dog (TAD/876)	Gavage 1-month	3	Lethal HNSTD	40	800	800 mg/m ² /day: one death (1/3 ♂); 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, partially closed eyes and sore in mouth; ↓ in cumulative b.w. 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% ♂); ↓ ALB (40-47%), A/G (39-48%), ALT (18-58%) and ALP (44-46%) on D30; ↑CHOL (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/m ² /day: no death or clinical signs, ↓ ALB (20-23%), A/G (16-19%), ALT (17-301%); ↓ urine Na+/creatinine (87-96%) on D30
				10	200	

* this is a reproductive toxicity study conducted on ♀ rats; ^b approximate LD₁₀ based on MTD for the ♀ rats, this 14 study did not have a recovery observation period.

The toxicokinetics results are summarized in the following table:

Species	Day	Dose (mg/m ² /day)	Tmax (h)		Cmax (µg/ml)		AUC _{0-8, 0-12, 0-24} ^a (µg·h/ml)		t _{1/2} (h)	
			♂	♀	♂	♀	♂	♀	♂	♀
Rat (TAR/2492)	1	60	5	6	0.2	0.2	0.97	1.85		
		300	2	3	1.5	1.7	12.7	17.6		
		750	2	6	4.6	4.9	30.9	41.3		
	14	60	3	3	0.4	0.4	2.1	3.0		
		300	6	5	2.1	1.9	14.1	17.4		
		750	5	2	3.4	3.5	23.5	ND ^b		
Rat (TAR/2570)	1	12	4	4	0.02	0.03	NC	NC	NC	
	28	12	4	0.5	0.05	0.06	NC	0.35	NC	6.8
		60	2	2	0.54	0.58	2.55	3.22	6.8	5.0
		240	8	1	1.46	1.69	22.8	27.3	7.1	6.7
Dog (TAD/870)	1	100	1	2	2.6	1.6	19	16	7.1	6.7
		1,000	4	4	8.7	11.8	102	142 177	4.8	7.6
		1,500	1	1	12.8	16	149		9.9	8.8
	14	100	2	4	2.0	1.1	20	15	7.2	6.7
		1,000	8	8	3.5	5.1	63	93	NC	NC
		1,500	6	NC ^c	9.6	NC	192	NC	NC	NC
Dog (TAD/876)	1	40	2.3		0.07		0.35		NC	
	28	40	3.0		0.14		0.65		NC	
		200	3.0		0.94		5.3		5.8	

		800	3.7	2.36	15.8	NC
Human ^d (1839IL/0001)	1	6.3	5.5	0.0028	0.086 ^e	14.8 ^e
		15.6	5.0	0.0079 ^f	0.126 ^f	12.9 ^f
		31.2	5.5	0.013	0.261 ^g	15.6 ^g
		46.9	5.0	0.026	0.424	12.1
Human ^d (1839IL/0010)	1	62.5 ^h	5.0	0.044 ^h	0.558 ^h	12.7 ^h
	3	62.5 ^h	6.0	0.068 ^h	1.078 ^h	31.5 ^h

^aAUC₀₋₈ for studies TAR/2570 and TAD/876, AUC₀₋₁₂ for study TAR/2492, AUC₀₋₂₄ for study TAD/870;
^bAUC₀₋₁₂ cannot be determined due to the lack of sampling points; ^cNC: not calculated; ^d healthy male volunteers (n = 4), assuming the body surface area = 1.6 m²; ^e n = 1, ^f n = 2, ^g n = 3, ^h n = 5

The AUC following single oral doses in humans, rats and dogs are plotted in the following graph and good correlation were seen in all three species (although the samples from human study were limited). Since the data for humans were derived at a much lower dose, it is not know what pattern will be for humans when higher doses are reached.

**APPEARS THIS WAY
 ON ORIGINAL**

AUC values are generally greater in the ♀ than the ♂ rats at a given dose. The gender difference in toxicity observed in the rat was possibly due to this PK pattern. Fourteen day exposure slightly increased the AUC exposure in rats for the same doses. After 28 days of dosing in separate study, the AUCs was double the 14 day values. It is difficult to draw substantive conclusions from studies in separate groups of animals. AUC values were similar in dogs after 14 days of dosing. A separate study showed markedly lower AUCs after 28 days of dosing, suggesting induction of metabolism. However, longer term drug administration seems to be more toxic in that the HNSTD was lower in dogs given ZD1839 for 28 days than that for 14 days.

APPEARS THIS WAY
ON ORIGINAL

The correlation of AUC with the dose are different in rats and dogs and the pattern depends on the length of drug exposure. The AUC values for 14 days ZD1839 administration was greater in the dog than in the rat; this pattern was reversed when exposure extended to 28 days.

APPEARS THIS WAY
ON ORIGINAL

ZD1839 was highly protein bound in all species investigated including the rat, dog, mouse and human, although binding was slightly lower in the rats (87%) than in the other species (> 90%). After a single oral dose of the radiolabeled ZD1839 (5 mg/kg) to the rat, the radioactivity was rapidly absorbed and well distributed into the tissues. High concentrations of radioactivity were observed in organs of metabolism and excretion (liver, kidney, lung, GI tract) and in glandular tissues. Radioactivity was also detected at in the melanin containing tissues (eye, pigmented skin) of the pigmented animals. After an intravenous or oral administration of radiolabeled ZD1839, the radioactivity was mainly recovered from the feces in both rats and dogs indicating the GI is the major route for drug excretion.

Metabolism studies revealed the presence of circulating metabolite(s) and suggested that the rate of metabolism of ZD1839 is slower in the female than in the male rats. This also could contribute to the gender difference in toxicity if the parent drug is more toxic than the metabolite(s). A major metabolite (56-74% of the sample radioactivity) recovered from the feces was not identified but seemed to suggest a labile conjugate that has been cleaved either during excretion or during sample preparation. A second component less polar than ZD1839 accounted for 8-15% of the sample radioactivity. The remainder of <8% of the radioactivity was accounted for by at least 6 minor components. *In vitro* metabolism study using rat, dog and human hepatocytes revealed an extensive metabolism for this compound. The majority of the components observed in the rat and dog were more polar than the parent compound and the profiles were qualitatively similar. However, at the end of incubation (180 min), the parent

compound of ZD1839 accounted for 28% and 55% of the incubate radioactivity in the dog and rat hepatocytes, respectively, indicating that the parent drug is more completely metabolized by the dog hepatocytes. Incubation of ZD1839 with human hepatocytes from three individuals revealed both qualitative and quantitative differences reflecting inter-individual differences in metabolizing activity. ZD1839 accounted for 62.1, 81.3 and 34.3% of the incubate radioactivity after three hour incubation. If the toxicity of ZD1839 is determined by the presence of the un-metabolized compound, then extra safety factors should be taken for the substantial individual variations.

ZD1839 was non-mutagenic in Ames tests in four *S. typhimurium* tester strains and two *E. Coli* strains in the presence or absence of S9-mix. ZD1839 at single oral doses of 1,200, 4,020 and 12,000 mg/m², did not increase the incidence of the micronucleated polychromatic erythrocytes in rats 24 or 48 hr post dose. ZD1839 at maternal toxicity dose levels (60, 120 and 240 mg/m²/day) had no effect on female fertility and embryonic survival when female rats were treated 2 weeks prior to pairing until day 7 of gestation, though increased occurrence in irregular estrous cycles were observed in rats given 120 mg/m²/day dose. ZD1839 at 240 mg/m²/day × 21 was lethal and 1/10 was killed at D9 and the rest 9/10 were killed at D11 due to clinical signs of toxicity. No results was obtained for reproductive toxicity for this dose level.

Two clinical trials were conducted in healthy male human volunteers. Single oral doses of up to 75 mg ZD1839 was given to human subjects. One volunteer at 75 mg dose (46.9 mg/m²) experienced an altered state of awareness commencing within 6 hrs after dosing and lasted for 4 hrs. It is noted that all 4 subjects experienced adverse events, of which one reported severe headache and one reported severe neck pain. Headache was also observed in subjects given placebo and ZD1839 at lower doses but were all mild to moderate in severity. At 46.9 mg/m², the human AUC level was higher than that for dogs at 40 mg/m² but lower than that in rat at 60 mg/m² and the correlation of AUC to dose was not evaluated for humans at higher doses or for rats or dogs at lower doses.

In another phase I study, healthy male volunteers were administered 3 consecutive daily doses of 100 mg (63 mg/m²) ZD1839. Adverse events reported were headache, flu-like symptoms, and ophthalmological abnormalities (medial corneal deposits for both eyes, conjunctival hyperemia, prominent nerve and blood vessel in right eye). The ophthalmological abnormalities were not seen in placebo-treated subjects. It is alarming that the AUC almost doubled after just 3 repeated daily dose of ZD1839 in human volunteers at 63 mg/m² compared to that after a single dose. This may suggest an increased absorption or drug accumulation due to slow clearance.

In this IND the sponsor only proposed a single starting human dose of 63 mg/m². Data from the mouse study would predict a human starting dose of 600 mg/m², and available previous human experience has used this dose for up to 3 days administration without causing severe toxicity. This dose is therefore acceptable as a safe starting dose for single dose trial. However, the sponsor also proposes a repeat dose trial at the same starting dose of 63 mg/m²/day for 14 days. The available human experience does not support this dose for a 14 consecutive days. Human volunteers dosed at this level for 3 consecutive days had already developed ophthalmological abnormalities. The animal data from rats did not derive a definitive LD₁₀ value for 14-day dosing. Taking 300 mg/m²/day as the approximate LD₁₀ from study TAR/2492, one would predict a safe starting dose of 30 mg/m²/day, which was not severely toxic to dogs. Dogs seems to be less sensitive than rats for the 14 day studies, however, both studies on dogs and rats did not include a recovery observation period and the reversibility of the toxicities are unknown. If predicted from 1/6 of the HNSTD from study TAD/876 in dogs, a starting dose of 33.3 mg/m²/day would be accepted. The human exposure (AUC) after multiple administration at doses of 63 mg/m²/day or higher is hard to predict. Human volunteers showed a doubled AUC (1.08 vs. 0.59 µg·h/ml) and tripled t_{1/2} (31.5 vs. 12.7 h) value after only 3 days of dosing with 63 mg/m²/day level compared to after a single dosing indicating saturation of metabolism and/or elimination pathway which is alarming. In addition, individual variations in the drug metabolism by human hepatocytes were substantial based on

limited *in vitro* analyses. These factors warrant that the prediction for a starting dose should be more conservative in order to be safe. Therefore, $30 \text{ mg/m}^2/\text{day} \times 14$ should be a safe starting dose in human.

Recommendations:

The planned clinical trial may not proceed as planned, unless the starting dose for the repeat dose trial is lowered to $30 \text{ mg/m}^2/\text{day} \times 14$.

Points discussed with medical officer: starting dose
human experience
gender difference in toxicity and PK
species difference in toxicity and PK
metabolism

Draft Reports: Yes, the summary of non-clinical pharmacology (including pharmacology and safety pharmacology) are draft reports without providing data for the individual studies.

Draft Letter, Request for Sponsor: Yes

Hold issue:

1. The starting dose for the 14 days multiple dose schedule is not supported by preclinical data. The multiple dose trial may not proceed until you lower the starting dose to 50 mg/day ($30 \text{ mg/m}^2/\text{day}$).

15/1

Hua Zheng, Ph.D. Date
Pharmacologist/Toxicologist

15/1

Paul Andrews, Ph.D
Pharm/Tox Team Leader

cc: IND [redacted] and Div. File
/HFD-150
/P Andrews
/K Kobayashi
/A Chapman
/H Zheng

*Appendix II, Dr. Zheng's second review***Division of Oncology Drug Products, HFD-150****REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA****Original Review**IND No. Type IND Serial No(s). 000Date(s) of Submission IND dated: 11/17/97
Received by CDER: 11/18/97

Information to be Conveyed to Sponsor: Yes (), No (X)

Reviewer: Hua Zheng, Ph.D.

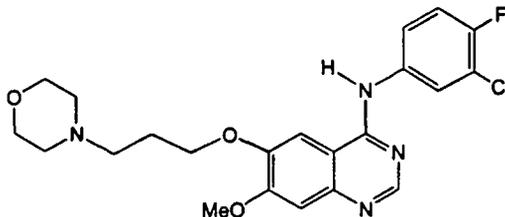
Date Review Completed: Jan 29, 1998

Sponsor: Zeneca Pharmaceuticals
Wilmington, DE 19850-5437

Drug Name: ZENECA ZD1839

Chemical Name: 4-(3-Chloro-4-fluorophenylamino)-7-methoxy-6-(3-(4-morpholinyl) propoxy)
quinazoline

CAS Number: None

StructureMolecular Formula: $C_{22}H_{24}ClFN_4O_3$

Molecular Weight: 446.91

Related INDs/NDAs/DMFs: IND

Class: Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor

Indication: Solid malignant tumors

Clinical Formulation: The finished product is presented as a range of round, biconvex,
brown film coated tablets containing 1 mg, 5 mg, 25 mg and 100 mg of ZD1839.

Qualitative and quantitative composition of drug product

Ingredient	5 mg (mg/tablet)	25 mg (mg/tablet)	100 mg (mg/tablet)	Function
Core				
ZD1839	5.0	25.0	100.0	Active ingredient
Lactose				
Microcrystalline cellulose				
Croscarmellose sodium				
polyvidone				
Sodium lauryl sulphate				
Magnesium stearate				
Core tablet weight				
Coating				
Methylhydroxypropylcellulose				
Polyethylene glycol, PEG 300				
Red iron oxide E172				
Yellow iron oxide E172				
Titanium dioxide				
Coated tablet weight	229.6	224.6	204.6	

Route of Administration: Oral

Proposed Clinical Protocol: This is a multi-center, phase I safety and pharmacokinetic clinical trial of orally administered ZD1839 with multiple rising dose and sequential block escalation in patients with solid malignant tumors known to commonly overexpress EGFR, which are refractory to other treatments.

Objective: To assess:

- a) the tolerability and toxicity,
- b) multiple dose PK,
- c) antitumor activity of ZD1829 in patients with advanced cancer, and
- d) effect on biological surrogates of antitumor efficacy (Ki67, c-fos mRNA and EGFR) in tumor biopsies

Starting Dose: a) Single dose: 100 mg (63 mg/m²)

b) Multiple dose: 100 mg/day (63 mg/m²/day)

Escalation: Multiple dose: 250, 500, 750 and 1,000 mg/day

(156, 313, 469 and 625 mg/m²/day)

Frequency and duration: a) Single dose: one day treatment followed by 6 days washout

b) Multiple dose: daily x 14, followed by 14 days off treatment

Age of patient population: ≥ 18 years

Previous Review(s), Date(s), and Reviewer(s): Safety Review, 12/16/97, Zheng

Studies reviewed for IND

I. Previous human experience

1. Clinical experience (Vol. 1, p101)

I. Pharmacology

2. Pharmacology Relevant to Cancer (Vol. 1, p265)

III. Safety Pharmacology

3. General pharmacology (Vol. 1, p143)

IV. Toxicology*Single dose Studies*

4. TLM/958 Acute toxicity (limit) study in mice: oral administration (Vol. 2, p1)
 5. TLR/2571 Acute toxicity (limit) study in rats: oral administration (Vol. 2, p47)

Multi-dose Studies

6. TAR/2492 Rat pilot toxicity study (Vol. 2, p112)
 7. TAD/870 Dog pilot toxicity study (Vol. 2, p167)
 8. TAR/2570 One month oral toxicity studies in rats (Vol. 3, p1)
 9. TAD/876 One month oral toxicity study in dogs (Vol. 4, p1)

V. Toxicokinetics/ADME The PK studies were integrated into the above toxicity studies

10. KMR/010 The tissue distribution of total radioactivity in the rat following oral administration of [¹⁴C]-ZD1839 (Quantitative whole body autoradiography) (Vol. 5, p45)
 11. KMR/007 The disposition of [¹⁴C]-zeneca ZD1839 in the rat (Vol. 5, p92)
 12. BIT/00401 The effects of ZD1839 on the hepatic microsomal mixed function oxidase enzymes of the male rat (Vol. 5, p111)
 13. KKR/008 The distribution of radioactivity in the blood after oral and intravenous administration of [¹⁴C]-zeneca ZD1839 to rats (Vol. 5, p132)
 14. KKD/009 The disposition of [¹⁴C]-zeneca ZD1839 in male dogs (Vol. 5, p164)
 15. KPJ/013 The binding of [¹⁴C]-ZD1839 to plasma proteins (Vol. 5, p191)
 16. KMN/012 Metabolism of zeneca ZD1839 in rat, dog and human hepatocytes (Vol. 5, p208)

VI. Special Toxicology*Reproductive Toxicity*

16. TGR/2616 Single female fertility study in rats: oral administration (Vol. 4, p307)

Genetic Toxicity

17. TMV/668 An evaluation of mutagenic potential using *S. typhimurium* and *E. Coli* (Vol. 4, p332)
 18. TQR/2573 Micronucleus test in the rat: oral administration (Vol. 4, p369)
 19. TYX/78 *In vitro* cytogenetic study using cultured human lymphocytes (Vol. 5, p1)

Studies not reviewed**III. Toxicology**

- TLM/958 Acute toxicity (limit) study in mice: intravenous administration (Vol. 2, p20)
 TLR/2571 Acute toxicity (limit) study in rats: intravenous administration (Vol. 2, p87)

IV. Toxicokinetics and ADME

- KPV/006 Validation of _____ method for the determination of zeneca ZD1839 concentrations in rats and dog plasma (Vol. 5, p234)
 KPV/014 Validation of _____ method for the determination of zeneca ZD1839 concentrations in human plasma (Vol. 5, p274)
 KML/002 The synthesis of N-(3-Chloro-4-fluorophenyl)-7-methoxy-6-(3-morpholino-[2,3-

¹⁴C]propoxy)quinazolin-4-amine hydrochloride (Vol. 5, p311)

Note that portions of this review were excerpted directly from the sponsor's submission.

Review

Introduction: The EGFR tyrosine kinase is activated by binding of a variety of ligands to the external domain which causes EGFR itself and a number of cellular substrates to become phosphorylated on tyrosine domains. Over-expression of EGFR has been shown in a significant percentage of human tumors, and has been correlated in many cases with poor prognostic features. Evidence suggests that blockade of the EGFR pathway, either by the dominant negative mutant or the antisense approach, reduces the proliferation and invasive properties of a human colon tumor cell line. Antibodies that block the EGF binding site of EGFR inhibit tumor cell proliferation in cell cultures and produce complete regressions of some human tumor xenografts. Mice with mutant EGFR representing a model of partial inhibition of EGFR *in vivo* are healthy and fertile, but have hair, skin and eye abnormalities. Recent studies have shown that EGFR can protect tumor cells from apoptosis and anti-EGFR antibody induces both G1 arrest and apoptosis. ZD1839, an inhibitor of EGFR, is being developed as an orally active anti-tumor agent for the treatment of a broad range of major human solid tumor types.

I. Previous human experience

Clinical experience (Vol. 1, p101). Two clinical trials were conducted in healthy male human volunteers. Single oral doses of up to 75 mg ZD1839 was given to human subjects. One volunteer at 75 mg dose (46.9 mg/m²) experienced an altered state of awareness commencing within 6 hrs after dosing and lasted for 4 hrs. It is noted that all 4 subjects experienced adverse events, of which one reported severe headache and one reported severe neck pain. Headache was also observed in subjects given placebo and ZD1839 at lower doses but were all mild to moderate in severity. At 46.9 mg/m², the human AUC level was higher than that for dogs at 40 mg/m² but lower than that in rat at 60 mg/m² and the correlation of AUC to dose was not evaluated for humans at higher doses or for rats or dogs at lower doses.

In another phase I study, healthy male volunteers were administered 3 consecutive daily doses of 100 mg (63 mg/m²) ZD1839. Adverse events reported were headache, flu-like symptoms, and ophthalmological abnormalities (medial corneal deposits for both eyes, conjunctival hyperemia, prominent nerve and blood vessel in right eye). The ophthalmological abnormalities were not seen in placebo-treated subjects. It is alarming that the AUC almost doubled after just 3 repeated daily dose of ZD1839 in human volunteers at 63 mg/m² compared to that after a single dose. This may suggest an increased absorption or drug accumulation due to slow clearance.

II. Pharmacology

Pharmacology Relevant to Cancer (Vol. 1, p265).

***In vitro* studies:** Enzyme assay showed that ZD1839 markedly inhibited the autophosphorylation of EGF-stimulated EGFR in KB oral squamous, A549 lung, DU145 prostate, and HT29 colorectal carcinoma cells in culture (IC₁₀₀ = 160 - 800 nM). ZD1839 selectively inhibited EGF-stimulated human oral tumor cell (KB) growth in culture; the IC₅₀ was 80-90 nM. Inhibition of non-EGF-stimulated basal cell growth by ZD1839 was 40 times less potent (IC₅₀ 3.64 μM). The selectivity of ZD1839 in growth inhibition were demonstrated in a panel of cells grown in medium with different growth factors. In a

series of experiments with NIH 3T3 mouse fibroblasts, ZD1839 was shown to inhibit proliferation induced by multiple growth factors.

Cells	Growth factor/Stimulation	ZD1839 IC ₅₀ (μM) ^a
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

^aCell proliferation assay; ^bhuman vascular endothelial cells

***In vivo* studies:** antitumor activity of ZD1839 in human tumor xenografts in nude mice was tested in a panel of tumor xenografts. Statically significant responses to ZD1839 have been demonstrated in 8/16 xenograft models (listed in the following table). For the other 8/16 xenografts tested, no statistically significant response were seen with KB oral squamous; HX147 non small cell lung and MiaPaCa2 pancreatic carcinomas; no effect was seen in P246 broncho-epithelial; MKN45 gastric; AR42J pancreatic, RT112 bladder and MDA-MB-231 breast carcinomas. Regression and continued suppression of tumor xenograft growth was also demonstrated in A431 human squamous vulval carcinoma xenografts in nude mice given 200 mg/kg/day > 100 days and the inhibition of tumor growth sustained for up to 4 months. Antitumor activity of ZD1839 (50 mg/kg/day) was also seen in the AO strain rat with transplantable rat uterine tumors, T169 and 3010, with tumor growth inhibition of 83% and 56%, respectively.

Tumor xenograft	Tumor type	Dose (mg/kg) ^a	Tumor age at start (days)	No. of doses	% Inhibition of tumor vol.	Significance (p value) ^b
A549	non small cell lung adeno-carcinoma	3.125	11	25	11.6	NS
		12.5	11	25	44.3	< 0.05
		50	0-11	21-35	50-76	< 0.01
		200	4-33	14-35	33 - 81	< 0.05
CR10	colorectal ca.	200	11	11	96	< 0.01
DU145	prostate ca.	3.125	15	48	7.7	NS
		12.5	15	48	45	< 0.05
		50	15	48	53	< 0.05
		200	15-49	14-48	58 - 85	< 0.01
HCT15	colorectal ca.	3.125	13	56	0	NS
		12.5	13	18-56	0-44	NS
		50	13	56	42	NS
		200	13	28-56	57 - 59	NS - <0.01
HT29	colorectal	3.125	12	34	31.3	NS

	ca.	12.5	12	34	8.5	NS
		50	12	34	21	NS
		200	12-14	34-35	43-75	NS - <0.01
HX62		200	27	38	67	< 0.01
LOVO	colorectal ca.	12.5	6	14	30.5	NS
		50	5-6	14-16	42-61	< 0.05
		200	5-14	14-16	33-80	< 0.05
MCF-7 ^c	breast ca.	200	14	22	26	NS
MCF-7		200	27	40-50	55-65	< 0.01

^a expressed as free base; ^b student's t test (2-tailed); ^c nude mice received estrogen-supplementation

Comment: the results indicated that the *in vitro* anti-proliferative efficacy of ZD1839 was not consistent and with the *in vivo* antitumor effect for the KB human oral squamous tumor although cells of this tumor express EGFR.

III. Safety Pharmacology

The review on this part is based on the integrated summary provided by the sponsor; no individual study report was provided.

Cardiovascular and respiratory function: *In vitro* testing of ZD1839 at 10 μ M revealed no agonist or antagonist activity in the guinea-pig right or left atria. Weak antimuscarinic and antihistamine effects were observed. ZD1839 (100 mg/kg po) had no significant effect on the blood-pressure or heart-rate in the spontaneous hypertensive rat over a period of 20 hrs, and had no bronchoconstrictor or bronchodilator activity in the anesthetized guinea-pig.

Gastrointestinal function: *In vitro* testing of ZD 1839 at 10 μ M revealed no agonist or antagonist activity in the guinea-pig ileum. ZD1839 (100 mg/kg po) had no effect on gastrointestinal motility in the mouse.

Central nervous system function: ZD1839 (100 mg/kg po) had no stimulant or depressant activity, or any effects on a range of other behavioral parameters. At a dose of 100 mg/kg (i.m.) ZD1839 did not produce local anesthesia in the mouse.

Renal function: ZD1839 at a dose of 100 mg/kg caused significant decreases in the excretion of water, sodium, chloride and urine osmolarity.

Immune function and inflammation: ZD1839 at a dose of 100 mg/kg po produced no effect on the delayed hypersensitivity response to Freund's adjuvant in the mouse; this dose, however, caused a weak, statistically significant inhibition (16%) of acute inflammatory response to carrageenin in the rat.

IV. Toxicology

Single dose Studies

TLM/958 Acute toxicity (limit) study in mice: oral administration (Vol. 2, p1). Conducted by the sponsor in England with compliance to the GLP in UK. The signed GLP and QA statements were provided. Single oral dose MTD of ZD1839 was found to be greater than 6,000 mg/m² in the mouse.

species: Alpk:AP₁CD-1 mice (5/sex)
 age; weight: 4-5 wks; 19.5-22.5 g (♂), 18.7-20.5 g (♀)
 drug: ZD1839 (Ref. No. ADM36757E96)
 vehicle: 0.5% (w/v) hydroxypropyl methylcellulose in 0.1% w/v aqueous polysorbate 80
 dosage: 2,000 mg/kg
 route: oral gavage at a volume of 20 ml/kg b.w.
 duration: single administration

Observations

Clinical signs twice daily for 13 days
 Body weights predose on D1, D8, and prior to necropsy on D15

Results

- a. Clinical Observations: no deaths occurred and no clinical signs observed throughout the study period.
 b. Body weight: all mice gained weight during the study period.

TLR/2571 Acute toxicity (limit) study in rats: oral administration (Vol. 2, p47). Conducted by the sponsor in England with compliance to the GLP in UK. The signed GLP and QA statements were provided. The target organs for acute toxicity of ZD1839 were identified to be the adrenal glands, heart, GI tract, liver, kidney, skin, spleen and trachea. No single oral LD₁₀ of ZD1839 was derived but it can be predicted to be less than 12,000 mg/m² in the rat. Deaths occurred only in ♀ animals.

species: Alpk:AP₁CD-1 rats (5/sex)
 age; weight: 4 wks; 104-125 g (♂), 102-121 g (♀)
 drug: ZD1839 (Ref. No. ADM36757E96)
 vehicle: 0.5% (w/v) hydroxypropyl methylcellulose in 0.1% w/v aqueous polysorbate 80
 dosage: 2,000 mg/kg
 route: oral gavage at a volume of 20 ml/kg b.w.
 duration: single administration

Observations

Clinical signs twice daily for 15 days
 Body weights predose on D1, D8, and prior to necropsy on D15
 Gross Pathology on D15
 Histopathology on D15

Results

- a. Clinical Observations: 4/10 deaths (euthanized in moribund condition): 3 ♀ (D3) and 1 ♀ (D6). The onset of the clinical signs began on D5. Clinical signs observed include hunched posture (10/10), loss of skin tone (10/10), piloerection (10/10), subdued behavior (6/10), trembling and shaking (1/10) and urinary staining (4/10). All signs were recovered by the end of the study on D15.
 b. Body weight: ↓ body weight by 7% in ♂ on D8 compared to the predose b.w. on D1. Only one ♀ survived by D8 which had only 2.3% increase from the predose b.w. measured on D1. The surviving 5 ♂ and 1 ♀ rats gained weight on D15 compared to D7 (63 g for the ♂ and 46 g for the ♀). No control animal was included in this study.
 c. Gross pathology: tissues were taken from 6 animals (2 survivors and 4 decedents) and the following changes were found: discoloration of the adrenal glands (3/6); content and discoloration of the GI tract (3/6); adhesion of duodenum to liver (1/6); softness and discoloration of the spleen (1/6), and brown material present on the

- surface of the stomach (2/6).
- d. Histopathology: the cause of death in 1/4 decedents was a perforated duodenal ulcer; test article-related findings were found in the following target organ/systems:
- Endocrine system: adrenal medullary vacuolation (3/6)
 - Cardiovascular system: myocarditis (2/6);
 - GI tract: necrosis or ulceration in duodenum (2/6); necrosis with ulceration in cecum (2/6); necrosis and ulceration in the stomach (1/6)
 - Respiratory system: mucosal atrophy or necrosis of the trachea (3/6), multifocal hemorrhage (1/6)
 - Liver: periportal hepatocyte vacuolation (2/6); multifocal hepatocyte necrosis (1/6)
 - Kidney: tubular dilatation (2/6) and basophilia (1/6)
 - Spleen: splenic atrophy (4/6) and macrophage vacuolation (1/6)
 - Skin: microabscess formation (2/6)

Multiple dose Studies

TAR/2492 Rat pilot toxicity study (Vol. 2, p112). Conducted by the sponsor in England with compliance to the GLP in UK. The signed GLP and QA statements were provided. Gender difference in sensitivity to ZD1839 was observed again. Repeat dose LD₁₀ for ♂ rats > 750 mg/m²/day × 14 while for ♀ rats the LD₁₀ < 750 mg/m²/day × 14. The approximate LD₁₀ based on the MTD in ♀ rats was 300 mg/m²/day × 14.

species: Alpk:AP,CD-1 rats (5/sex/group for toxicity study; 4/sex/group for PK).
 age; weight: 5-6 wks; 124 - 159 g
 drug: ZD1839 (batch Ref. No. 019)
 vehicle: 0.5% (w/v) hydroxypropyl methylcellulose in 0.1% w/v aqueous polysorbate 80
 dosage: 0, 10, 50 and 125 mg/kg/day
 route: oral gavage at a volume of 5 ml/kg b.w.
 duration: 14 consecutive days

Observations

Clinical signs daily
 Body weights daily
 Food consumption daily

Clinical Pathology

Hematology: D15
 Blood chemistry: D3, D15

Ophthalmology D15

Pharmacokinetics at 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 18 and 24 hrs after dosing on D1 and D14

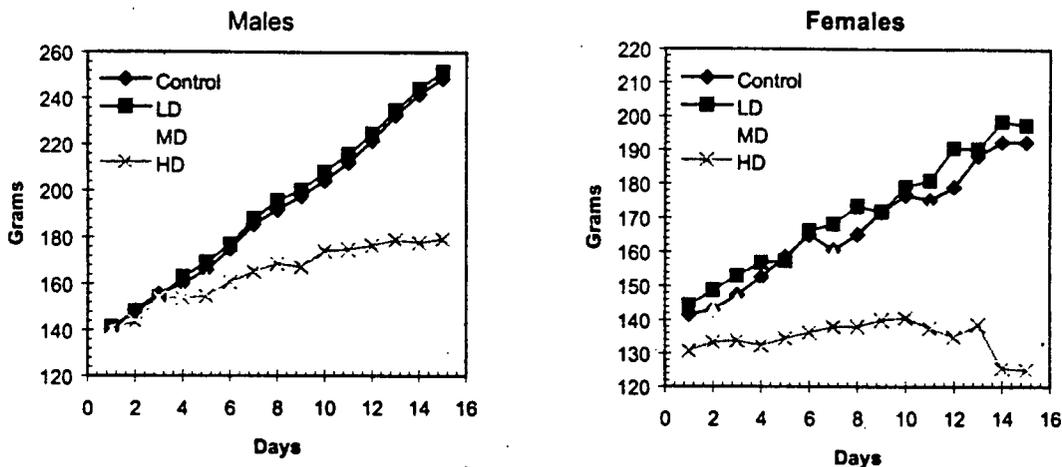
Gross Pathology on D15

Histopathology on D15

Results

- a. Clinical Observations: Mortality: 3/5 ♀ at HD died (1 on D12 and 2 on D15)
 Control and LD: no clinical signs;
 MD: stains around muzzle (5/5 ♂; 5/5 ♀), red exudate around the eyes (2/5 ♂; 5/5 ♀), hair loss (1/5 ♀), hunched posture (4/5 ♀), loss of skin tone (1/5 ♀), subdued behavior (1/5 ♀);
 HD: stains around muzzle (5/5 ♂; 5/5 ♀), red exudate around the eyes (4/5 ♂; 5/5 ♀), hunched posture (5/5 ♂; 5/5 ♀), loss of skin tone (4/5 ♂; 5/5 ♀), partially closed eyes (4/5 ♂; 5/5 ♀), and subdued behavior (4/5 ♀)

b. Body weight:- The group mean body weights are presented in the following graphs:



↓ body weight gains were obvious for HD group, reaching maximum by the end of the experiment; changes were more significant in the females. MD group also had a reduced weight gain compared to the controls.

c. Food Consumption:

Changes (%) in Food Consumption During the Study (relative to the control)						
Days	LD		MD		HD	
	♂	♀	♂	♀	♂	♀
4-5	-	↓9	-	↓8	↓7	↓24
9-10	-	-	-	↓12	↓12	↓19
14-15	-	-	-	-	↓29	↓69

*-: changes ≤ 5%

d. Clinical Pathology

Changes (%) in hematology and blood chemistry on Day 15 ^a						
Parameters	LD		MD		HD	
	♂	♀	♂	♀	♂	♀
PLT	- ^b	-	-	↑25	↑41	↑70
WBC	↓6	-	↑19	↑117	↑155	↑130
NEUT	-	-	↑210	↑724	↑655	↑713
Urea	-	-	-	↑7	↑24	↑197
ALP	↑11	-	-	-	↓11	↑162
ALT	↑28	↓14	↑44	-	↑52	↑37
AST	↑12	-	↑18	↑25	↑44	↑55
TG	↓16	-	↓21	↑39	↑55	↑51

^aonly 2 HD ♀ were included in hematology assay and 1 HD ♀ was included for blood chemistry analysis; ^b-: changes ≤ 5%

- e. Ophthalmology: HD and MD females had slightly dry, rough corneal surfaces.
- f. Pharmacokinetics: systemic exposure to ZD1839 was demonstrated at all dose levels with maximum plasma concentration occurring between 2 and 6 hours after dosing.

The AUC₀₋₁₂ for ♀ were 23%-44% higher than that of males.

Plasma concentrations and derived parameters on D1						
Time after dose (h)	ZD1839 concentration (µg/ml)					
	LD		MD		HD	
	Male	Female	Male	Female	Male	Female
0.5						
1						
2						
3						
4						
5						
6						
8						
12						
18						
24						
AUC ₀₋₁₂ (µg•h/ml)	0.971	1.85	12.7	17.6	30.9	41.3
C _{max} (µg/ml)	0.206	0.210	1.54	2.14	4.58	4.93
t _{max} (h)	5	6	2	3	2	6

^aNQ: below the assay limit of quantification (ng/ml); ^bNR: no result

Plasma concentration derived parameters on D14						
Time after dose (h)	ZD1839 concentration (µg/ml)					
	LD		MD		HD	
	Male	Female	Male	Female	Male	Female
Pre-dose						
0.5						
1						
2						
3						
4						
5						
6						
8						
12						
18						
24						
AUC ₀₋₁₂ (µg•h/ml)	2.11	3.03	14.1	17.4	23.5	NA ^b
C _{max} (µg/ml)	0.408	0.408	2.05	1.89	3.44	3.50
t _{max} (h)	3	3	6	5	5	2

^aNQ: below the assay limit of quantification (mg/ml);

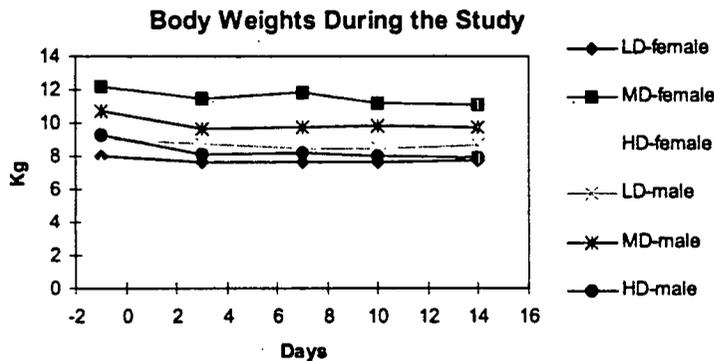
^bAUC₀₋₁₂ cannot be determined due to the lack of sufficient sample points

- g. Gross pathology: enlarged adrenals (3/5 HD ♀), enlarged LN (HD: 5/5 ♂ and 3/5 ♀; MD: 1/5 ♂ and 4/5 ♀), red exudate on eyelids or muzzle (HD: 4/5 ♂ and 4/5 ♀; MD: 4/5 ♀), small thymus (HD: 1/5 ♂ and 2/5 ♀)
 changes in absolute organ weights in HD: liver: ↓7% (♂) and ↓21% (♀), adrenals: ↑19% (♂) and ↑13% (♀), kidney: ↓13% (♂) and ↓10% (♀);
 changes in relative organ weights in HD: liver: ↑7% (♂) and ↑20% (♀), adrenals: ↑45% (♂) and ↑79% (♀), kidney ↑14% (♂) and ↑42% (♀).

Hematology: D15
 Blood chemistry: D3, D15
 Ophthalmology: predose, D15
 Pharmacokinetics: at 0.5, 1, 2, 4, 6, 8 and 24 hrs after dosing on D1 and D14
 Gross Pathology: on D15
 Histopathology: on D15

Results

- a. Clinical signs: Mortality: 1 ♀ at HD was killed in moribund condition on D11
 HD: loose feces, emesis, reddened eyes, reddened inside of mouth, thin, eyes partially closed;
 MD: reddened inside of mouth, emesis, loose feces (♀); reddened eyes (♀), eyes partially closed (♀);
 LD: red liquid in feces (♀)
- b. Body weight: no body weight gains were observed for all treated animals during the study, compared to the predose values,
 for ♀: body weight ↓ 11% on D7 and ↓22% on D10 for HD group,
 for ♂: body weight ↓ 12-13% (HD) and 9-10% (MD) on D3-7,
 ↓ 15% (HD) and 9% (MD) on D14
 Body weight were not measured after 14 days



c. Food consumption:

Week	Changes (%) in Food Consumption ^a					
	LD		MD		HD	
	♂	♀	♂	♀	♂	♀
1	↓23	↓25	↓60 ^b	↓43 ^b	↓59 ^b	↓61 ^b
2	↓24	↓12	NR	↓21 ^b	↓66 ^b	NR ^c

^ano control animal was included in this study, the comparison was made to the predose value at week -1 for each individual animal;

^bindicates a mean omitting supplemented diet(s) which were offered between D4-6 of dosing until D15; ^cNR =no record

- d. Clinical Pathology: increases in WBC and neutrophils and decreases in RBC and HB were seen in almost all groups. ↓ in blood levels of albumin and ALP were seen in all treated animals. ↑ urea was only seen in HD animals. ALT was ↓ in LD and MD, but ↑ in HD

Changes (%) in hematology and blood chemistry on D7 and D14*												
Parameter s	LD				MD				HD			
	♂		♀		♂		♀		♂		♀	
	D7	D14	D7	D14								
RBC	-	↓9	-	↓18	-	-	-	-	↓11	↓15	↓8	↓18
HB	↓8	↓15	-	↓16	-	↓9	-	↓10	↓10	↓15	↓8	↓19
WBC	-	↑47	-	-	↑59	↑6	-	↓8	-	-	-	↑21
NEUT	-	↑72	↓8	↑13	↑50	-	-	↓18	-	↑16	-	↑30
ALB	↓10	↓17	↓13	↓20	↓24	↓41	↓13	↓28	↓25	↓41	↓28	↓28
ALP	↓22	↓28	↑41	↓23	↑16	↓39	-	↓29	-	↓19	-	↓36
ALT	↓14	↓29	↓13	↓33	↓38	↓51	↓27	↓25	↑30	↑70	↑183	↑155
UREA	-	↑8	↓20	↓8	↑18	-	↑16	↓21	-	↑32	-	↑83

*comparisons were made to the respective predose value for each specific parameter

- e. Ophthalmology:
 - HD: diffuse staining of the whole cornea surface of both eyes with Rose Bengal; the tear films had a speckled appearance and moved slowly after blinking;
 - MD: Rose Bengal staining in one area of one eye of each animal; the ♀ dog had a rough appearance to the surface of both corneas and tear films moved slowly after blinking.

f. Pharmacokinetics:

PK Parameters on D1						
Parameter	LD		MD		HD	
	Male	Female	Male	Female	Male	Female
t _{1/2} (h)	7.1	6.7	4.8	7.6	9.9	8.8
AUC _{0-∞} (µg•h/ml)	20.5	17.6	105.1	163.1	184.9	210.9
AUC ₀₋₁₂ (µg•h/ml)	18.9	17.6	101.5	141.7	148.7	177.2
C _{max} (µg/ml)	2.56	1.62	8.69	11.8	12.8	16.0
t _{max} (h)	1	2	4	4	1	1

^aNQ: non quantifiable

PK Parameters on D14						
Parameter	LD		MD		HD	
	Male	Female	Male	Female	Male	Female
t _{1/2} (h)	7.1	6.7	NC ^b	NC	NC	NC
AUC _{0-∞} (µg•h/ml)	22.9	17.0	NC	NC	NC	NC
AUC ₀₋₁₂ (µg•h/ml)	20.3	15.3	62.7	94.2	192.6	NC
C _{min} (µg/ml)	0.257	0.158	1.43	2.57	6.86	NC
C _{max} (µg/ml)	2.04	1.11	3.53	5.11	9.56	NC
t _{max} (h)	2	4	8	8	6	NC

^aThe animal was sacrificed on D11; ^bNC: not calculated

- g. Histopathology: HD: corneal epithelial atrophy (1/1 ♀), mild foamy alveolar macrophages (2/2), germinal center vacuolation in LN (2/2) and spleen (2/2); diffuse atrophy of the thymus (2/2), multifocal epidermal micro-abscesses (1/1 ♂)
- MD: corneal epithelial atrophy (2/2), germinal center vacuolation in LN (2/2) and spleen (1/1 ♀), diffuse atrophy of the thymus (1/1 ♂), multifocal epidermal micro-abscesses (1/1 ♀)

TAR/2570 One month oral toxicity studies in rats (Vol. 3, p1). Conducted by _____ with compliance to the GLP in UK. The signed GLP and QA statements were provided. No LD₁₀ was derived from this study. The MTD in rats for 4-weeks repeated daily oral administration of ZD1839 was 240 mg/m²/day, which can be taken as the approximate LD₁₀.

- species: Alp:AP,SD-1 (Wistar derived) rats (10 or 15/sex/group for toxicity study; 9 or 18/sex/group for PK)
- age; weight: 6 wks; 134 - 227 g (♂) and 119-174 g (♀)
- drug: ZD1839 (batch Ref. No. ADM36757E96)
- vehicle: 0.5% (w/v) hydroxypropyl methylcellulose in 0.1% w/v aqueous polysorbate 80
- dosage: 0, 2, 10 and 40 mg/kg/day
- route: oral gavage at a volume of 5 ml/kg b.w.
- duration: 29 consecutive days; 5 animals of each sex in Control and HD groups were followed up un-dosed for a further 29 days recovery period

Observations

- Clinical signs twice daily
- Body weights predose, and once a week
- Food consumption once daily continuously from predose at D-7
- Clinical Pathology predose and D29/30 for hematology and blood chemistry; D26 and D54 for the coagulation test in Control and HD animals; D23/24 for urine analysis (D53 for the Control and HD recovery groups)
- Ophthalmology D15
- Pharmacokinetics at 0.5, 1, 2, 4, 8 and 24 hrs after dosing on D1 and D28
- Gross Pathology on completion of the dosing (D30-D33) or withdrawal period (D58)
- Histopathology on completion of the dosing (D30-D33) or withdrawal period (D58)

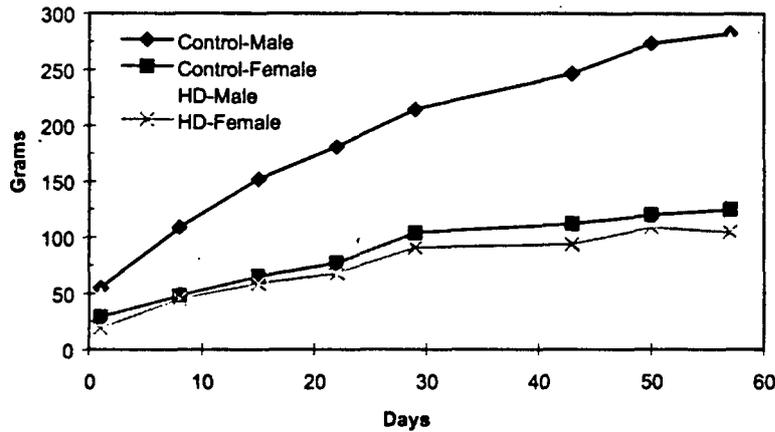
Results

- a. Clinical Observations: Mortality: 1/5 ♀ at MD group designed for PK sampling died on D1. No description was made to address the cause of this premature death and no histopathology data was provided for this animal. For the main toxicity study, no death occurred at HD and no test article-related clinical signs were observed LD and MD groups.

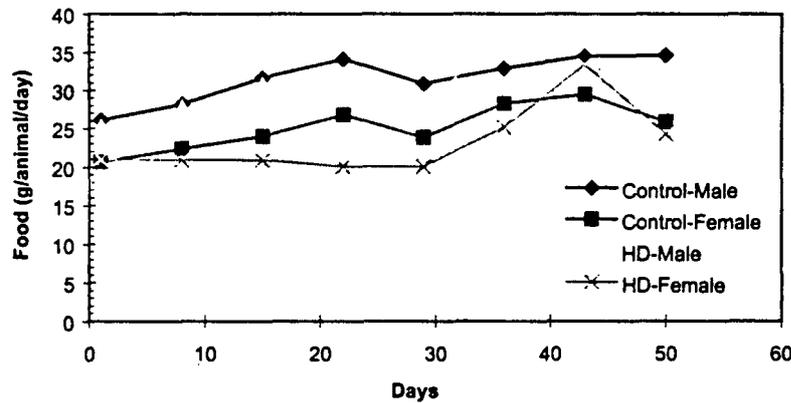
Clinical Signs in the High Dose Group (40 mg/kg/day × 29)				
Findings	♂	Weeks	♀	Weeks
Deposits on nose	12/15	2-5	9/15	3-5
Eyes part closed	1/15	5	5/15	2-5
Limbs/ joints*	—	—	5/15	2-6
Red exudate	1/15	2-3	5/15	2-5
Scabs on mouth	13/15	2-7	15/15	2-6
Ungroomed	—	—	9/15	2-6

*From weeks 2-6, 5 HD ♀ showed slight inflammation and encrustation of the ankle and toe joints of both fore and hind paws.

b. Body weight: HD animals showed a reduction in cumulative body weight gain compared to controls from day 8, ↓ by 19% and 12% for ♂ and ♀, respectively, on D29. This decrease was recovered for the male by D57 but not for the ♀ rats (↓ by 16%). The cumulative body weight gains for control and HD groups are presented in the following graph:



c. Food consumption:



d. Clinical Pathology:

Changes (%) in Hematology and Blood Chemistry in HD animals				
Parameters	Male		Female	
	D29/30	D57	D29/30	D57
RBC	↓7	—	↓10	—
HB	—	—	↓12	—
WBC	↑142	↑44	↑126	↑72
NEUT	↑546	↑72	↑400	↑158

ALB	↓16	-	↓15	↓9
A/G	↓22	↓11	↓24	↓19
ALT	↑46	↑32	↑32	↑33
AST	↑55	↑35	↑29	↑13

e. Ophthalmology:

Incidence of Granular Appearance of the Cornea in Rats (Numbers/Eyes examined)								
Time	Control		LD		MD		HD	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 4	4/30	0/30	0/20	0/20	3/20	3/20	2/30	8/30
Week 8	0/10	0/10	-	-	-	-	0/10	0/10

f. Pharmacokinetics: after repeated dosing for 29 days, the half life ($t_{1/2}$) increased from 5-7 h for MD group to ~24 h for the HD females; the t_{max} was 1 h for HD ♀ but 8 h for HD ♂.

Summary of the PK Parameters on D1		
Parameter	Male	Female
$t_{1/2}$ (h)	NC ^b	6.81
AUC ₀₋₂₄ (ng•h/ml)	NC	NC
AUC ₀₋₈ (ng•h/ml)	NC	350
C _{max} (ng/ml)	21.3*	28.7*
t_{max} (h)	4	4

^aNQ: not quantified; ^bNC: not calculated; *p < 0.05 between male and female

Summary of the PK Parameters on D29						
Parameter	LD		MD		HD	
	Male	Female	Male	Female	Male	Female
$t_{1/2}$ (h)	NC ^b	6.81	5.03	7.30	NC	23.8 ^c
AUC ₀₋₂₄ (ng•h/ml)	NC	NC	4611	6765	22753	27264
AUC ₀₋₈ (ng•h/ml)	NC	350	2547	3216	8937	10662
C _{max} (ng/ml)	52.0	63.9	535	578	1463	1690
t_{max} (h)	4	0.5	2	2	8	1

^aNQ: not quantified; ^bNC: not calculated; ^cestimated value

g. Gross Pathology: HD: discolored papilla in kidney: 2/10 ♀, enlarged LN: 8/10 ♂ and 9/10 ♀, hair loss: 2/10 ♂ and 4/10 ♀, discoloration of skin 4/10 ♂ and 4/10 ♀, scab formation in the skin: 4/10 ♂ and 1/10 ♀, enlarged spleen: 1/10 ♂ and 3/10 ♀; enlarged LN were also seen in 3/10 LD ♂ and 1/10 control ♂.
Changes (%) in the relative organ weights in HD group compared to the controls are summarized in the following table:

Organs	Male		Female	
	D29/30	D57	D29/30	D57
Adrenals	↑19	↑14	-	↓8
Liver	-	↑9	↑15	↑17
Ovary	NA	NA	↓21	↓17

Prostate	↓12	-	NA	NA
Uterus	NA	NA	↓24	↓28
Kidney	↑13	↑7	↑10	↑8
Thymus	↑13	↑9	↑16	↑16

h. Histopathology:

- HD:** 10/10 ♀ showed bilateral corneal epithelial atrophy; corneal epithelial atrophy had completely reversed by the end of the withdrawal period; papillary necrosis of kidney: 6/10 ♀ and 1/10 ♂; papillary inflammation of the kidney: 4/10 ♀ and 1/10 ♂; kidney tubular dilatation 2/10 ♀ and 1/10 ♂; reduced corpora lutea: 9/10 ♀; scab formation in inguinal region of the skin: 10/10 ♀ and 3/10 ♂; focal inguinal folliculitis: 2/10 ♀ and 2/10 ♂; eyelid scab formation: 2/10 ♀ and 8/10 ♂; eyelid folliculitis: 6/10 ♀ and 2/10 ♂; eyelid abscess: 6/10 ♀; muzzle scab formation: 6/10 ♀ and 5/10 ♂; muzzle folliculitis: 8/10 ♀ and 6/10 ♂; muzzle abscesses: 2/10 ♀ and 2/10 ♂; extramedullary hematopoieses: 8/10 ♀ and 3/10 ♂; reactive lymphoid hyperplasia in mandibular LN: 10/10 ♀, 8/10 ♂;
- MD:** reduced corpora lutea: 3/10 ♀; eyelid scab formation: 1/10 ♀; muzzle scab formation: 1/10 ♀; muzzle folliculitis: 1/10 ♀; extramedullary hematopoieses: 3/10 ♀ and 1/10 ♂;
- LD:** extramedullary hematopoieses: 1/10 ♂; scab formation in inguinal region of the skin: 1/10 ♀ and 2/10 ♂; focal inguinal folliculitis: 1/10 ♀;
- Control:** extramedullary hematopoieses: 1/10 ♀.

TAD/876 One month oral toxicity study in dogs (Vol. 4, p1). Conducted by _____ with compliance to the GLP in UK. The signed GLP and QA statements were provided. Oral administration of ZD1839 at 800 mg/m²/day × 30 was lethal and severely toxic dose for the dog. The multiple oral HNSTD for dogs was 200 mg/m²/day × 30.

- species:** _____ Beagle dogs (3/sex/group for LD and MD; 6/sex/group for Control and HD)
- age; weight:** 36-47 wks; 8.6-13.8 kg (♂) and 8.1-10.7 kg (♀)
- drug:** ZD1839 (batch Ref. No. ADM36757E96)
- vehicle:** 0.5% (w/v) hydroxypropyl methylcellulose in 0.1% w/v aqueous polysorbate 80
- dosage:** 0, 2, 10 and 40 mg/kg/day
- route:** gastric intubation once daily at a volume of 1 ml/kg b.w.
- duration:** 30 consecutive days; 3 animals of each sex in the Control and HD groups were followed up for a 4-week withdrawal phase

Observations

- Clinical signs** twice daily
- Body weights** predose, and once a week
- Food consumption** once daily continuously from predose at D-7
- EKG** predose, D2-4 and D24-26 of dosing, during the 4th wk of the withdrawal period
- Clinical Pathology**
- Hematology and blood chemistry:** predose and D29/30; D53 for groups in recovery phase;
- Urinalysis:** predose and on D29/30
- Ophthalmology** predose, wks 2, and 4; the 4th week of the withdrawal period
- Pharmacokinetics** at 0.5, 1, 2, 4, 8 and 24 hrs after dosing on D1 in LD only and on D30 in all dose groups
- Gross Pathology** on completion of the dosing (D31-D33) or withdrawal period (D59-60)
- Histopathology** on completion of the dosing (D31-D33) or withdrawal period (D59-60)

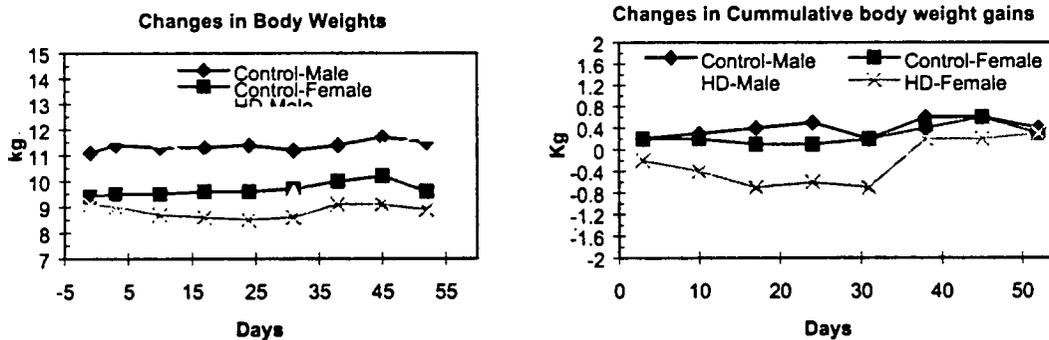
Results

- a. Clinical Observations: one death (♂, premature kill) in HD on D18; no test article-related clinical signs were observed for the control, LD and MD groups.

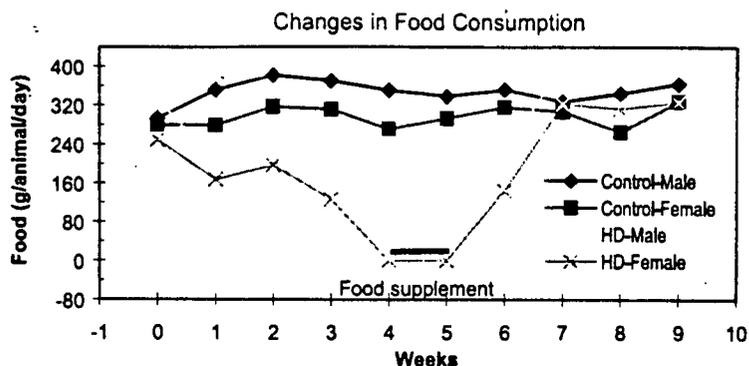
Clinical Signs in the High Dose Group (40 mg/kg/day× 30) (main test)					
Findings	♂	♀	Findings	♂	♀
Loose feces	6/6	6/6	Emesis	1/6	0/6
Abnormal feces	6/6	6/6	Eyelids swollen	1/6	0/6
Thin	3/6	2/6	Sores in mouth	1/6	0/6
Reddened mouth	1/6	2/6	Reddened skin on hind feed	1/6	0/6
Reddened eye(s)	2/6	1/6	Cold	1/6	0/6
Discharge from eye(s)	1/6	3/6	Subdued	1/6	0/6
Partially closed eye(s)	1/6	2/6	Trembling/shaking	1/6	0/6
			Loss of skin tone	1/6	0/6
Clinical Signs in the High Dose Group (40 mg/kg/day× 30) (withdrawal)					
Loose feces	1/3	1/3	Discharge from eye(s)	1/3	2/3
Thin	2/3	1/3	Partially closed eye(s)	1/3	1/3
Reddened mouth	1/3	1/3	Sores in mouth	1/3	0/3
Reddened eye(s)	1/3	1/3			

Comment: the premature kill on D18 (for animal #834) was not mentioned in the summary of this study.

- b. Body weight: HD animals showed a reduction in body weight and cumulative body weight gain compared to controls. The changes in body weights and cumulative body weight gains for control and HD groups are presented in the following graph:



- c. Food consumption: Food consumption was most affected for the HD group. Due to inappetance in HD group, supplements were provided as necessary from D20 to necropsy (D31/33) for main test or until D38 (withdrawal).



d. EKG: HD: 2/12 animals (1 ♀ and 1 ♂) had lengthened PR intervals with large variations (> 30 milliseconds) between PR interval measurements at wk4; the ♂ animal also had one incident of second degree A-V block 2 h after dosing. By the 4th week of the withdrawal period, the ECG of both these animals had returned to normal.

e. Clinical pathology:

Hematology

Changes (%) in Hematology in HD animals				
Parameters	Male		Female	
	D29	D53	D30	D53
RBC	↓8	↓6	↓11*	↓25
HB	↓7	↓7	↓13*	↓21
HCT	↓9	--	↓11*	↓17
WBC	↑27**	--	↑6	--
NEUT	↑30**	--	↓10	--

* p ≤ 0.05; **p ≤ 0.01

Blood chemistry

Changes (%) in Blood Chemistry						
Parameters	MD		HD			
	Male D29	Female D30	Male D29	D53	Female D30	D53
ALB	↓20*	↓23**	↓47**	↓11	↓40**	↓14
A/G	↓16	↓19	↓48**	↓30	↓39**	↓36
ALP	↓8	↓12	↓44**	↑7	↓46**	↑40
ALT	↓31	↓17	↓18	↓32	↓58**	↓29
CHOL	--	↑10	↑36**	↑15	↑20	↑69
TRG	--	↓13	↑58**	↑58	↑10	↑92

* p ≤ 0.05; **p ≤ 0.01

Urinalysis:

Changes (%) in Urinalysis								
Parameters	LD		MD		HD			
	Male D29	Female D30	Male D29	Female D30	Male D29	D53	Female D30	D53

NAG ^a	↑124	↑>248	↑374	↑>244	↑329*	↑185**	↑>539**	--
K ⁺ /creatinine	↑52	↓52	↑36	↓52	↓77	↓43	↓42	↑139
Na ⁺ /creatinine	↓90	--	↓96	↓87	↓92	↓84	↓79	↓27

* p ≤ 0.05; **p ≤ 0.01; ^a n-acetylglucosaminidase

f. Ophthalmology:

Incidence of ophthalmological observations in the HD group (occurrence/number of eyes examined)										
Observations	Time point		Week 2		Week 3		Week 4		Week 8*	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Area of diffuse corneal translucency	0/1	0/1	0/2	NA	3/10**	7/12	4/6	3/6		
	2	2								
Rough appearance to whole cornea	0/1	0/1	0/0	NA	2/10	0/12	0/6	0/6		
	2	2								
Retention of Rose Bengal stain by cornea	0/1	NA	0/2	NA	5/10	7/12	0/6	0/6		
	2									
Speckled appearance to tear film or surface of cornea	6/1	2/1	2/2	NA	8/10	2/12	0/6	0/6		
	2	2								
Tear films moves slowly on blinking	4/1	0/1	2/2	NA	4/10	0/12	0/6	0/6		
	2	2								
Conjunctival reddening	4/1	1/1	2/2	NA	2/10	2/12	0/6	0/6		
	2	2								

* the drug withdrawal group; ** due to lost of 1 one ♂ dog on D18

Ocular changes due to the administration of ZD1839 were restricted to the HD group mainly during the week 4. During the withdrawal period, most ocular changes were reversed except an area of faint diffuse corneal translucency was persistent.

g. Pharmacokinetics:

Summary of pharmacokinetic parameters				
Parameter	Day 1	Day 29		
	LD	LD	MD	HD
t _{1/2} (h)	NC ^a	NC	5.8	NC
AUC ₀₋₈ (ng•h/ml)	352	652	5300	15800
AUC ₀₋₁₄ (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

^aNC: not calculated

- h. Gross Pathology: HD: bilateral papilla discoloration in kidney: 2/3 (♂), dark red discoloration in mesenteric LN: 1/3, discoloration ileum mucosal surface: 1/3 (♂),
 MD: bilateral papilla discoloration in kidney: 2/3 (♂), dark red discoloration in mesenteric LN: 1/3 (♂);
 LD: dark red discoloration in thymus 2/3 (♂).

Changes (%) in the relative organ weights in HD group compared to the controls:

Organs	Male		Female	
	D31/33	D53	D29/30	D53

Adrenals	↑33	-	-	-
Liver	-	↑18	↑15	↑17
Testis	-	↓41	NA	NA
Prostate	↑88	↓24	NA	NA
Ovary	NA	NA	-	↑56
Uterus	NA	NA	-	↑314
Spleen	↑132	↑11	↑52	↑15
Brain	↑37	↑8	↑19	-
Kidney	↑36	-	↑16	-
Thymus	not examined			

i. Histopathology:

Incidence of lesions in HD group		
Histological Finding	Males	Females
Corneal epithelial ulceration	0/3	1/3
Corneal epithelial atrophy	2/3	1/3
Eyelid epidermal microabscesses	2/3	2/3
Unilateral papillary necrosis in kidney	0/3	1/3
Germinal center vacuolation (Bronchial LN)	1/3	0/3
Germinal center vacuolation (Cervical LN)	3/3	1/3
Germinal center vacuolation (Mensenteric LN)	1/3	0/3
Spleen lymphoid germinal center vacuolation	1/3	0/3
Thymus atrophy	2/3	0/3

Summary of Toxicology Studies

The target organs of ZD1839 in preclinical single dose toxicity studies were identified to be the skin, adrenal glands, heart, GI tract, liver, kidney, spleen and trachea. Single dose studies revealed a lethal oral dose of 12,000 mg/m² on the rat while ZD 1839 at 6,000 mg/m² in the mouse was not frankly toxic.

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 death occurred and no clinical signs observed for up to 13 days. Histopathology was not examined.
Rat (TLR/2571)	Gavage	5	Lethal	2,000	12,000	12,000 mg/m ² /day: 4/10 deaths (3 ♀ and 1 ♂). clinical signs occurred by D5: hunched posture (10/10), loss of skin tone (10/10); piloerection (10/10), subdued behavior (6/10), trembling and shaking (1/10) and urinary staining (4/10). Histopathology revealed discoloration of adrenal glands and adrenal medullary vacuolation; myocarditis; necrosis and ulceration in GI tract; mucosal atrophy and/or necrosis in the trachea; multifocal hemorrhage in lung; multifocal hepatocyte necrosis; microabscess formation on the skin; splenic atrophy; etc.

Multiple dose studies confirmed the target organs for ZD1839 toxicity are similar to those observed in the acute toxicity studies; target organ toxicity was also observed on cornea of the eyes and thymus in both rats and dogs. The approximate LD₁₀ of 14-day multiple oral dose in rats were 300 mg/m²/day in one study but was < 240 mg/m²/day in pregnant rats. The approximate LD₁₀ of 30-day repeat oral dose in rats was 240 mg/m²/day. The HNSTD for dogs were 1,000 mg/m²/day × 14 or 200 mg/m²/day × 30. Gender difference in sensitivity was seen in rodents but not obvious in dogs; females rats were generally more sensitive than the males to ZD1839 toxicity.

Summary of the Multiple Dose Toxicology Studies						
Species (Study No.)	Route & Duration	N/sex/dose	Critical Doses	mg/kg /day	mg/m ² /day	Significant findings
Rat (TAR/2492)	Gavage 14-days	5	LD ₁₀ ^b NOAE L	50 10	300 60	750 mg/m ² /day: 3/5 ♀ 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 ♀); ↓ b.w. (28% for ♂ and 35% for ♀), ↓ food intake (29% for ♂ and 69% for ♀) by D15; ↑ WBC, NEUT, PLT; ↑ blood urea, ALP (♀), ALT, TG and AST; dry and rough corneal surface; enlarged adrenals; ↑ organ weight for adrenals; thymus atrophy; abnormal histopathologic findings in the adrenals, small intestine, kidneys, liver, lungs, LN, ovaries, skin, spleen and thymus. 300 mg/m ² /day: no deaths, stains around muzzle (5/5 ♀), red exudate around eyes (7/10), loss of skin tone (1/5 ♀), subdued behavior (1/5 ♀); minor decrease in b.w. gain (6%) and food intake (12-19%) by the end of the dosing; ↑ WBC and NEUT, dry and rough corneal surfaces in ♀ rats; abnormal histopathology findings in small intestine, kidneys (♀ only), liver, LN and skin.
Rat (TGR/2616)* Seg. I	Gavage 21-days	10 ♀	LD ₁₀	< 40	< 240	240 mg/m ² /day: 1/10 was euthanized on D9 and 9/10 were euthanized on D11; clinical signs: urine staining, loose feces, hunched posture, piloerection, discharge from eyes, and partially closed eyes; ↓ food intake (21%)
Rat (TAR/2570)	Gavage 1-month	10-15	LD ₁₀ ^b	40	240	240 mg/m ² /day: no death occurred; clinical signs: deposit on nose, eyes partially closed, slight inflammation and encrustation of the ankles and toe joints; red exudate, and scab on mouth; ↓ b.w. (12-19%) on D29; ↓ b.w. by 16% for ♀ 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 ♀); enlarged LN (17/20); discoloration of the skin (8/20); scab in skin (5/20); enlarged spleen (4/20); ↑ relative organ weight: adrenals (19% in ♂); kidney (10-13%); thymus (13-16%); liver (15% in ♀); ↓ 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 LN.
Dog (TAD/870)	Gavage 14-days	1	Lethal NHSTD	75 50	1,500 1,000	1,500 mg/m ² /day: 1 ♀ died on D1. Loose feces, emesis, reddened eyes, reddened inside of the mouth, thin, eyes partially closed; ↓ b.w. by 22% for ♀ on D10 and by 13% for the ♂ on D3; ↓ food intake by 59-61% during wk1 and 66% (♂) during wk2; ↓RBC and HB (8-19%); ↑ WBC and NEUT (16-30%); ↓ ALB (25-41%) and ALP (19-36%); ↑ ALT (30-70% for ♂ and 183-155% in ♀) and blood urea (32-83%); diffuse staining of the cornea surface, corneal epithelial atrophy; germinal center vacuolation in LN and spleen; thymus atrophy, and multifocal epidermal microabscesses. 1,000 mg/m ² /day: emesis, loose feces, reddened eyes and reddened inside of the mouth; ↓ b.w. (10%) for ♂ 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 LN & spleen; diffuse thymus atrophy, & multifocal skin microabscesses.
Dog (TAD/876)	Gavage	3	Lethal	40	800	800 mg/m ² /day: one death (1/3 ♂); 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

	1-month		HNSTD	10	200	<p>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, partially closed eyes and sore in mouth; ↓ in cumulative b.w. 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% ♂); ↓ ALB (40-47%), A/G (39-48%), ALT (18-58%) and ALP (44-46%) on D30; ↑CHOL (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.</p> <p>200 mg/m²/day: no death or clinical signs, ↓ ALB (20-23%), A/G (16-19%), ALT (17-301%); ↓ urine Na+/creatinine (87-96%) on D30</p>
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* this is a reproductive toxicity study conducted on ♀ rats; ^b approximate LD₁₀ based on MTD for the ♀ rats, this 14 study did not have a recovery observation period.

Histopathology Inventory for IND #

<i>Studies</i>	TAR/2570	TAD/876
Species	rat	dog
Adrenals	X	X
Aorta	X	X
Bone Marrow Smear		X
Bone (femur, sternum)	X	X
Brain	X	X
Cervix	X	X
Epididymis	X	X
Esophagus	X	X
Eyes with eyelids	X	X
Fallopian tubes		
Gall bladder	X	X
Gross lesions	X	X
Harderian gland	X	
Heart	X	X
Hyphophysis		
Kidneys	X	X
Lachrymal gland		X
Large Intestine (cecum, colon, rectum)	X	X
Larynx		
Liver	X	X
Lungs and Bronchi	X	X
Lymph nodes, bronchial		X
Lymph nodes, cervical		X
Lymph nodes mandibular	X	
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Optic nerves		
Ovaries	X	X
Pancreas	X	X
Peripheral nerve (brachial)	X	
Pharynx		
Pituitary	X	X
Prostate	X	X
Rib		X
Salivary gland	X	X
Sciatic nerve	X	X

Seminal vesicles	X	
Skeletal muscle	X	X
Skin	X	X
Small intestine (duodenum, ileum, jejunum)	X	X
Spinal cord	X	X
Spleen	X	X
Sternum	X	X
Stomach	X	X
Testes	X	X
Thymus	X	X
Thyroid/parathyroid		X
Tongue	X	X
Tonsil		
Trachea	X	X
Urinary bladder	X	X
Uterus	X	X
Vagina	X	X
Zymbal gland		

V. Pharmacokinetic and ADME

Summary of Pharmacokinetics/ADME:

After single i.v. dosing, the volume of distribution were almost the same in both sexes of rats: 9.2 v.s 9.8 l/kg in the ♂ and ♀, resp., which were ~14-fold of the volumes of total body water and ~300-fold of the volumes of plasma indicating that ZD1839 is extensively distributed in the tissues. The AUC value in ♀ rats was 1.7-fold of the ♂ value and the half life (t_{1/2}) was 2.1 h longer (5.3 vs. 3.2 hrs) in ♀ rats than that in the ♂. Clearance was faster in ♂ than in ♀ rats. After oral administration of single doses of the radiolabeled ZD1839, higher values of C_{max}, AUC, t_{1/2} and bioavailability were seen in the ♀ rats than the ♂ rats. These differences may be responsible for the gender difference observed with the sensitivity to ZD1839 toxicity. It is shown that at lower dose (30 mg/m² for rats and 100 mg/m² for dogs, the bioavailability after single oral dose was about 50%. Bioavailability increased with the increase in dose. The PK profiles in rats and dogs after single doses of radiolabeled ZD1839 are summarized below:

PK parameters derived following administration of [¹⁴ C]-ZD1839 to rats and male dogs													
Species	Dose (mg/m ²)	C _{max} (ng/ml)		AUC _{0-∞} (ng•h/ml)		t _{1/2} (hrs)		V _{dss} (L/kg)		Clearance (ml/min/kg)		Bioavailability (%)	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Rats (KKR008)	30 (i.v.)	—	—	1900	354	3.2	5.3	9.2	9.8	42.0	23.6	NC	NC
	30 (oral)	127	23	NC	178	NC	4.1	NC	NC	NC	NC	NC	50
	75 (oral)	381	59	3520	716	4.7	5.7	NC	NC	NC	NC	77	88
♂ Dogs (KKR008)	100 (i.v.)	6163		7851		3.4		2.1		10.6		—	
	100 (oral)	357		3882		4.5		—		—		49	

After repeated dosing, there are some gender differences in T_{max} in the rat at high dose, T_{max} are

shorter for the ♀ rat than the ♂ rat: 2 h vs. 5 h when ZD1839 was given at 750 mg/m²/day × 14, and 1 h vs. 8 h at 240 mg/m²/day × 14. T_{1/2} were similar at different dose levels for the rat. AUC values are generally greater in the ♀ than the ♂ rats at a given dose. Longer term drug exposure dramatically increased the AUC exposure in the rat for the same doses, suggesting a saturation of the elimination (include metabolism) pathways. The AUC following single oral doses in humans, rats and dogs are plotted in the following graph and good correlation were seen in all three species (although the samples from human study were limited). Since the data for humans were derived at a much lower dose, it is not know what pattern will be for humans when higher doses are reached.

The toxicokinetics results obtained from the repeat dose toxicology studies are summarized in the following table:

Species	Day	Dose (mg/m ² /day)	Tmax (h)		Cmax (µg/ml)		AUC _{0-8, 0-12, 0-24} ^a (µg·h/ml)		t _{1/2} (h)	
			♂	♀	♂	♀	♂	♀	♂	♀
Rat (TAR/2492)	1	60	5	6	0.2	0.2	0.97	1.85		
		300	2	3	1.5	1.7	12.7	17.6		
		750	2	6	4.6	4.9	30.9	41.3		
	14	60	3	3	0.4	0.4	2.1	3.0		
		300	6	5	2.1	1.9	14.1	17.4		
		750	5	2	3.4	3.5	23.5	ND ^b		
Rat (TAR/2570)	1	12	4	4	0.02	0.03	NC	NC	NC	
	28	12	4	0.5	0.05	0.06	NC	0.35	NC	6.8
		60	2	2	0.54	0.58	2.55	3.22 27.3	6.8	5.0
240		8	1	1.46	1.69	22.8		7.1	6.7	
Dog (TAD/870)	1	100	1	2	2.6	1.6	19	16	7.1	6.7
		1,000	4	4	8.7	11.8	102	142 177	4.8	7.6
		1,500	1	1	12.8	16	149		9.9	8.8
	14	100	2	4	2.0	1.1	20	15	7.2	6.7
		1,000	6	8	3.5	5.1	63	93	NC	NC
		1,500	8	NC ^c	9.6	NC	192	NC	NC	NC
Dog (TAD/876)	1	40	2.3		0.07		0.35		NC	
	28	40	3.0		0.14		0.65		NC	
		200	3.0		0.94		5.3		5.8	
800		3.7		2.36		15.8		NC		
Human ^d (1839IL/0001)	1	6.3	5.5		0.0028		0.086 ^e		14.8 ^e	
		15.6	5.0		0.0079 ^f		0.126 ^f		12.9 ^f	
		31.2	5.5		0.013		0.261 ^g		15.6 ^g	
		46.9	5.0		0.026		0.424		12.1	
Human ^d (1839IL/0010)	1	62.5 ^h	5.0		0.044 ^h		0.558 ^h		12.7 ^h	
	3	62.5 ^h	6.0		0.068 ^h		1.078 ^h		31.5 ^h	

^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; ^c NC: not calculated; ^d healthy male volunteers (n = 4), assuming the body surface area = 1.6 m²; ^e n = 1, ^f n = 2, ^g n = 3, ^h n = 5

APPEARS THIS WAY
ON ORIGINAL

AUC values are generally greater in the ♀ than the ♂ rats at a given dose. The gender difference in toxicity observed in the rat was possibly due to this PK pattern. Fourteen day exposure slightly increased the AUC exposure in rats for the same doses. After 28 days of dosing in separate study, the AUCs was double the 14 day values. It is difficult to draw substantive conclusions from studies in separate groups of animals. AUC values were similar in dogs after 14 days of dosing. A separate study showed markedly lower AUCs after 28 days of dosing, suggesting induction of metabolism. However, longer term drug administration seems to be more toxic in that the HNSTD was lower in dogs given ZD1839 for 28 days than that for 14 days.

APPEARS THIS WAY
ON ORIGINAL

The correlation of AUC with the dose are different in rats and dogs and the pattern depends on the length of drug exposure. The AUC values for 14 days ZD1839 administration was greater in the dog than in the rat; this pattern was reversed when exposure extended to 28 days.

APPEARS THIS WAY
ON ORIGINAL

ZD1839 was highly protein bound across all species investigated including the rat, dog, mouse and human (87-90%). The plasma protein binding did not change with increasing concentrations of ZD1839. The binding of ZD1839 to purified human serum albumin was 83% and to α -1 acid glycoprotein declined from 83% to 69% when concentrations of ZD1839 increased from 0.05 to 8.0 μ g/ml.

Binding of ZD1839 to plasma proteins of rabbit, rat and mouse									
Nominal Spiked Conc. (μ g/ml)	Rabbit ♀	Rat		Mouse		Dog		Human	
		♂	♀	♂	♀	♂	♀	♂	♀
0.05									
0.20									
1.0									
2.0									
4.0									
5.0									
6.0									
8.0									
Mean	91.1	87.2		91.3		91.2		90.8	

Recovery of radioactivity (%) in excretion following administration of single dose of [14 C]-ZD1839 to rats*					
Sample Type	Times after dose (hrs)	Intravenous		Oral	
		Rats	Dogs	Rats	Dogs
Feces	0-120	96.27	80.9	97.53	72.6
Urine	0-24	3.50	1.08	2.54	0.52
	0-120	4.30	3.23	3.24	1.61
Cage Wash	NA	0.64	1.44	1.20	2.29
Total Recovery	0-120	104.9	84.2	105.4	76.5

*Each value shows the means obtained from 2-3 animals, the SE was not shown

After a single oral dose of the radiolabeled ZD1839 (5 mg/kg) to the rat, the radioactivity as measured by quantitative whole body autoradiography (QWBA) was rapidly absorbed and well distributed into the tissues. High concentrations of radioactivity were observed in organs of metabolism and excretion (liver, kidney, lung, GI tract) and in glandular tissues (lacrimal gland, salivary gland and adrenals).

Radioactivity was also detected in the melanin containing tissues (eye, pigmented skin) of the pigmented animals. After an intravenous or oral administration of radiolabeled ZD1839, the radioactivity was mainly recovered from the feces in both rats and dogs indicating the GI is the major route for drug excretion. No apparent difference was found in excretion between male and female animals. ZD1839 administered to ♂ rats at 12, 60 and 240 mg/m²/day × 14, had no enzyme inducing potential for relative weight, cytochrome p450 concentration, and activities several hepatic metabolic enzymes (NADPH cytochrome c reductase, ethoxycoumarin O-deethylase, testosterone 6β-, 2α-, 16 α-hydroxylase, and pentoxifyresorufin O-dealkylase).

Metabolism studies revealed the presence of circulating metabolite(s) and suggested that the rate of metabolism of ZD1839 is slower in the female than in the male rats. This also could contribute to the gender difference in toxicity if the parent drug is more toxic than the metabolite(s). A major metabolite (56-74% of the sample radioactivity) recovered from the feces was not identified but seemed to suggest a labile conjugate that has been cleaved either during excretion or during sample preparation. A second component less polar than ZD1839 accounted for 8-15% of the sample radioactivity. The remainder of <8% of the radioactivity was accounted for by at least 6 minor components. *In vitro* metabolism study using hepatocytes from rat, dog and human revealed an extensive metabolism for ZD1839. The majority of the components observed in the rat and dog were more polar than the parent compound and the profiles were qualitatively similar. However, at the end of incubation (180 min), the parent compound of ZD1839 accounted for 28% and 55% of the incubate radioactivity in the dog and rat hepatocytes, respectively, indicating that the parent drug is more completely metabolized by the dog hepatocytes. Incubation of ZD1839 with human hepatocytes from three individuals revealed both qualitative and quantitative differences reflecting inter-individual differences in metabolizing activity. The parental ZD1839 accounted for 62.1, 81.3 and 34.3% of the incubate radioactivity after three hour incubation. If the toxicity of ZD1839 is determined by the presence of the un-metabolized compound, then extra safety factors should be taken for the substantial individual variations.

Profiles Obtained Following Incubation of [¹⁴ C]-ZD1839 (20 μM) with Hepatocytes ^a						
Peak No.	Relative Retention Time ^b	Rat	dog	Human-1	Human-2	Human-3
1	0.055-0.056	5.0	1.7	1.9	0.8	1.4
2	0.324-0.327	--	--	0.6	--	--
3	0.330	--	0.5	--	--	0.8
4	0.393-0.398	1.0	2.6	--	--	--
5	0.462	0.4	2.2	1.3	0.4	--
6	0.471-0.478	--	1.6	--	--	1.7
7	0.500-0.506	--	1.1	--	--	--
8	0.532-0.535	0.8	3.9	--	--	--
9	0.622-0.632	--	--	1.3	--	1.7
10	0.719-0.722	--	--	0.7	--	--
11	0.729-0.732	1.5	--	0.6	--	--
12	0.809	--	--	--	1.0	12.2
13	0.819-0.824	--	1.3	--	--	--
14	0.849-0.851	5.3	5.5	--	4.2	19.2
15	0.925-0.932	0.9	3.3	--	--	--
16	0.952-0.956	14.1	18.7	13.3	2.0	10.9
17	0.992-1.090	55.4	27.9	62.1	81.3	34.3
18	1.080-1.090	2.6	14.9	--	--	--

19	1.112-1.115	--	--	--	--	--
20	1.131-1.146	2.2	--	4.7	--	3.5
21	1.162-1.168	--	--	1.5	--	--
22	1.210-1.212		2.2	4.4	1.3	8.3
23	1.361-1.364	4.4	--	--		--
24	1.367-1.369	--	2.2	--		--
25	1.372-1.384	--	--	1.7		1.8

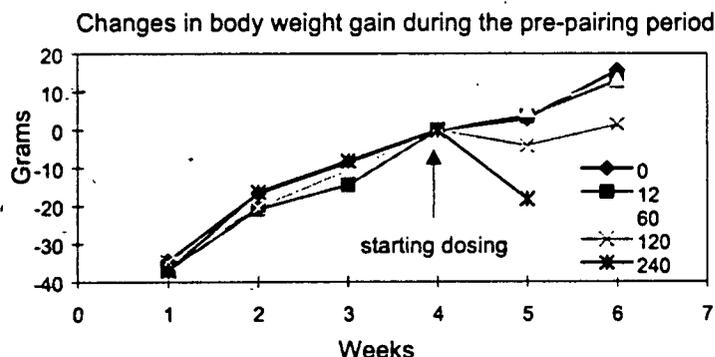
^a after 180 min incubation; ^b relative retention times are expressed relative to the mean [¹⁴C]-ZD1839 retention time

VI. Special Toxicology

Reproductive Toxicity

TGR/2616 Single female fertility study in rats: oral administration (Vol. 4, p307). Fifty rats ♀ (10 each group) were treated with ZD1839 orally by gastric intubation (dose volume 0.5 ml/ 100 g) at dose levels of 0, 12, 60, 120 and 240 mg/m²/day for at least 21 days (from 2 weeks prior to pairing until day 7 of gestation). ZD1839 at 240 mg/m²/day was lethal to pregnant rats: 1/10 was killed on D9 and 9/10 were killed on D11 due to toxicity. Clinical signs seen in this group included: urine staining, scabs around the mouth, loose feces, hunched posture, piloerection and partially closed eyes. Maternal food consumption ↓ 21% in this group. The body weight gain was significantly reduced in 240 and 120 mg/m²/day dose groups during the pre-pairing dosing period. Scabs around the mouth/nose/muzzle and loose feces were also observed in animals given 120 mg/m²/day ZD1839. ZD1839 was associated with a disruption to regular estrous cyclicity at doses of 60 mg/m²/day and above. However, there were no effects on female fertility or on embryonic survival at dose levels up to 120 mg/m²/day.

Reproduction Performance	Dose (mg/m ² /day × 21)				
	0	12	60	120	240
No. of ♀ paired	10	10	10	10	0
Mean Pre-coital period (Mean)	1.4	3.8	2.2	2.2	0.0
(S.D.)	0.7	6.5	1.6	1.2	0.0
No. with positive smear/plug	10	10	9	10	0
No. pregnant	8	10	8	10	0
Female fertility index	80.0	100.0	80.0	100.0	0.0
Male fertility index	80.0	90.0	80.0	100.0	0.0



Genetic Toxicity

TMV/668 An evaluation of mutagenic potential using *S. typhimurium* and *E. coli* (Vol. 4, p332). The study was conducted by the sponsor according to UK GLP with signed GLP and QA

compliance statement. ZD1839 gave a negative, i.e. non-mutagenic response in *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 and *E. coli* strains WP2P and WP2P *uvrA* in the presence or absence of S9-mix. The positive controls were daunomycin HCl (TA98), ENNG (WP2P *uvrA*), mitomycin C (WP2P), sodium azide (TA1535 and TA100) and acridine mutagen ICR191 (TA1537) for tests without S9. The positive control for all strains in tests with S9-mix was 2-aminoanthracene.

TQR/2573 Micronucleus test in the rat: oral administration (Vol. 4, p369). The study was conducted by the sponsor according to UK GLP with signed GLP and QA compliance statement. Male rats were administered ZD1839 orally at a single doses of 0, 1200, 4020 and 12000 mg/m² at a dosing volume of 20 ml/kg. Cyclophosphamide (at dose of 12,00 mg/m²) was used as positive control for the test. Animals were treated for 24 or 48 hr and bone marrow smears were taken for evaluation. No clinical signs or deaths observed during the study. No increased incidence of micronucleated polychromatic erythrocytes and percentages of polychromatic erythrocytes were observed in ZD1839-treated rats at any dose level.

Incidence of micronucleated polychromatic erythrocytes in rat bone marrow				
Dose levels (mg/m ²)	Mean No. of micronucleated polychromatic erythrocytes in 2000 polychromatic erythrocytes		Mean percentage of polychromatic erythrocytes	
	24 hr	48 hr	24 hr	48 hr
0 (vehicle control)	0.9	1.7	67.1	65.7
1200	1.1	1.3	62.5	65.8
4020	1.4	1.3	67.0	63.5
12000	1.1	0.9	64.9	65.0
Cyclophosphamide (120 mg/m ²)	43.3***	--	58.7	--

***p < 0.001

TYX/78 *In vitro* cytogenetic study using cultured human lymphocytes (Vol. 5, p1). The study was conducted by the sponsor according to UK GLP with signed GLP and QA compliance statement. ZD1839 was formulated as a stock solution in DMSO, from which serial dilutions were prepared with culture medium. Human lymphocytes were obtained from whole blood drawn aseptically from healthy donor on the days of culture initiation. Cyclophosphamide was used as the positive control substance requiring metabolic activation with the S9-fraction and mitomycin C was used as the direct-acting positive control. The mitotic activity was almost totally suppressed at 64 µg/ml ZD1839 and therefore the concentrations of ZD1839 tested in the main cytogenetic assay were 1 to 64 µg/ml. No statistically or biologically significant increases in the numbers of cells with chromosomal aberrations were observed in cultures treated with ZD1839 in the absence of S9. In the presence of S9, there was a small increase in the number of chromosomally abnormal cells following treatment at 32 mg/ml ZD1839. However, this increase was not seen in a repeat assay.

Chromosomal abnormalities (68 h Sampling) ^a					
Compound	Conc. (µg/ml)	% abnormal Cells (+gaps)	No. of aberration s per cell (+gaps)	% abnormal cells (-gaps)	No. of aberrations per cell (+gaps)
		-S9			
DMSO	10	6.50	0.065	3.50	0.035
Mitomycin C	0.5	34.48	0.552	24.14**	0.345

	ZD1839	16 ^b	1.23	0.012	0.62	0.006
		8	3.14	0.031	2.52	0.025
		4	6.00	0.065	4.00	0.045
		2	5.00	0.050	2.00	0.020
+S9	DMSO	10	2.00	0.020	1.00	0.010
	Cyclophosphamide	30	42.00	0.520	26.00*	0.300
	ZD1839	32 ^b	9.00	0.095	8.00*	0.085
		32 ^{b,c}	4.00	0.040	2.00	0.020
		24 ^{b,c}	6.00	0.080	4.00	0.045
		16 ^b	2.00	0.020	2.00	0.020
		12	2.50	0.025	1.00	0.010
		8 ^b	3.50	0.040	3.00	0.030

^a continuous treatment; ^b 3 h treatment; ^c repeat assay; * p < 0.05; ** p < 0.01

Summary of Special Toxicology

ZD1839 was non-mutagenic in Ames tests in four *S. typhimurium* tester strains and two *E. Coli* strains in the presence or absence of S9-mix. ZD1839 at single oral doses of 1200, 4020 and 12000 mg/m², did not increase the incidence of the micronucleated polychromatic erythrocytes in rats 24 or 48 hr post dose. ZD1839 had no effect on female fertility and embryonic survival, though increased occurrence in irregular estrous cycles were observed in rats given 120 mg/m²/day dose. ZD1839 at 240 mg/m²/day × 21 was lethal and 1/10 was killed at D9 and the rest 9/10 were killed at D11 due to clinical signs of toxicity. No results was obtained for reproductive toxicity for this dose level.

Overall Summary and Evaluation

ZD1839, an inhibitor of EGFR, is being developed as an orally active anti-tumor agent for the treatment of a broad range of major human solid tumor types. Safety pharmacology studies showed that in vitro ZD 1839 at 10 μM had no agonist or antagonist activity in: 1) the guinea-pig right or left atria, 2) the guinea-pig ileum. Rats given ZD1839 at 100 mg/kg po had significant decreases in the excretion of water, sodium, chloride and urine osmolality, and a weak, statistically significant inhibition (16%) of acute inflammatory response to carrageenin. However, ZD1839 at 100 mg/kg po had: 1) no significant effect on the blood-pressure or heart-rate in the spontaneous hypertensive rat; 2) not effect on gastrointestinal motility in the mouse; 3) no stimulant or depressant activity, or any effects on a range of other behavioral parameters in the mouse; 4) no bronchoconstrictor or bronchodilator activity in the anesthetized guinea-pig; and 5) no effect on the delayed hypersensitivity response to Freund's adjuvant in the mouse. ZD1839 was non-mutagenic in Ames tests in the presence or absence of S9-mix. ZD1839 at single oral doses of 1200, 4020 and 12000 mg/m², did not increase the incidence of the micronucleated polychromatic erythrocytes in rats. ZD1839 had no effect on female fertility and embryonic survival at up to maternally lethal doses (240 mg/m²/day).

The target organs of ZD1839 in preclinical toxicity studies are the eyes, skin, adrenal glands, heart, GI tract, liver, kidney, spleen and trachea. The approximate LD₁₀ of 14-day multiple oral dose in rats was 300 mg/m²/day in one study, but was < 240 mg/m²/day in pregnant rats. The approximate LD₁₀ of 30-day repeat oral dose in rats was 240 mg/m²/day. Gender difference in sensitivity was seen in rodents but were not obvious in dogs; female rats were generally more sensitive than the males to ZD1839 toxicity. The HNSTD for dogs were 1,000 mg/m²/day × 14 or 200 mg/m²/day × 30.

In this IND the sponsor only proposed a single starting human dose of 63 mg/m². Data from the mouse

study would predict a human starting dose of 600 mg/m². Previous human experience has used 63 mg/m² for up to 3 days administration without causing severe toxicity. This dose is therefore acceptable as a safe starting dose for the single dose trial. However, the sponsor also proposes a repeat dose trial at the same starting dose of 63 mg/m²/day for 14 days. The available human experience does not support this dose for a 14 consecutive days. Human volunteers dosed at this level for 3 consecutive days had already developed ophthalmological abnormalities. The animal data from rats did not derive a definitive LD₁₀ value for the 14-day dosing schedule. Taking 300 mg/m²/day as the approximate LD₁₀ from study TAR/2492, one would predict a safe starting dose of 30 mg/m²/day, which was not severely toxic to dogs. Dogs seems to be less sensitive than rats for the 14 day studies, however, both studies on dogs and rats did not include a recovery observation period and the reversibility of the toxicities are unknown. If predicted from 1/6 of the HNSTD from study TAD/876 in dogs, a starting dose of 33.3 mg/m²/day would be accepted. The human exposure (AUC) after multiple administration at doses of 63 mg/m²/day or higher is hard to predict. Human volunteers showed a doubled AUC (1.08 vs. 0.59 µg·h/ml) and tripled t_{1/2} (31.5 vs. 12.7 h) values after only 3 days of dosing with 63 mg/m²/day level compared to after a single dosing, indicating saturation of metabolism and/or elimination pathway. In addition, individual variations in the drug metabolism by human hepatocytes were substantial based on limited *in vitro* analyses. These factors warrant that the prediction for a starting dose should be more conservative in order to be safe. Therefore, 30 mg/m²/day × 14 is considered to be a safe starting dose in human.

Recommendations:

The planned clinical trial may not proceed as planned, unless the starting dose for the repeat dose trial is lowered to 30 mg/m²/day × 14. This recommendation was conveyed to the sponsor in our safety review and the sponsor agreed to lower the starting dose as requested.

Points discussed with medical officer: starting dose
human experience
gender difference in toxicity and PK
species difference in toxicity and PK
metabolism

Draft Reports: No

Draft Letter, Request for Sponsor: No

IS/

Hua Zheng, Ph.D. Date
Pharmacologist/Toxicologist

IS/

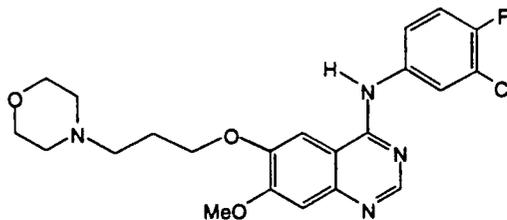
Paul Andrews, Ph.D

Pharm/Tox Team Leader

cc: IND [redacted] and Div. File
/HFD-150
/P Andrews
/K Kobayashi
/A Chapman
/H Zheng

*Appendix III, Dr. Zheng's third review.***Division of Oncology Drug Products, HFD-150****REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA****Review No. 2**

IND No.	SE No(s).	Type	Dated	CDER Stamp Date
	002	IT	01/29/98	02/02/98
	003	RD, PC	03/02/98	03/03/98

Information to be Conveyed to Sponsor: Yes (), No (X)**Reviewer:** Hua Zheng, Ph.D.**Date Review Completed:** April. 9, 1998**Sponsor:** Zeneca Pharmaceuticals
Wilmington, DE 19850-5437**Drug Name:** ZENECA ZD1839**Chemical Name:** 4-(3-Chloro-4-fluorophenylamino)-7-methoxy-6-(3-(4-morpholinyl) propoxy) quinazoline**CAS Number:** None**Structure****Molecular Formula:** C₂₂H₂₄ClFN₄O₃**Molecular Weight:** 446.91**Related INDs/NDAs/DMFs:** IND [redacted]**Class:** Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor**Indication:** Solid malignant tumors**Clinical Formulation:** The finished product is presented as a range of round, biconvex, brown film coated tables containing 1 mg, 5 mg, 25 mg and 100 mg of ZD1839.

Qualitative and quantitative composition of drug product

Ingredient	5 mg (mg/tablet)	25 mg (mg/tablet)	100 mg (mg/tablet)	Function
Core				
ZD1839	5.0	25.0	100.0	Active ingredient
Lactose	.	.	.	
Microcrystalline cellulose	.	.	.	
Croscarmellose sodium	.	.	.	
polyvidone	.	.	.	
Sodium lauryl sulphate	.	.	.	
Magnesium stearate	.	.	.	
Core tablet weight				
Coating				
Methylhydroxypropylcellulose				
Polyethylene glycol, PEG 300				
Red iron oxide E172				
Yellow iron oxide E172				
Titanium dioxide				
Coated tablet weight	229.6	224.6	204.6	

Route of Administration: Oral

Proposed Clinical Protocol: This is a multi-center, open, phase I tolerability trial (1839IL/0005) of orally administered ZD1839 with multiple rising dose and sequential block escalation in patients with solid malignant tumors known to commonly overexpress EGFR, which are refractory to other treatments.

- Objective: To assess:**
- a) the tolerability and toxicity
 - b) single and multiple dose PK
 - c) antitumor activity of ZD1829 in patients with advanced cancer, and
 - d) effect on biological surrogates of antitumor efficacy (Ki67, *c-fos* mRNA and EGFR) in tumor biopsies
- Starting Dose:**
- a) Single dose: 50 mg (32 mg/m²)
 - b) Multiple dose: 50 mg/day (32 mg/m²/day)
- Escalation:** Multiple dose: 100, 150, 225, 300, 400, 525, 700 and 925 mg/day (62.5, 93.8, 141, 188, 250, 328, 437.5 and 578 mg/m²/day)
- Frequency and duration:**
- a) Single dose: one day treatment followed by 6 days washout
 - b) Multiple dose: daily x 14, followed by 14 days off treatment
- Age of patient population:** ≥ 18 years

Previous Review(s), Date(s), and Reviewer(s): Safety Review, 12/16/97, Zheng
Original Review, 01/29/97, Zheng

Studies reviewed for this submission

1. TMV/669 L5178Y +/- mouse lymphoma mutation assay (10/IG/1031493) (Vol. 2.1, Sec.1)

2. KMX/024 The effects of ZD1839 on P450-dependent activities in human hepatic microsomes and determination of the human microsomal P450 isozymes involved in [¹⁴C]ZD1839 metabolism (11/IG/1031564) (Vol. 2.1, Sec. 2)
3. TTR/2590 Teratology sighting study in rats: oral administration (12/IG/1031318) (Vol. 2.1, Sec. 3)
4. TRB/725 Teratology sighting study in rabbits: oral administration (12/IG/1032277) (Vol. 2.1, Sec. 4)
5. TRB/696 Teratology sighting study in rabbits: oral administration (12/IG/1032279) (Vol. 2.1, Sec. 5)
6. TGR/2575 Fertility study in rats: oral administration (12/IG/1032379) (Vol. 2.1, Sec. 6)

Studies previously reviewed

I. Previous human experience

1. Clinical experience (Vol. 1.1, p101)

I. Pharmacology

2. Pharmacology Relevant to Cancer (Vol. 1.1, p265)

III. Safety Pharmacology

3. General pharmacology (Vol. 1.1, p143)

IV. Toxicology

Single dose Studies

4. TLM/958 Acute toxicity (limit) study in mice: oral administration (Vol. 1.2, p1)
5. TLR/2571 Acute toxicity (limit) study in rats: oral administration (Vol. 1.2, p47)

Multi-dose Studies

6. TAR/2492 Rat pilot toxicity study (Vol. 1.2, p112)
7. TAD/870 Dog pilot toxicity study (Vol. 1.2, p167)
8. TAR/2570 One month oral toxicity studies in rats (Vol. 1.3, p1)
9. TAD/876 One month oral toxicity study in dogs (Vol. 1.4, p1)

V. Toxicokinetics/ADME The PK studies were integrated into the above toxicity studies

10. KMR/010 The tissue distribution of total radioactivity in the rat following oral administration of [¹⁴C]-ZD1839 (Quantitative whole body autoradiography) (Vol. 1.5, p45)
11. KMR/007 The disposition of [¹⁴C]-zeneca ZD1839 in the rat (Vol. 1.5, p92)
12. BIT/00401 The effects of ZD1839 on the hepatic microsomal mixed function oxidase enzymes of the male rat (Vol. 1.5, p111)
13. KKR/008 The distribution of radioactivity in the blood after oral and intravenous administration of [¹⁴C]-zeneca ZD1839 to rats (Vol. 1.5, p132)
14. KKD/009 The disposition of [¹⁴C]-zeneca ZD1839 in male dogs (Vol. 1.5, p164)
15. KPJ/013 The binding of [¹⁴C]-ZD1839 to plasma proteins (Vol. 1.5, p191)
16. KMN/012 Metabolism of zeneca ZD1839 in rat, dog and human hepatocytes (Vol. 1.5, p208)

VI. Special Toxicology

Reproductive Toxicity

17. TGR/2616 Single female fertility study in rats: oral administration (Vol. 1.4, p307)

Genetic Toxicity

18. TMV/668 An evaluation of mutagenic potential using *S. typhimurium* and *E. Coli*

(Vol. 1.4, p332)

- 19. TQR/2573 Micronucleus test in the rat: oral administration (Vol. 1.4, p369)
- 20. TYX/78 *In vitro* cytogenetic study using cultured human lymphocytes (Vol. 1.5, p1)

Note that portions of this review were excerpted directly from the sponsor's submission.

Review

Introduction: This IT (002) submission provided additional information on some toxicology studies completed since the original IND submission. These include 1 *in vitro* metabolism study, 1 genetic toxicity study, 3 Segment II developmental toxicity studies and 1 fertility study. The RD/PC submission (003) provided the sponsor's responses to FDA facsimiles of Dec. 11, 1997 and Dec. 16, 1997. A revised protocol is also provided in which the starting dose for multiple dose study is lowered to 50 mg/day, as requested. In addition, the starting dose for the single dose administration is also changed to 50 mg, which was not requested by the agency. The escalation scheme was also modified to a less aggressive scheme as described above.

1. TMV/669 L5178Y +/- mouse lymphoma mutation assay (10/IG/1031493) (Vol. 2.1, Sec. 1).

Conducted by _____ Signed statement of U. K. GLP and dated QA statement compliance are provided. L5178Y TK⁺ mouse lymphoma cells were treated *in vitro* with various concentrations of ZD1839 (Batch reference: C185/1), both in the presence and absence of a rat liver derived metabolic system (S9-mix) in three independent experiments. The control substance and solvent for the test sample and positive control was DMSO; the positive controls were ethyl methanesulphonate (EMS) and benzo- α -pyrene (BP). Mutant frequencies were assessed by cell growth in the presence of trifluorothymidine after 48 h exposure. The cytotoxicity of the test substance is assessed by post-treatment cloning efficiency which were >50% for the solvent control viability plates. No significant increase in mutation frequency were obtained in cultures treated with ZD1839 at any concentration tested in either the presence or absence of S9-mix. The *in vitro* mouse lymphoma mutation assay confirmed the previous results obtained from the *in vitro* mutation assay on bacteria and micronucleus assay on rats, indicating that ZD1839 is not genotoxic.

Summary of the results for experiment 1					
Test Substances	Conc. (μ g/ml)	-S9 Mix		+S9 Mix	
		Relative Survival (%)	Mean Mutant Frequency ($\times 10^{-4}$)	Relative Survival (%)	Mean Mutant Frequency ($\times 10^{-4}$)
ZD1839	200	a	a	a	a
	100	a	a	a	a
	50	a	a	0	b
	25	64	0.6	37	0.6
	13	74	0.4	82	1.4
	6	84	0.9	99	1.4
DMSO	10	100	1.3	100	1.7
EMS	750	53	8.7	--	--
BP	3	--	--	37	6.9

a = not plated due to excessive toxicity; b = not counted due to "excessive toxicity" (not defined)

Summary of the results for experiment 2					
Test Substances	Conc. (μ g/ml)	-S9 Mix		+S9 Mix	
		Relative Survival (%)	Mean Mutant Frequency ($\times 10^{-4}$)	Relative Survival (%)	Mean Mutant Frequency ($\times 10^{-4}$)

ZD1839	50	0	b	0	b
	40	0	b	0	b
	30	7	b	1	b
	20	73	1.3	32	1.1
	10	77	1.6	80	0.8
	5	81	2.6	77	0.9
DMSO	10	100	2.4	101	1.2
EMS	750	29	15.7	--	--
BP	3	--	--	10	14.6

b = not counted due to "excessive toxicity" (not defined)

Summary of the results for experiment 3					
Test Substances	Conc. (µg/ml)	-S9 Mix		+S9 Mix	
		Relative Survival (%)	Mean Mutant Frequency (× 10 ⁻⁴)	Relative Survival (%)	Mean Mutant Frequency (× 10 ⁻⁴)
ZD1839	35	0	1.8	2	b
	30	0	1.0	11	1.6
	25	7	1.1	36	2.1
	20	73	1.3	59	1.6
	15	77	1.0	70	2.0
	10	81	1.6	85	1.6
DMSO	10	100	1.1	101	1.6
EMS	750	39	15.4	--	--
BP	3	--	--	10	13.4

b = not counted due to excessive toxicity

2. **KMX/024 The effects of ZD1839 on P450-dependent activities in human hepatic microsomes and determination of the human microsomal P450 isozymes involved in [¹⁴C]ZD1839 metabolism (11/IG/1031564) (Vol. 2.1, Sec. 2).** Conducted by the sponsor according U. K. GLP regulation with the signed and dated GLP and QA statements. This study was designed to evaluate the potential of ZD1839 (Batch ADM 37551D96) to cause drug interactions through inhibition of the human hepatic cytochrome P450 isozymes and to investigate which P450 isozymes were involved in the metabolism of [¹⁴C]-ZD1839 (Batch 1R1) by human hepatic microsomes. For the later purpose, effect of P450-selective inhibitors and recombinant P450 on metabolism of [¹⁴C]-ZD1839 (10 µM) were evaluated. ZD1839 produced limited inhibition (<10%) of CYP 1A2, 2C9 and 3A4 enzyme activity. Although inhibition of CYP 2C19 and 2D6 was more pronounced, this did not exceed 50% at the maximum concentrations. ZD1839 underwent extensive CYP 3A4-mediated metabolism to a number of components. Three metabolites of [¹⁴C]-ZD1839 were isolated after incubation with hepatic microsomal protein (2 mg/ml) in the presence of NADPH (2 µM) for 30 min. Two of these metabolites were identified by NMR and MS to be products of oxidative defluorination and *O, N*-dealkylation products. The formation of all metabolites was markedly reduced in a concentration-dependent manner by ketoconazole and inhibited to a less extend by omeprazole.

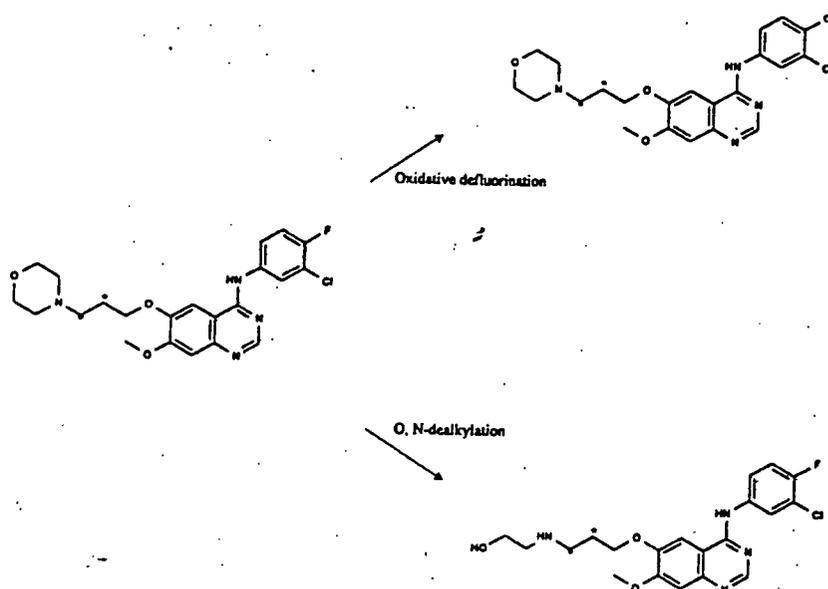
Effect of ZD1839 on P450 substrate activities (% of Control)			
P450 Substrate	Product formed	Measurement	P450 isozyme monitored
Phenacetin <i>O</i> -deethylase	4-acetamidophenol		CYP 1A2
Tolbutamide 4'-hydroxylase	hydroxytolbutamide		CYP 2C9
S-mephenytoin 4-hydroxylase	4-hydroxymephenytoin		CYP 2C19

Dextromethrophan O-demethylase.	dextrophan	Fluorimetric	CYP 2D6
Testosterone 6 β -hydroxylase	6 β -hydroxytestosterone		CYP 3A4

Results:

Effect of ZD 1839 on P450 marker substrate activities (% of Control)					
Conc. (μ M)	Phenacetin O-deethylase (CYP 1A2)	Tolbutamide 4'-hydroxylase (CYP 2C9)	S-mephenytoin 4-hydroxylase (CYP 2C19)	Dextromethrophan O-demethylase (CYP 2D6)	Testosterone 6 β -hydroxylase (CYP 3A4)
ZD1839					
0.004	106.4	99.8	88.7	99.0	107.7
0.02	104.7	99.1	78.4	109.1	108.2
0.11	111.7	100.0	82.5	81.3	104.2
0.56	98.3	100.9	82.5	89.0	103.8
2.24	107.6	101.2	80.4	87.1	104.5
11.2	104.7	91.3	76.3	56.9	92.7

Structures of the [¹⁴C]-ZD1839 and identified metabolites (Peaks 3 and 4, the oxidative defluorination and O, N-dealkylation products, respectively):



Effect of P450-selective chemical inhibitors on [¹⁴ C]-ZD1839 metabolism by human liver microsomes						
P450	Selective chemical inhibitor	Conc. (μ M)	Peak 2	Peak 3	ZD1839	Peak 4
	Control	0	9.0	32.1	30.3	9.3
1A2	Furafylline	1	10.8	34.3	25.2	6.5
		5	13.6	34.1	27.6	8.8
		25	13.2	33.7	26.1	7.1

2C9	Sulphaphenazole	1	14.3	29.0	25.2	8.1
		5	10.3	33.1	27.1	9.4
		25	9.3	29.1	34.2	8.2
2C19	Omeprazole	6	11.1	32.7	44.0	6.7
		12	9.6	27.3	44.1	5.8
		20	8.6	23.1	55.5	4.4
2D6	Quinidine	0.05	11.2	37.9	26.0	9.2
		0.1	11.3	35.7	25.4	8.3
		1.0	11.7	39.5	25.3	7.7
3A4	Ketoconazole	0.04	9.9	32.1	33.3	9.3
		0.1	10.5	10.5	35.8	10.1
		1.0	1.3	3.5	3.5	1.4

3. TTR/2590 Teratology sighting study in rats: oral administration (12/IG/1031318) (Vol. 2.1, Sec. 3). Conducted by the sponsor according U. K. GLP regulation with the signed GLP statement. ZD1839 did not cause maternal toxicity and had no adverse effects on embryonic and fetal survival and development at doses up to 120 mg/m²/day when administered to pregnant rats during the period of major organogenesis.

species: Alp:APSD (Wistar derived) female rat (6/group)
 age; weight: age not specified; 228 - 301 g
 drug: ZD1839 (Batch not specified)
 vehicle: 0.5% (w/v) hydroxypropyl methylcellulose in 0.1% w/v aqueous polysorbate 80
 dosage: 0, 1, 2.5, 5, 10 and 20 mg/kg
 route: oral gavage at a volume of 5 ml/kg b.w.
 duration: From day 7 to day 16 of gestation

Observations

Clinical signs daily
 Maternal body weights Days 1, 7, 10, 13, 16, 19 and 22 of gestation
 Food consumption D7-14, and D14-21 of gestation
 Necropsy D22 of gestation,
 gross macroscopic examination was done for each animal and fetus
 no histological examination was performed

Results

- a. Clinical Observations: no deaths occurred and no significant clinical signs observed during the course of the study
- b. Maternal body weight: all rats gained weight during the study period and there were no difference between control and ZD1839-treated groups
- c. Food Consumption: no change was observed among groups
- d. Necropsy: no abnormality was found in fetal development

D22 Uterine Examination-Summary (median values)						
Parameters Examined	Dose of ZD1839 (mg/kg)					
	0	1	2.5	5	10	20
No. of live fetuses/litter	14	15	14	14	14	14
No. of implants/dam	15	15	15	14	16	14
No. of corpora lutea/dam	16	15	16	16	16	14
Pre-implantation loss/dam	1	0	0	2	0	0

Post-implantation loss/dam	0	0	0	0	1	0
Mean fetal bwt/litter (g)	5.0	4.9	5.0	5.1	5.1	5.1
Mean-placental bwt./litter (g)	0.6	0.6	0.6	0.6	0.5	0.5
Male proportion/litter	0.4	0.5	0.5	0.5	0.5	0.6
Empty uterus weight (g)	6.4	6.3	6.3	8.2	6.0	6.8

Comment: The highest dose (120 mg/m² /day × 10) tested in this study was not significantly maternally toxic. In a 14-day repeat oral dose toxicity study (TAR/2492) the NOAEL in rats was 60 mg/m²/day.

4. TRB/725 Teratology sighting study in rabbits: oral administration (12/IG/1032277) (Vol. 2.1, Sec. 4; Summary Report). Conducted by the sponsor according U. K. GLP regulation with the signed GLP statement. No developmental toxicity data were obtained in this study for ZD1839 at doses between 1180 and 2360 mg/m²/day administered to pregnant rabbits during the period of organogenesis due to severe maternal toxicity.

species: HsdPoc:NZW rabbits (6/group)
 age; weight: sexually mature (not specified); 2.82 - 3.51 kg
 drug: ZD1839 (Batch not specified)
 vehicle: 0.5% (w/v) hydroxypropyl methylcellulose in 0.1% w/v aqueous polysorbate 80
 dosage: 0, 100, 150 and 200 mg/kg
 route: oral gavage
 duration: day 7 to day 19 of gestation; the day of mating was designed day 1 of gestation

Observations

Clinical signs: daily
 Maternal body weights: Days 1, 7, 10, 13 and 16 of gestation
 Food consumption: D7-14 of gestation
 Necropsy: D11-D16 of gestation,
 gross macroscopic examination was done for each animal
 histological examination was performed for tissues preserved from animals found dead or prematurely killed

Results

a. **Clinical Observations:** study was terminated for all dose levels due to maternal toxicity, reduced defecation were seen in all treated animals
 HD: all animals were killed on D11/12 of gestation
 MD: all animals were killed on D13/15 of gestation
 LD: 1 found dead (D15), the rest LD and control animals were killed on D15/16 of gestation

b. **Maternal body weight:** ZD1839 caused a dose-dependent ↓ in b.w. (see table)

Groups	Dose (mg/kg)	Mean Body Weight (g)						
		D7	D10	D11	D13	D14	D15	D16
Control	0	3381	3356	--	3318	--	3408 ^a	3279 ^a
LD	100	3302	3085	--	2865	--	2257 ^c	3070 ^a
MD	150	3167	2878	--	2621	2590 ^a	2568 ^b	--
HD	200	3195	2857	2841	--	--	--	--

^a mean of 3 animals; ^b mean of 2 animals; ^c data from only 1 animals

c. **Food Consumption:** no data reported
 d. **Necropsy:** severe villous atrophy was found in duodenum of the decedent rabbits

examined and this was considered by the sponsor as the major cause of the death in ZD1839 treated animals; gastric erosions/gastritis, mucosal erosions of cecum and spleen lymphoid atrophy were seen in all groups of drug treated animals

5. TRB/696 Teratology sighting study in rabbits: oral administration (12/IG/1032279) (Vol. 2.1, Sec. 5; Summary Report). Conducted by the sponsor according U. K. GLP regulation with the signed GLP statement. Oral administration ZD1839 to pregnant rabbits during the period of organogenesis caused maternal toxicity at 2950 mg/m²/day dose level. There was no evidence of embryo-lethality at doses up to 590 mg/m²/day. The dose range to be used for definitive study should be between 600 and 1,000 mg/m²/day in rabbits.

species: HsdPoc:NZW rabbits (6/group)
 age; weight: sexually mature (not specified); 2.48 - 3.39 kg
 drug: ZD1839 (Batch not specified)
 vehicle: 0.5% (w/v) hydroxypropyl methylcellulose in 0.1% w/v aqueous polysorbate 80
 dosage: 0, 1, 5, 10, 50 and 250 mg/kg
 route: oral gavage
 duration: day 7 to day 19 of gestation, the day of mating was designed day 1 of gestation

Observations

Clinical signs daily
 Maternal body weights Days 1, 7, 10, 13, 16, 19, 22, 25 and 29 of gestation
 Food consumption D7-14, D14-21 and D21-28 of gestation
 Necropsy D29 of gestation
 gross macroscopic examination was done for each animal and fetus
 detailed uterine examination was conducted where fetuses were present
 histological examination was performed for tissues preserved from animals of premature decedents and controls

Results

- a. Clinical Observations: premature kill: 1/6 in control, 1/6 in 50 mg/kg/day group and 6/6 in 250 mg/kg/day group;
 reduced defecation were seen in all treated animals
- b. Maternal body weight: no noticeable changes in animals bearing living fetuses
- c. Food Consumption: no noticeable changes in animals bearing living fetuses
- d. Necropsy: severe villous atrophy was found in duodenum of the all decedent rabbits given ZD1839 and this was considered by the sponsor as the major contributory factor in the cause of the death in ZD1839 treated animals;
 ↑ in the empty uterus weight (37%) for animals given 50 mg/kg/day group compared to the control group
 no external abnormalities were observed for all live fetuses obtained

Summary of failed pregnancies

Pregnancy Status	Dose groups (mg/kg/day)					
	0	1	5	10	50	250
Pregnant ^a	6	5	6	6	5	6
Resorption ^b	0	1	0	0	1	0
Aborted ^b	0	0	0	0	0	0

^aPregnant (include animals killed prior to scheduled termination and found to have at least one live implant

^bnumber of litters

Summary of Cesarean Data (median values)						
Parameters Examined	Dose groups of ZD1839 (mg/kg)					
	0	1	5	10	50	250
No. of live fetuses/litter	7	7	6	6	8	--
No. of implants/dam	7	8	8	8	8	--
No. of corpora lutea/dam	8	8	9	8	9	--
Pre-implantation loss/dam	0	0	1	0	0	--
Post-implantation loss/dam	0	0	0	1	0	--
Mean fetal bwt/litter (g)	33.5	33.5	35.3	32.7	36.3	--
Mean placental bwt./litter (g)	4.7	5.2	4.7	5.3	5.1	--
Male proportion/litter	0.4	0.6	0.6	0.4	0.5	--
Empty uterus weight (g)	47.0	55.0	55.4	52.0	64.3	--

6. TRB/2725 Fertility study in rats: oral administration (12/IG/1032379) (Vol. 2.1, Sec. 6).

Conducted by the sponsor according U. K. GLP regulation with the signed GLP and dated QA statement. At high dose of 120 mg/m² /day, ZD1839 induced maternal toxicity reflected by decrease in maternal body weight gain. Although it was shown in study TGR/2616 that ZD1839 was associated with a disruption of regular estrous cyclicity at doses of 60 mg/m² /day and above when dosing for 21 days, in the current study, no dose-dependent increase in the cases of irregular estrus cycles was seen in ZD1839 treated female rats. The fertility indices were slightly lower (5-10%) in all ZD1839 dosed females and males compared to the untreated controls, however, no dose-dependent pattern were seen. Significant decreases were found in the number of corpora lutea, uterine implants and live embryos per litter in female rats given 120 mg/m²/day ZD1839 upon uterine examination. No drug related changes were identified in the sperm function in male rats. No pathologically significant findings were seen in the reproductive organs for animals of the infertile cases.

species: Alpk:APSD rats (22/sex/group)
age; weight: 10-11 wks; 309 - 446 g (♂), 215 - 289 g (♀)
drug: ZD1839 (Ref. No.: ADM3657E96)
vehicle: 0.5% (w/v) hydroxypropyl methylcellulose in 0.1% w/v aqueous polysorbate 80
dosage: 0, 2, 10 and 20 mg/kg/day
route: oral gavage at a dosing volume of 5 ml/kg b.w.
frequency
& duration: ♀s were dosed once daily from 2 weeks prior to pairing and through pairing to pregnancy day 7
♂s were dosed once daily from 4 weeks prior to pairing, and through pairing to scheduled termination

Observations

Clinical signs daily
Body weights twice weekly
Food consumption weekly
Vaginal smears daily for each ♀ commencing 2 weeks prior to the start of dosing and continuing through dosing and pairing to pregnancy day 6
Mating detected by finding a sperm positive vaginal smear or a vaginal plug
Uterine examination at scheduled necropsy on putative pregnancy day 13
General pathology on scheduled necropsy on D13 of gestation for ♀,

on scheduled necropsy on D72-75 on sperm function assay dates (D71-74) for ♂s

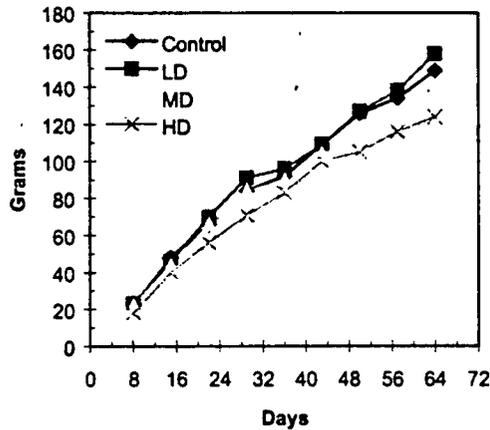
Results

a. Clinical Observations: 1/22 HD ♂ (D32) was found dead; 1/22 MD ♀ (D43) was killed moribund

↓ defecation: 1/22 HD ♂ (D2) and 1/22 LD ♀ (D2)
 hair loss (dorsal + ventral): 7/22 HD ♀, 8/22 MD ♀, 4/22 LD ♀;
 scabs on mouth: 19/22 HD ♀, 10/22 MD ♀, 2/22 LD ♀;
 stain surround the muzzle: 3/22 HD ♀; urine staining: 2/22 HD ♀

b. Body weight: no changes in body weights in F0 ♀ prior to pairing
 ↓ by 17% in HD ♂ after 64 days

F0 Males: Cummulative body weight gain



F0 maternal body weight gain during pregnancy (median value)				
Days	Dose groups			
	C	LD	MD	HD
1-7	36	42	41	37
7-10	14	14	17	19*
10-13	23	21	25	28

*p < 0.05 compared to the control group

c. Food Consumption: no changes in F0 ♂ animals and in F0 ♀ prior to pairing
 no record on food consumption for both male and female animals after pairing

d. Estrous cycles no pattern of changes was seen after treatment

Summary of the Changes in Estrous Cycles					
Before dosing (14 days)	During dosing (14 days)	Dose groups			
		C	LD	MD	HD
Regular	Regular	11	12	10	13
Regular	Irregular	2	4	6	3
Irregular	Regular	4	5	3	1
Irregular	Irregular	5	1	3	5

Total No. of animals examined	22	22	22	22
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e. Mating and fertility

Summary of mating and fertility in females				
Parameters	Dose groups			
	C	LD	MD	HD
No. paired	22	22	22	22
No. mated	22	22	22	22
No. pregnant at D13 necropsy	20	18	19	19
No. with live embryos-	20	18	19	19
No. with resorptions only-	0	0	0	0
No. no pregnant at scheduled necropsy	2	4	2	3
Copulation index (%) ^a	100	100	100	100
Fertility Index ^b	90.9	81.8	86.4	86.4

^acopulation index = (No. paired/No. mated) × 100%;

^bfertility index = (No. fertile/No. mated) × 100%

Summary of mating and fertility in males				
Parameters	Dose groups			
	C	LD	MD	HD
No. paired	22	22	22	22
No. mated	22	22	22	22
No. fertile	20	18	19	18
No. involved in infertile matings	2	4	2	3
Copulation index (%) ^a	100	100	100	100
Fertility Index ^b	90.9	81.8	86.4	85.7

^acopulation index = (No. paired/No. mated) × 100%;

^bfertility index = (No. fertile/No. mated) × 100%

- f. Uterine examination: statistically significant decrease in the number of corpora lutea, uterine implants and live embryos were seen at HD group.

Summary of Day 13 Uterine examination (Median Values/No. animals)				
Dose groups	C	LD	MD	HD
No. of live fetuses/litter	16/20	15/18	14/18	12/19*
No. implants/dam	16/20	16/18	14/18	12/19*
No. corpora lutea/dam	18/20	18/18	17/18	16/19*
Pre-implantation loss/dam	2/20	1/18	2/18	3/19
Post-implantation loss/dam	0/20	0/18	0/18	0/19

*p < 0.05 .

- g. Sperm function: no drug related changes
- h. Necropsy: Reproductive organs (testes, epididymides, ovaries and uterus) from animals of the infertile cases were examined by necropsy and histology, no underlying cause was found.

Overall Summary and Evaluation

ZD1839 is not genotoxic in an *in vitro* mouse lymphoma mutation assay which confirmed the previous results obtained from bacteria mutation assay. ZD1839 is extensively metabolized mainly by CYP 3A4 and three metabolites were identified; two of which are products of oxidative defluorination and O, N-dealkylation. The metabolism of ZD1839 can be inhibited by ketoconazole and omeprazole. In a pilot teratology study in rats (TTR/2950), no developmental or maternal toxicity was observed at the highest dose tested, 120 mg/m²/day. The first pilot teratology study in rabbits (TRB/725) was terminated before any developmental toxicity data was obtained, due to significant maternal toxicity at doses \geq 1180 mg/m²/day. In a second teratology study in rabbits (TBR/696), ZD1839 at 2950 mg/m²/day dose level caused significant maternal toxicity (100% lethality), no Cesarean data was obtained from this group. ZD1839 at a dose of 590 mg/m²/day was not embryo-toxic or teratogenic. This dose is expected to be used in clinical trial (up to 625 mg/m²/day). The dose range to be used for a definitive teratology study in rabbits is recommended to be between 600 to 1,000 mg/m²/day. In a Segment I study in rats, ZD1839 at maternally toxic dose (120 mg/m²/day) is embryo-fetal toxic as demonstrated by \downarrow No. of corpora lutea, uterine implants and live embryos per litter. Although ZD1839 at doses up to 120 mg/m²/day did not impair the sperm function in male rats or induce any histopathologic changes in the reproductive organs in both genders, the fertility indices were 5-10% lower in both male and female rats of all dose groups than the untreated controls.

Recommendation: No regulatory action is necessary at this time

NDA issues:

Since genotoxicity tests and fertility study are completed, based on the available data, the label section for corresponding section (Carcinogenesis, Mutagenesis, and Impairment of Fertility) can be indicated as follows:

Draft Letter, Request for Sponsor: No

Histopathology table

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NDA 21,399 IRESSA		TAD/877 EDR July 30	TAD/876 EDR July 30	TPR2576 EDR July 30
Study Submission volume Species		Dog	Dog	Rat
Adrenals		X	X	X
	Cortex			
	Medulla			
Aorta		X	X	X
Bone		X	X	X
Bone Marrow smear		X	X	X
Brain		X	X	X
Brain Stem				
Bronchi		X	X	X
Cecum				
Cerebellum				
Cerebrum				
Cervix		X	X	X
Chest wall				
	Right			
	left			
Colon		X	X	X
Crista iliaca				
Diaphragm				
Duodenum		X	X	X
Epididymis		X	X	X
Esophagus		X	X	X
Eye		X	X	X
Eye lids		X	X	X
Falopian tube				
Femur		X		X
Foot				
Gall bladder		X	X	
Hind Limb				
Heart		X	X	X
Harderian gland				X
Hypophysis				
Ileum		X	X	X
Ileo-cecal colic junction		X	X	
Injection site				
Jejunum-		X	X	X
Kidneys		X	X	X
Lachrymal gland		X	X	
Large Intestine				
lymph node		X	X	X
Liver		X	X	X
Lungs		X	X	X
	Apical & diaphragmatic lobes right			
	Apical & diaphragmatic lobes left			
Macroscopic lesions				
Mammary Gland		X	X	X
Mesenteric lymph glands		X	X	X
Nervus ischiadicus				
Optic Nerve				
Ovaries		X	X	X
Pancreas		X	X	X

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this page is the manifestation of the electronic signature.

/s/

William McGuinn
10/15/02 12:08:58 PM
PHARMACOLOGIST

John Leighton
10/15/02 12:14:29 PM
PHARMACOLOGIST
substituting for David Morse, secondary reviewer for this NDA

Executive CAC

Date of Meeting: March 12, 2002

Committee: Joseph Contrera, Ph.D., HFD-901, Acting Chair
Abby Jacobs, Ph. D., HFD-540, Alternate Member
Joe Sun, Ph. D., HFD-570, Alternate Member
David Morse, Ph. D, HFD-150, Supervisory Pharmacologist
David McGuinn, Ph. D., D.A.B.T., HFD-150, Presenting Reviewer

Author of Draft: David McGuinn

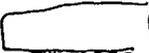
The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual reviews.

The committee did not address the sponsor's proposed statistical evaluation for the 2-year carcinogenicity bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from Agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND #

Drug Name:

Sponsor:


ZD 1839, gefitinib, IRESSA
AstraZeneca Pharmaceuticals LP.

Mouse Dose Selection:

The data from a 13-week toxicity study in the mouse demonstrated that a dose of 175-mg/kg/day (PO) caused toxicity that would be unacceptable in a longer-term study. These toxicities included decreased weight gain, closed and swollen eyes, microscopic (macrophage and/or lymphocyte infiltration) and macroscopic changes in the liver, lungs and spleen, chronic folliculitis, and corneal hyperplasia and retinal atrophy. The mid-dose of 125-mg/kg/day caused similar but considerably less severe toxicities. The animals in this group gained weight near normally through the course of the study. The low dose of 50-mg/kg/day was not considered a 'no effect' level.

Rat Dose Selection:

The data from a six-month oral toxicity study in the rat demonstrated that a dose of 25-mg/kg/day caused unacceptable toxicity by week-9. This toxicity manifest as decreased weight gain and the clinical appearance of rapidly declining health, with two apparently drug related deaths during weeks 8 and 11. The high dose in this study was lowered to 15-mg/kg/day in week-9. The animals recovered and two deaths that occurred after this reduction were considered incidental. At the end of the study, the high dose animals weighed as much as 17% less than controls. They showed signs of significant liver damage manifest as increased serum concentrations of liver function enzymes to as much as 2.5-times the mean control value. Macroscopic changes in the liver, kidney, spleen and heart, correlated with microscopic damage. The mid-dose of 5-mg/kg/day caused similar, but considerably less severe toxicities. The animals in this group gained weight near normally through the course of the study. The low dose of 1-mg/kg/day was not considered a 'no effect' level.

Executive CAC Recommendations and Conclusions:

Mouse:

Based on these results, the committee concurred with the sponsor's selection of doses, those being 10, 50 and 125 mg/kg/day (PO). The committee expressed some concern that the high dose of 125-mg/kg/day might be too high to assure adequate long-term survival, but felt that it would be possible to lower the dose during the study if it proved to cause unacceptable toxicity. It was therefore recommended that the sponsor contact the Food and Drug Administration before making any changes to the dosage levels or study protocol during the 'in-life' phase of the study. The committee recommended that the sponsor split the control mice into two independent control groups. Because the committee's concurrence with the sponsor's dose selection is based on a draft-unaudited report, final Agency concurrence is contingent on the audited report agreeing with the draft report regarding details relevant to dose selection.

Rat:

Based on the results of the 6-month study of oral toxicity in the rat, the committee recommended that the sponsor use doses of 1, 5 and 10 mg/kg/day for the 2-year rat carcinogenicity study. Again, the committee expressed some concern that the 10-mg/kg/day dose might prove too high, and result in unacceptable morbidity and premature mortality. As stated above, should this become the case the sponsor should contact the Food and Drug Administration prior to making changes to the dose levels or study protocol. The committee recommended that the sponsor split the control rats into two independent control groups.

If the sponsor plans histological evaluation of tissues from only control and high dose treatment groups, they will also need to conduct histopathological examination of other dose groups under any of the following circumstances:

- (a) For any macroscopic findings in the low and mid dose groups for a given tissue, they will need to look at that tissue for all of the dose groups.
- (b) For an increase in the incidence of tumors (rare or common) in the high dose group for a tissue, even if not statistically significant, they will also need to look at the next lower dose group.
- (c) For an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma, etc., see McConnell et al., JNCI 76:283, 1986) they should look at all relevant tissues for that dose level and the next lower dose level.
- (d) For an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

Joseph Contrera, Ph.D.
Acting Chair, Executive CAC

cc:\

/Division File, HFD-150
/D Morse, HFD-150
/WD McGuinn, HFD-150
/A Baird, HFD-150
/A Seifried, HFD-024

**This is a representation of an electronic record that was signed electronically and
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/s/

Joe Contrera

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