CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

20-541/S-010

Pharmacology Review(s)

MEMORANDUM

Date: August 9, 2002
From: John K. Leighton, Ph.D., DABT
Supervisory Pharmacologist, HFD-150
To: File for NDA #20-541, supplement 10
Re: Approvability for Pharmacology and Toxicology
Arimidex (anastrozole)

Arimidex is an aromatase inhibitor approved for first-line treatment of postmenopausal women with hormone receptor positive or receptor unknown locally advanced or metastatic breast cancer. Arimidex is also indicated for the treatment of advanced breast cancer in postmenopausal women with disease progression following tamoxifen therapy. The sponsor, AstraZeneca, is seeking approval with this supplemental application for the treatment of breast cancer in the adjuvant setting. The active agent in Arimidex is anastrozole, also known as ZD 1033. The submission contained preclinical studies on reproductive toxicity (fertility study); the propensity of ZD 1033 to form hepatic DNA adducts alone and in the presence of tamoxifen; and studies on the mechanism of tumor formation. Dr. Brower has provided a comprehensive review of the submitted studies. Label changes are proposed only for the reproductive toxicity section.

Carcinogenicity studies had been conducted by the sponsor and reviewed in the original NDA application. The sponsor conducted additional studies submitted with this supplemental NDA to investigate the mechanism of some of the tumor findings (thyroid and liver but not uterine). No changes were requested or needed for the carcinogenicity section of the label; thus, an Executive CAC review was not requested at this time.

Comparison to human dosing for the reproductive toxicity (fertility) studies are made on an exposure (AUC) basis, except as noted by Dr. Brower in her review. Dr. Brower provided adequate justification in her review for those instances where dose comparisons (body surface area) are used.

Recommendations: The pharmacology and toxicology data supports approval of this supplemental NDA. There are no outstanding issues.

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

/s/

John Leighton
8/9/02 01:04:25 PM
PHARMACOLOGIST

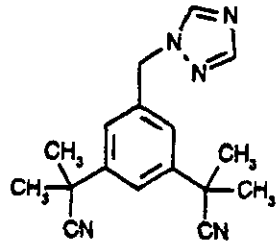
PHARMACOLOGY/TOXICOLOGY COVER SHEET

sNDA number: 20,541
Review number: NDA review #2
Sequence number/date/type of submission: Supplement 10 /December 2001-March 2002/sNDA
Information to sponsor: Yes (X) No ( )
Sponsor: AstraZeneca
Manufacturer for drug substance : AstraZeneca

Reviewer name: Margaret E. Brower, Ph.D.
Division name: Oncology Drug Products
HFD #: 150
Review completion date: July 9, 2002

Drug:

Trade name: Arimidex
Generic name: anastrozole
Code name: ZD1033
Chemical name: 2,2'-[5-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-phenylene]bis(2-methylpropiononitrile)
CAS registry number: 120511-73-1
Mole file number: n/a
Molecular formula/molecular weight: 293.4, $C_{17}H_{19}N_5$
Structure:



Relevant INDs/NDAs: IND — NDA 20,541

Drug class: Aromatase inhibitor

Indication: "Adjuvant treatment in postmenopausal women with early breast cancer"

Clinical formulation:

Ingredient	Amount (mg)
ZD 1033	1.0
Lactose	
Povidone	
Sodium starch glycolate	
Magnesium stearate	
Hydroxypropylmethylcellulose	
Polyethylene glycol	
Titanium dioxide	

Route of administration: oral tablet

Proposed use: Treatment of adjuvant breast cancer

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

Executive Summary

I. Recommendations

A. Recommendation on Approvability:

The sNDA for Arimidex is approvable from a Pharmacology/Toxicology perspective with label changes as described under **Recommendations on Labeling**.

B. Recommendation for Nonclinical Studies:

The additional preclinical toxicology studies submitted with the sNDA contributed to the complete Arimidex database, but were not required studies with the exception of study TKR/2749. This study was recommended by the division to determine the potential of the Arimidex/tamoxifen combination to alter the level of DNA adducting in comparison to adducting with tamoxifen alone. Results of the fertility study in rodents are incorporated into the label under **PRECAUTIONS** as indicated below. The **Impairment of Fertility** section of the label has been changed from the proposed label submitted by the sponsor.

C. Recommendations on Labeling:

Comment: Reference to C_{ssmax} and $AUC_{0\text{-}24hr}$ in label below (Impairment of Fertility) is taken from toxicokinetics submitted with a 6-month oral toxicity study in rats (Study #TPR/1992) which was reviewed for NDA 20,541, completed in 1995. The referenced clinical (Study #0002) data was taken at day 7 in post-menopausal volunteers, and also referenced in the original NDA. The rat strain utilized for the 6-month rat study and the fertility study indicated below (Alpk:Ap$_f$SD Wistar) was identical. However, since rodent toxicokinetics were not conducted for doses below 1mg/kg/day, AUC data were not available for the 0.02 mg/kg dose level. Therefore, comparative dosing + AUC data were used for rodent doses of 1mg/kg and only comparative dosing was used for the lower dose level.

*Teratogenicity and fetotoxicity findings indicated under **WARNINGS** section of label unchanged.*

PRECAUTIONS
Impairment of Fertility:

Draft

Pregnancy: Pregnancy Category *unchanged*

Nursing Mothers: *unchanged*

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings (current sNDA)

When rats were pretreated with 25mg/kg (150mg/m^2) ZD 1033 for 2 weeks prior to administration of ZD 1033 (25mg/kg) in combination with tamoxifen (20mg/kg, 120mg/m^2) for 2 weeks, the number of DNA adducts was reduced by >50% compared to adducting by tamoxifen alone. These findings were attributed to enzyme induction of

ZD 1033 and the resultant reduction in the number of tamoxifen metabolites in the liver. Thyroid tumors exhibited in male rats administered 25mg/kg ZD 1033 in the 2-year carcinogenicity study may be mechanistically related to an increased clearance of T_4 resulting from enzyme induction, conclusions were based on an assessment of thyroid function in rats administered 25mg/kg (150mg/m^2) ZD 1033 for 30 days. In addition, the increased incidence of liver tumors observed in 25mg/kg/day female rats in the 2-year carcinogenicity study may be the result of a non-genotoxic process of liver induction; conclusions were based on an assessment of hepatocyte proliferation in female rats administered 5 (30mg/m^2) and 25mg/kg (150mg/m^2) ZD 1033 for 7 and 28 days, and evidence of an increased incidence of enzymatic induction following ZD 1033 administration.
Fertility and subsequent numbers of live births were significantly reduced in dams administered 1mg/kg/day ZD 1033 for 2w; the fertility index was depressed 82% compared to a 5% depression in dams administered 0.002 and 0.02mg/kg/day. Reduced number of implants/dam and an increased pre-implantation loss of ova or fetus were also observed at the HD; the effect was observed to a lesser extent at the LD and MD. HD females dosed for 3w and allowed to recover for 5w exhibited recovery of fertility; the fertility index was not significantly reduced compared to controls.

B. Pharmacologic Activity

ZD 1033 is a potent and specific inhibitor of aromatase with few other pharmacological effects at therapeutic concentrations.The majority of its inhibitory activity can be attributed to the parent drug and not to its metabolites. The ability to inhibit estrogen synthesis and deplete estrogens in plasma should confer antitumor activity in estrogen-dependent malignancies.

C. Nonclinical Safety Issues Relevant to Clinical Use (current sNDA):

1. The number of DNA adducts was reduced by >50% when animals were pretreated with 150mg/m^2 ZD 1033 followed by administration of ZD 1033 (150mg/m^2) in combination with tamoxifen (120mg/m^2).These findings were attributed to enzyme induction of ZD 1033 and the resultant reduction in the number of tamoxifen metabolites in the liver.
2. Thyroid tumors exhibited in male rats administered 25mg/kg ZD 1033 in the 2-year carcinogenicity study may be mechanistically related to an increased clearance of T_4 resulting from enzyme induction, conclusions were based on an assessment of thyroid function in rats administered 25mg/kg (150mg/m^2) ZD 1033 for 30 days. There were no proposed labeling changes regarding this issue; therefore, Executive CAC concurrence is not required at this time regarding these carcinogenicity findings.
3. The increased incidence of liver tumors observed in 25mg/kg/day female rats in the 2-year carcinogenicity study may be the result of a non-genotoxic process of liver induction; conclusions were based on an assessment of hepatocyte proliferation in female rats administered 5 (30mg/m^2) and 25mg/kg (150mg/m^2) ZD 1033 for 7 and 28 days and evidence of an increased incidence of enzymatic induction following ZD 1033 administration. There were no proposed labeling changes regarding this issue; therefore, Executive CAC concurrence is not required at this time regarding these carcinogenicity findings.
4. Fertility and subsequent numbers of live births were significantly reduced in dams administered 1mg/kg/day (6mg/m^2/day)ZD 1033 for 2w; the fertility index was depressed 82% compared to a 5% depression in dams administered 0.002 (0.012mg/m^2/day) and 0.02mg/kg/day (0.12mg/m^2/day). Reduced number of implants/dam and an increased pre-implantation loss of ova or fetus were also observed at the HD; the effect was observed to a lesser extent at the LD and MD. HD females dosed for 3w and allowed to recover for 5w exhibited recovery of fertility; the fertility index was not significantly reduced compared to controls.

III. Administrative

A. Reviewer signature: /S/

B. Supervisor signature: Concurrence - /S/

Non-Concurrence - /S/
(see memo attached)

C. cc: list:
LeightonJ

CortazarP
FarrellA
BrowerM
BairdA

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

INTRODUCTION AND DRUG HISTORY:
In February, 1997, following the proposal by Zeneca to combine treatment of Arimidex and tamoxifen in post-menopausal women with node-positive or node-negative breast cancer, the division asked that a preclinical *in vitro* study be initiated to determine the potential of the combination to alter the level of DNA adducting generated by rat liver microsomes treated with tamoxifen alone. We recommended comparison of microsomes from rats induced with vehicle, tamoxifen alone, ZD 1033 alone, and the combination of ZD 1033 and tamoxifen. We recommended that rats be predosed with ZD 1033 for sufficient time and at sufficient dose for enzyme induction (14d at >5mg/kg/day in pk data submitted with NDA).

In addition, we recommended that a proposed six-month oral study in Wistar rats using ZD 1033 and tamoxifen in combination (with doses and dosing schedule comparable to that proposed for clinical trial), include DNA adducting. We recommended that a group administered tamoxifen alone at the same dose be included for comparative purposes and satellite groups be used to assess DNA adducting (as well as pharmacokinetics and liver pathology) at one and three months.

In March, 1997, Zeneca proposed conducting a two-week *in vivo* adducting study with ZD 1033 and tamoxifen in lieu of the *in vitro* adducting study with the drug combination, due to the perceived difficulty in completing the latter study in rat liver microsomes. In addition, they indicated that they would "contemplate" the recommendation of including human hepatocytes to an *in vitro* study to allow comparison to the adducting potential of rat liver microsomes. At the same time, Zeneca agreed to conduct the six-month oral study in Wistar rats, detailed above. We indicated that results of the two-week study could potentially modify the recommendation for the six-month study.

The protocol for the *in vivo* DNA adducting study was received July 21, 1997. The protocol was acceptable, with recommendations to pretreat ZD 1033 groups for sufficient time prior to the tamoxifen exposure and include analysis of rat plasma for tamoxifen and tamoxifen metabolites in order to provide preliminary data on drug interaction for Biopharmaceutics.

Following submittal of the 2 week *in vivo* adducting study in June, 1999, Zeneca requested and was granted a waiver for the six-month study. This was based on a reduction in the number of adducts in animals administered the drug combination compared to tamoxifen alone.

The recommended *in vitro* adducting study comparing the adducting potential of rat liver microsomes to human hepatocytes and the 6-month oral study in rats were not conducted. It is not known if differences exist in the adducting potential of the combination following 6 months exposure. Previously, when tamoxifen was administered to rodents as a single drug, tamoxifen metabolites were observed to increase over extended periods of dosing.

Previous Clinical Experience: The worldwide 5-year ATAC clinical trial is currently being conducted comparing anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with breast cancer. Following three years of treatment, results with the combination were not significantly different from those with tamoxifen alone. (Lancet, 6/22/02; 359(9324):2131.)The incidence of bone fragility and bone breakage does appear to be increased in anastrozole-treated women; these findings were not observed preclinically.

I. PHARMACOLOGY: NONE

II. SAFETY PHARMACOLOGY: NONE

III. PHARMACOKINETICS/TOXICOKINETICS: NONE

IV. GENERAL TOXICOLOGY:

TKR/2749 28 day investigative study in rats: oral administration

TKR/2749(a)	Study to assess the potential of anastrozole to affect Tamoxifen-DNA adduct formation in female rat liver
TKR/2749(b)	ZD1033: The effects of ICI 46,474 alone or in combination with ZD1033 on the hepatic microsomal mixed function oxidase enzymes of the female rat. P450 assessment.
TKR/2904	Zeneca ZD1033: Assessment of thyroid function in rats
TKR/2963	Zeneca ZD1033: Investigation of liver tumors in rats
TGR/2857	Zeneca ZD1033:Fertility study in female rats: oral administration

Study title: 28 day investigative study in rats: oral administration

Key study findings:
• Number of DNA adducts reduced in animals predosed with 25mg/kg ZD 1033 for 2 weeks followed by 2 additional weeks of combined administration of 25mg/kg ZD 1033 and 20mg/kg tamoxifen
• Predosing with ZD 1033 for 2 weeks altered enzyme induction, the metabolism of tamoxifen and the formation of DNA adducts.
• The cytochrome P450 enzyme induction (primarily CYP2B) of 25mg/kg/day ZD 1033 for 28 days was significantly increased.

Study no: TKR/2749 + appendices a and b
Volume #, and page #: Electronic submission: January 18, 2002 (Original date of submission: June 10, 1999; Original date of review: February 7, 2000)
Conducting laboratory and location: Zeneca Pharmaceuticals, Cheshire, England
Date of study initiation: August-September, 1997
GLP compliance: UK GLP
QA report: yes (X) no ( )
Drug, lot # and % purity: ZD 1033 (anastrazole), batch # 005ARPV; ICI 46,474 (tamoxifen), batch #618TFP; purity not provided
Formulation/vehicle: 0.5% w/v hydroxypropyl methylcellulose solution in 0.1% w/v polysorbate 80
Methods: Combination drug dosing: ZD 1033 administered d1-28; TAM administered d15-28)
Dosing:
Species: EAlpk:Ap$_f$SD (Wistar derived) rats
#/group: 5
age: 81-88d
weight: 244-310g
drug, lot#, and % purity: ZD1033 (anastrazole), batch#005ARPV; ICI 46,474 (tamoxifen), batch#618TFP
formulation/vehicle: 0.5%w/v hydroxypropyl methylcellulose solution in 0.1% w/v polysorbate 80
dosage groups/dosing interval: 20mg/kg tamoxifen/d15-28 (dosed with vehicle d1-14), 1mg/kg ZD 1033 + 20mg/kg tamoxifen (ZD 1033 d1-28, TAM d15-28), 25mg/kg ZD 1033 + 20mg/kg tamoxifen (ZD 1033 d1-28, TAM d15-28)
route, volume: oral gavage, 0.5ml/100g with exception of vehicle control dosed at 1ml/100g

Observation and times
Mortality: 2X/day
Physical examination: weekly
Body weights: prior to study initiation, dosing d1, weekly thereafter
Food consumption: prior to study initiation, weekly
Gross pathology: necropsy d29, external features, abdominal cavity examined
Organ weights: at necropsy d29
Organs weighed: liver
Histopathology: d29
Assessment of microsomal enzyme activity: Frozen liver samples sent to [redacted] where hepatic microsomal fractions were prepared using differential centrifugation and total protein content, cytochrome P450 concentration and CYP2B and CYP3A activities were determined
Assessment of DNA adducts: [redacted]
Determination of tamoxifen and tamoxifen metabolites in liver and plasma: [redacted]

Results
Mortality: none
Clinical observations: Red staining of fore paws, salivation: ZD 1033 + tamoxifen animals d22-29 (individual data not submitted)

Body weight and food consumption:
% change from control

Dose Group	Body weight		Food consumption	
	d22	d26/29	d22	d26/29
20mg/kg TAM	11	14	42	40
1mg/kg ZD 1033 + 20mg/kg TAM	7	10	23	33
25mg/kg ZD 1033 +20mg/kg TAM	15	16	8	8

Microsome MFO and testosterone hydroxylase enzyme determination:

Dose Group	Cytochrome P450 (nmol/mg protein)	Pentoxyresorufin O-depentylase (pmol/min/mg)	6β-hydroxytestosterone (nmol/min/mg)	16β-hydroxytestosterone (nmol/min/mg)
Control	0.74	1.7	<LOQ	<LOQ
20mg/kg TAM	0.87[a]	7.92	0.86	<LOQ
1mg/kg ZD 1033 + 20mg/kg TAM	0.71	13.2	1.01	0.36
25mg/kg ZD 1033 + 20mg/kg TAM	1.00[b]	507	2.45	1.23

N=5
LOQ: — nmol/mg/min
[a]CYP2B, CYP3A
[b]Addition of ZD 1033 at 25mg/kg for 28d induced primary increase of CYP2B

Assessment of DNA adducts:
Mean number of adducts/10^8 nucleotides:

Dose Group	Mean # adducts/animal	Mean # adducts/group
Control	2.2 6.4 1.4 14.6 6.2	6.17±5.24
20mg/kg TAM	2042.6 344.8 349.1 1800.9 345.3	976.55±867.1
1mg/kg ZD 1033 + 20mg/kg TAM	992.4 1376.3 1219.8 1158.9 1687.3	1286.93±262.63
25mg/kg ZD 1033 + 20mg/kg TAM	462.7 153.8 502.0 779.7 303.1	440.23±234.74

3

Animals administered 25mg/kg ZD 1033 + 20mg/kg TAM exhibited a significantly lower number of adducts compared to animals administered TAM alone or TAM combined with 1mg/kg ZD 1033. There was a large variation in the number of adducts detected for individual animals in groups administered TAM alone (range — — adducts/10^8 nucleotides) and TAM combined with 25mg/kg ZD 1033 (range — adducts/10^8nucleotides).

Determination of tamoxifen and tamoxifen metabolites in plasma and liver:

Dose Group	TAM in plasma (ng/ml)	TAM in liver (nmoles/g)	N-desmethylTAM in liver (nmoles/g)	4-hydroxyTAM in liver (nmoles/g)
20mg/kg TAM	184	23.6	122.5	16.9
1mg/kg ZD 1033 + 20mg/kg TAM	116	12.4	56.7	7.2
25mg/kg ZD 1033 + 20mg/kg TAM	66	3.0	13.0	3.8

A reduction in the concentration of TAM in plasma and liver, and the concentration of metabolites of tamoxifen in liver was observed in animals administered TAM in combination with 25mg/kg ZD 1033. Due to enzyme induction and this resultant reduction in the number of tamoxifen metabolites in the liver, the number of DNA adducts was reduced in this dose group as indicated above.

Organ weights:
Liver weights of rats administered 25mg/kg ZD 1033 + 20mg/kg TAM were 37%.
Gross pathology:
25mg/kg ZD 1033 + 20mg/kg TAM: liver enlarged and discolored

Histopathology:

Liver findings	20mg/kg TAM	1mg/kg ZD 1033 + 20mg/kg TAM	25mg/kg ZD 1033 + 20mg/kg TAM
Hepatocellular hypertrophy			5/5
hepatocellular glycogen	1/5	1/5	

Study title: Zeneca ZD1033: Assessment of thyroid function in rats
Addendum to study: Zeneca ZD1033: The effects of Zeneca ZD1033 on hepatic mixed function oxidase enzymes of the male rat (Project # 88/250)

Key study findings:
• Male rats administered 25mg/kg/day ZD 1033 exhibited increased TSH activity, increased plasma clearance of ^{125}I-T_4 in association with hepatocellular hypertrophy, increase in CYP2B and T_4UDPGT activities and thyroid follicular epithelial cell hypertrophy.

Study no: TKR/2904
Volume #, and page #: Electronic submission: January 18, 2002
Conducting laboratory and location: Zeneca Pharmaceuticals, Cheshire, England; Addendum study conducted at —
Date of study initiation: August, 1998; October, 1998
GLP compliance: UK GLP
QA report: yes (X) no ( )
Drug, lot #, radiolabel and % purity: ZD 1033 (anastrazole), batch # 005ARPV; ^{125}I radiolabel for thyroxine clearance studies; purity not provided
Formulation/vehicle: 0.5% w/v hydroxypropyl methylcellulose solution in 0.1% w/v polysorbate 80
Methods: Unlabeled ZD 1033 males used for plasma hormone measurements, pathology and assessment of liver enzyme induction; labeled ZD 1033 males used for thyroxine clearance study. Satellite groups used for ^{125}I-thyroxine clearance studies: 20♂/group; 20uCi/kg ^{125}I-throxine administered iv (tail vein) 4h following final ZD 1033 dosing (Na I solution administered ip immediately following administration of ^{125}I-T_4 to

4

block uptake of radiolabelled material into thyroid, additional doses of Na I administered at 12 and 24h)

Dosing:

Species/strain: ♂ Alpk:ApfSD (Wistar derived)
#/sex/group (main study): 10♂/group
Age: 30d
Weight: 134-211g
Doses: 25mg/kg (labeled and unlabeled groups)
Route: gavage
Duration of dosing: 30d (unlabeled animals sacrificed d31)

Results (Observation times)

Mortality/clinical obs (2x daily)	None/UR
Body weights(d-7, d1, weekly)	UR
Hematology (main study: prestudy, d31; thyroxine clearance: 0.5, 1, 2, 4, 8, 12, 24, 30h following ^{125}I-T_4 admin)	Plasma hormone levels (total thyroxine [T_4], thyroid stimulating hormone, free T_4, luteinising hormone, follicle stimulating hormone) measured in animals of main study (see table below) Plasma ^{125}I-thyroxine, plasma levels (antibody binding assay), ^{125}I-T_4 clearance, half-life measured in animals of satellite group (see table below)
Organ weight (liver only)	↑23% relative liver weight compared to concurrent controls[a]
Gross pathology (d30/31)	Tissues examined:Thoracic and abdominal cavities, liver (cytochrome P450 analysis), esophagus, thyroid, parathyroid, trachea, tissues with gross abnormalities Results: Enlarged liver in 7/10 ZD 1033-dosed ♂
Histopathology (d30/31)	Liver, esophagus, thyroid, parathyroid, trachea, tissues with gross abnormalities (see table below)

[a]Absolute liver weights not submitted

Changes in the hepatic microsomal mixed function oxidase enzymes of the male rat following administration of 25mg/kg ZD 1033

Dose (mg/kg/day)	Cytochrome P450 (nmol/ mg protein)	EROD[a] (pmol/ min/mg)	PROD[b] (pmol/ min/mg)	6β-OH Testosterone hydroxylase (pmol/min/mg)	16β-OH Testosterone hydroxylase (pmol/min/mg)	T_4UDPGT[c] (pmol/min/mg)
0	0.606	32.16	8.62	626	206	1.634
25	1.281***	51.8*	157.15***	2190***	577***	2.106

[a]Ethoxyresorufin O-deethylase
[b]Pentoxyresorufin O-dependentylase
[c]Thyroxine UDP glucuronyl transferase
*, *** = $p<0.05$, 0.001

ZD 1033 administered at 25mg/kg induced CYP2B; this isoform of cytochrome P450 was expressed as significant increases in total P450, EROD, PROD, and 16β-OH testosterone. CYP3A was weakly induced and expressed as an increase in 6β-OH testosterone. The increase in T_4UDPGT following administration of ZD 1033, although not statistically significant, was considered by the study author to be biologically significant. The magnitude of increase in these values were compared to phenobarbital, the model CYP2B inducer, since phenobarbital administration results in increased incidence of thyroid tumors. ZD 1033-induced CYP2A and CYP2B isoforms were considered to be characteristic of a moderate phenobarbital-like effect on the thyroid-pituitary hypothalamus axis.

Plasma hormone levels (N at d31= 10)

Index	Day	control	25mg/kg ZD 1033
TSH (ng/ml)	-3	2.34	2.0
	31	3.26	4.44
LH (ng/ml)	-3	1.5	1.3
	31	2.45	1.5
FSH (nmol/L)	-3	16.05	14.55
	31	9.1	9.75

5

Plasma ^{125}I-thyroxine clearance and half-life (N=20)

Parameter	Control	25mg/kg ZD 1033
Plasma T4 clearance (ml/min)	23.2	28.4***
Half-life (h)	20.3	19.5

***p<0.001

LH and FSH were not elevated 30d following ZD 1033 administration; the median LH of dosed animals was depressed 39%, but was not considered to be toxicologically significant. ZD 1033 did not appear to interfere with the negative feedback regulation of gonadotrophin secretion from the pituitary glands of male rats. The plasma ^{125}I-thyroxine of dosed animals was depressed ~15-20% from 1 to 30h after dosing, compared to concurrent controls, which was reflected in the ~22% increase in ^{125}I-thyroxine clearance; there was no effect on the plasma half-life of ^{125}I T_4 in dosed animals compared to concurrent controls. Plasma TSH levels of dosed animals doubled from prestudy to day 30 but the increase was not significant compared to concurrent controls.

Histopathological findings

Tissue/finding	Control	25mg/kg ZD 1033
Liver/centrolobular hepatocyte hypertrophy (mild-moderate)		9/10
/reduced centrilobular hepatocye glycogen vacuolation	1/10	8/10
/reduced hepatocyte glycogen vacuolation	1/10	2/10
Thyroid/follicular cell hypertrophy	1/10	7/10

The thyroid tumors exhibited in male rats administered 25mg/kg ZD 1033 in the 2-year carcinogenicity study may be mechanistically related to an increased clearance of T_4 resulting from enzyme induction.

Study title: Zeneca ZD1033: Investigation of liver tumors in rats

Key study findings:
- Intact rats dosed with 25mg/kg/day ZD 1033 exhibited a significant increase in hepatocyte proliferation at d8, which reversed by d28.
- Ovariectomized rats administered 25mg/kg/day ZD 1033 and sacrificed on d8 exhibited a comparable increase in hepatocyte proliferation.
- Hepatocellular hypertrophy was observed in animals administered 25mg/kg/day ZD 1033 for 8days and 5 and 25mg/kg/day for 28d

Study no: TKR/2963
Volume #, and page #: Electronic submission dated January 18, 2002
Conducting laboratory and location: AstraZeneca UK Limited, Cheshire, England
Date of study initiation: February, 1999
GLP compliance: OECD and UK GLP
QA report: yes (X) no ( )
Drug, lot #: ZD1033, batch #005ARPV
Formulation/vehicle: 0.5% w/v hydroxypropyl methylcellulose and 0.1% w/v polysorbate 80

Methods: BrdU labelling of hepatocyte nuclei (incorporation into replicating DNA) to assess hepatocyte proliferation accomplished by implantation of minipump. Apoptosis was assessed using a terminal deoxytransferase mediated dUTP-fluorescein nick end labelling (TUNEL) procedure to label DNA strand breaks.

3 study phases:
Phase 1: 7-day ZD 1033 study with intact females, minipump containing BrdU (5-bromo-2'-deoxyuridine) implanted on study day 1;

6

Phase 2: 7-day ZD 1033 study with ovariectomized females, minipump containing BrdU implanted on study day 1;
Phase 3: 28-day ZD 1033/Letrozole comparative study with intact females; minipump containing BrdU implanted on study day 22.

Dosing:

Species/strain: AP rats (Alpk:ApfSD Wistar)
#/sex/group or time point (main study): 7-day studies: 6♀/group; 28-day study: 20♀/group
Satellite groups used for toxicokinetics or recovery: none
Age: 50d
Weight: 182-219g (intact), 200-230g (ovariectomized)
Doses in administered units:
7-day intact ♀: 0, 5, 25mg
7-day ovariectomized ♀: 0, 25mg
28-day intact ♀: 0, 5, 25 ZD1033, 10mg/kg/day letrozole
Route, volume: gavage, 0.5ml/100gBW dose volume

Observations (times)

Mortality/clinical observations (2x/day)	1/20 LD ZD 1033 28-day (Phase 3) study ♀ terminated d24 as result of poor condition of BrdU minipump/ no clinical observations recorded for study animals
Body weight (prestudy, d1, weekly)	Ovariectomized ♀ 11%↑ BW compared to intact ♀prior to dosing and 13%↑ d8 *(Ovariectomized animals necropsied d8)* D29 as compared to concurrent controls: LD ZD 1033 ↑7%, HD ZD 1033 ↑11%, Letrozole ↑16%
Plasma hormone levels (Phase 1,2: d8 necropsy/blood collection; Phase 3: blood collection 4, 16h postdose d28, necropsy/blood collection d29)	See tables below
Gross pathology (d8, d29)	Liver: enlarged: 5/6, 6/6 intact and ovariectomized HD ZD 1033 ♀ treated for 7d; 5/6 ovariectomized controls;2/20, 10/20 LD and HD ZD 1033 ♀ treated for 28d Uterus: thin: 1/6, 2/6 intact LD, HD ZD 1033 ♀ 7d; 3/20, 4/20 LD, HD ZD 1033 ♀ 28d; 6/6, 5/6 ovariectomized control and HD ZD 1033 ♀; 20/20 letrozole ♀ Vagina: small: 1/6, 6/6 ovariectomized control and HD ZD 1033 ♀ Ovaries: enlarged: 2/6, 3/6 LD, HD ZD 1033 ♀ 28d; 1/6 letrozole ♀28d
Organ weights	Liver, ovary, uterus (see table below)
Histopathology (d8, d29)	Liver, ovaries, uterus, cervix, vagina, mammary glands (see table below)

Estrous cycling dosing from d7 to d21 Phase 3 animals (N=20; # with estrous cycling described/# study animals examined)

Dosing	Regular cycling	Irregular cycling	Not cycling Occasional proestrus or late estrus	Not cycling Persistent diestrus
Control	10/20	10/20		
LD ZD 1033	1/20	17/19	2/19	
HD ZD 1033		9/20	11/20	
Letrozole			14/20	6/20

Note: rats monitored were immature at 7-10w from dosing initiation to termination; therefore, a high spontaneous rate of estrus cycle irregularity is expected.

7

Day 8 Plasma hormone levels

Hormone	Ovariectomized		Intact ♀		
	Control	HD ZD 1033	Control	LD ZD 1033	HD ZD 1033
Estradiol(pmol/L)	6.0	6.3	10.8	10.8	12.4
Testosterone (nmol/L)	0.1	0.1	0.1	0.1	0.1
Progesterone (nmol/L)	1.3	1.2	9.8	3.5**	3.4*
FSH (U/L)	34.3	31.1	4.2	6.9	4.2
LH (ng/ml)	14.5	16.7	3.2	3.8	3.8

*p<0.05, **p<0.01

As expected, plasma levels of estradiol and progesterone of ovariectomized animals were significantly decreased compared to intact animals; plasma levels of FSH and LH of these animals were significantly elevated. The administration of ZD 1033 did not effect hormone levels of ovariectomized animals. Progesterone levels of ZD 1033-treated intact animals were significantly decreased compared to concurrent control levels. Other hormone levels of ZD 1033-treated animals were similar to concurrent controls.

Day 28 Plasma hormone levels (16h post-dose/24h post-dose)

Hormone	Control[a]	LD ZD 1033	HD ZD 1033	Letrozole
Estradiol(pmol/L)	-/6.0	12.8/6.0	10.4/11.3***	22.3/6.0
Testosterone (nmol/L)	0.3/0.1	3.3***/0.3	4.4***/0.3	1.0**/2.3***
Progesterone (nmol/L)	24.8/12.0	26.2/7.9**	26.6/6.9***	16.0*/4.2***
FSH (U/L)	7.4/4.2	8.4/3.0*	11.8/5.1	-[b]/12.5***
LH (ng/ml)	1.0/2.9	2.7*/3.0	4.2**/3.7*	2.0/4.7***

[a]Comparative control values for estradiol noted to be insufficient at 16h post-dose
*** p<0.001
[b] Insufficient sample size

Plasma levels of progesterone were significantly depressed 24h following dosing in ZD 1033 and letrozole-treated animals. FSH levels of ZD 1033-treated animals were similar to controls, while LH levels appeared to be elevated. Comparatively, FSH and LH levels were significantly increased in letrozole-treated animals at 24h post dose. Testosterone levels of ZD 1033 and letrozole-treated animals were elevated at 16h post-dose, compared to concurrent controls; levels appeared to return to the control level at 24h in ZD 1033-treated animals only. This testosterone increase is an expected result of aromatase inhibition.

Sample size of control animals used for estradiol comparison were insufficient for analysis at 16h post dose; in addition, samples sizes of treated groups were indicated to be "less than ideal" at 16 and 24h post dose. As a result, no meaningful interpretation for estradiol can be made.

Absolute organ weight changes of ZD 1033 and comparative Letrozole-dosed animals[a]

Organ	HD ZD 1033 Phase 1*	HD ZD 1033 Phase 2**	LD ZD 1033 Phase 3***	HD ZD 1033 Phase 3***	Letrozole Phase 3***
Liver	↑20	↑20	↑11	↑41	↑24
Ovary	UR	Not determined	↑20	↑25	↑26
Uterus	UR[b]	[c]	↓22	↓10	↓71

[a]% organ weight change as compared to concurrent control
[b]Uterine weights of LD ZD 1033 dosed intact ♀ ↓25%
[c] Uterine weights of ovariectomized controls ↓80% compared to intact controls; uterine weights of ZD 1033-dosed ♀ UR compared to ovariectomized controls
* Phase 1; 8d study intact ♀ - weights determined d8
**Phase 2; 8d study ovariectomized ♀- weights determined d8
*** Phase 3; 28d study – weights determined d29
UR unremarkable

8

Hepatocyte proliferation was assessed in sections of liver stained immuno-histochemically using anti-BrdU antibodies. Apoptosis was assessed using a terminal deoxytransferase mediated dUTP-fluorescein nick end labelling (TUNEL) procedure to label DNA strand breaks.

Assessment of hepatocyte proliferation (BrdU labelling index) and apoptosis (TUNEL labelling index)
7-day study

Parameter	Intact			Ovariectomized	
	Control	LD ZD 1033	HD ZD 1033	Control	HD ZD 1033
BrdU index (%)	15.0	23.9	45.3***	12.0	32.6**
TUNEL index (%)	0.72	0.48	0.45	0.62	0.76

Assessment of hepatocyte proliferation (BrdU labelling index) and apoptosis
(TUNEL labelling index) 28-day study

Parameter	Control	LD ZD 1033	HD ZD 1033	Letrozole
BrdU index (%)	7.6	7.7	10.3	2.2***
TUNEL index (%)	0.48	0.43	0.56	0.66*

A dose-related increase in hepatocyte proliferation was observed in intact and ovariectomized ZD 1033-treated ♀dosed for 8 days, as measured by the BrdU labelling index. The increase in hepatocyte proliferation was significant at 25mg/kg ZD 1033 (intact and ovariectomized). There was no increase in hepatocyte proliferation after 28 days. There were no significant alterations in the rate of apoptosis, as measured by the TUNEL labelling index, in ZD 1033-treated animals of the 8 or 28day studies; letrozole-treated animals exhibited increased apoptosis at 28 days.

Note: The TUNEL index of the intact controls appears to be unusually high; there was no explanation for this result in the study report. The study author indicated that the pattern of immunostaining for BrdU was different in control and treated animals. There is some question regarding the reliability of both staining techniques.

Histopathological findings

Tissue/finding	LD Ph 1*	HD Ph 1*	HD Ph 2**	LD Ph 3***	HD Ph 3***	L Ph 3***
Liver/centrilobular hepatocellular hypertrophy Minimal Mild moderate		1/6	4/6	1/20	11/20 6/20 3/20	4/20
/hepatocellular eosinophilia		1/6	1/6	7/20	14/20	4/20
/↑ mitotic figures	2/6	3/6	3/6		3/20	
/centrilobular single cell necrosis: minimal-mild					7/20	
Ovaries/Graffian follicle enlargement Occasional Multifocal				2/20 1/20	2/20 3/20	
/↑ # Graffian follicles	2/6	1/6		15/20	19/20	17/20
/↓ corpora lutea				2/20	9/20	13/20
Uterus/atrophy			6/6[a]		1/20	19/20
/occasional mitotic figures				3/20	1/20	
Vagina/atrophy			6/6[a]	1/20		18/20
/↑ apoptosis		1/6	6/6	8/20	15/20	3/20

*Phase 1: 8-day study intact LD, HD-ZD 1033-dosed ♀
**Phase 2: 8-day study ovariectomized HD-ZD 1033-dosed ♀
***Phase 3: 28-day study LD, HD-ZD 1033-dosed, Letrozole-dosed ♀
[a] incidence of 6/6 atrophy of uterus and vagina also observed in ovariectomized control group

9

Hepatic enzyme induction may be the cause of the increase in incidence of liver tumors observed in 25mg/kg/day ZD 1033-treated female rats in the 2-year carcinogenicity study as proposed by the sponsor. However, data from this study alone are not sufficient to reach that conclusion. Data indicating the increased incidence of enzyme induction following ZD 1033 administration, included in study #TKR/2904, must be included in the study assessment.

Toxicology summary (sNDA):

The number of DNA adducts was reduced by >50% in female rats administered 20mg/kg tamoxifen in combination with 25mg/kg ZD 1033, compared to administration of 20mg/kg tamoxifen alone. Predosing with ZD 1033 for 2 weeks altered the metabolism of tamoxifen and the number of hepatic metabolites. The cytochrome P450 enzyme induction potential of ZD 1033 (primarily CYP2B) at a dose level of 25mg/kg/day for 28d was increased; induction was observed to a lesser extent at the lower dose of 1mg/kg ZD 1033.

In a separate study, male rats administered 25mg/kg ZD 1033 exhibited increased TSH activity, increased plasma clearance of ^{125}I-T_4 in association with hepatocellular hypertrophy, increase in CYP2B and T_4UDPGT activities and thyroid follicular epithelial cell hypertrophy. The thyroid tumors exhibited in male rats administered 25mg/kg ZD 1033 in the 2-year carcinogenicity study may be mechanistically related to an increased clearance of T_4 resulting from enzyme induction.

In another study, intact rats dosed with 25mg/kg/day ZD 1033 exhibited a significant increase in hepatocyte proliferation at d8, which reversed by d28. Liver weights of 25mg/kg/day ZD 1033-dosed ♀ were increased by 40% following 28d of dosing; hepatocellular hypertrophy and eosinophilia was observed histologically. Hepatic enzyme induction *may* be the cause of increase in incidence of liver tumors observed in 25mg/kg/day ZD 1033-treated female rats in the 2-year carcinogenicity study. However, in order to reach this conclusion, data indicating the increased incidence of enzyme induction following ZD 1033 administration, included in study #TKR/2904, must be included in the study assessment.

Toxicology conclusions (sNDA):

When rats were pretreated with 25mg/kg (150mg/m^2) ZD 1033 for 2 weeks prior to administration of ZD 1033 (25mg/kg) in combination with tamoxifen (20mg/kg, 120mg/m^2) for 2 weeks, the number of DNA adducts was reduced by >50% compared to adducting by tamoxifen alone. These findings were attributed to enzyme induction of ZD 1033 and the resultant reduction in the number of tamoxifen metabolites in the liver. The sponsor proposes that the thyroid tumors exhibited in male rats administered 25mg/kg ZD 1033 in the 2-year carcinogenicity study may be mechanistically related to an increased clearance of T_4 resulting from enzyme induction, conclusions were based on an assessment of thyroid function in rats administered 25mg/kg (150mg/m^2) ZD 1033 for 30 days. We agree with this assessment. In addition, the sponsor proposes that the increased incidence of liver tumors observed in 25mg/kg/day female rats in the 2-year carcinogenicity study are the result of a non-genotoxic process of liver induction; conclusions were based on an assessment of hepatocyte proliferation in female rats administered 5 (30mg/m^2) and 25mg/kg (150mg/m^2) ZD 1033 for 7 and 28 days. We agree that this increased incidence of hepatic tumors *may* be the the result of an increased incidence of liver induction, however, the study assessment must include data indicating the increased incidence of enzymatic induction following ZD 1033 administration. These data are included in study #TKR/2904. Since there were no proposed labeling changes regarding these issues, Executive CAC concurrence is not required at this time.

Histopathology Inventory for sNDA #20,541

Study	TKR/ 2749	TKR/ 2904	TKR/ 2963	TGR/ 2857
Species	rat	rat	rat	rat
Adrenals				
Aorta				
Bone Marrow smear				
Bone (femur)				
Brain				
Cecum				
Cervix			X	
Colon				
Duodenum				

10

Epididymis				X
Esophagus		X		
Eye				
Fallopian tube				
Gall bladder				
Gross lesions		X		
Harderian gland				
Heart				
Ileum				
Injection site				
Jejunum				
Kidneys				
Lachrymal gland				
Larynx				
Liver	X	X	X	
Lungs				
Lymph nodes, cervical				
Lymph nodes mandibular				
Lymph nodes, mesenteric				
Mammary Gland			X	X
Nasal cavity				
Optic nerves				
Ovaries			X	X
Pancreas				
Parathyroid		X		
Peripheral nerve				
Pharynx				
Pituitary				
Prostate				
Rectum				
Salivary gland				
Sciatic nerve				
Seminal vesicles				
Skeletal muscle				
Skin				
Spinal cord				
Spleen				
Sternum				
Stomach				
Testes				X
Thymus				
Thyroid		X		
Tongue				
Trachea		X		
Urinary bladder				
Uterus			X	X
Vagina			X	
Zymbal gland				

X, histopathology performed
*, organ weight obtained

V. GENETIC TOXICOLOGY: NONE

VI. CARCINOGENICITY: NONE

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

11

Study title: Zeneca ZD1033:Fertility study in female rats: oral administration

Key study findings:
•HD (1mg/kg/day): fertility index depressed 82%, significant reduction in numbers of live births and implants/dam with increase in pre-implantation loss of ova or fetus
•Recovery of fertility at HD following 3w of dosing and 5w of recovery
•LD (0.002mg/kg/day) and MD (0.02mg/kg/day) affected to lesser extent

Study no: TGR/2857
Volume #, and page #: Electronic submission: March 19, 2002
Conducting laboratory and location: Zeneca Pharmaceuticals, Safety of Medicines Dept., Cheshire, England
Date of study initiation: June 8, 1998
GLP compliance: Conducted in compliance with UK GLP and OECD GLP
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: ZD1033, batch #005ARPV, purity not provided
Formulation/vehicle: aqueous 0.5% w/v hydroxypropyl methylcellulose solution + 0.1% w/v polysorbate 80/0.5% polysorbate 80 vehicle

Methods: (See study design) Ovaries and uterus excised on pregnancy d13
Dosing:
Species: Alpk:ApfSD Wistar rats
#/sex/group (main study): 22
Satellite groups used for recovery mating: 22/sex/group
Age: 13-14w
Weight: 233-344g
Doses: 0.002, 0.02, 1mg/kg/day (♀ only dosed)
Route: oral (♀)
Study design: Phase 1:LD, MD and HD ♀ compared to controls following 2w of dosing prior to mating to pregnancy d7; Phase 2:HD♀ compared to controls following 3w of dosing and 5w of recovery prior to mating.

Results (Observation times)

Mortality/clinical obs (daily)	None/ no comment on clinical observations other than reproductive indices
Body weights(2X/w until mating; pregnancy d 1, 7, 10, 13)[a]	Phase 1: ↑ BW gain prior to pairing at MD and HD; the HD was slightly depressed compared to LD and MD during pregnancy days 7-13. Phase 2: ↑ BW gain prior to pairing and during pregnancy compared to controls. The effect on BW gain was lessened following 5w of recovery.
Food consumption (weekly during dosing)	Phase 1: UR Phase 2: 8-16% ↑ FC of HD compared to controls.
Gross pathology (♀ from all dosed groups and ♂ involved in infertile matings or to provide reference tissues)	Ovaries, uterus excised pregnancy d13 UR
Histopathology (epididymides, testes, ovaries, uterus, mammary only)[b]	♂: UR ♀: mammary gland adenocarcinoma 1/22 LD Phase 1 study; considered to be incidental finding; was not observed at higher doses ↑ ovarian corpora lutea HD Phase 1 (13/18) and 1/6 (Phase 2), corpus luteum cyst 1/6HD (Phase 2)

[a] Group tabulated BW were not available
[b] Histological examination of uteri reported to be difficult due to introduction of artifact in necropsy and staining

Additional parameters examined:
Vaginal smears (daily from 2w prior to dosing-pregnancy d6)
Fertility index (#fertile/#mated) x 100%
Copulation index (#mated/#paired) x 100%

12

Irregular estrous cyclicity of ZD 1033 and control dams (N=22; #animals with finding/#study animals)

Group	Predosing	Dosing[a]	Recovery				
	2w	2 or 3 wk	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5
Control							
Phase 1	1/22	9/22					
Phase 2	2/22	9/22	4/22	8/22	9/22	4/22	5/22
LD	1/22	10/22					
MD	4/22	12/22					
HD							
Phase 1	3/22	21/22					
Phase 2	3/22	22/22	6/22	4/22	3/22	13/22	11/22

[a] Estrous cycles were assessed over the 2w dosing period prior to pairing (Phase 1) or over the 3w period prior to cessation of dosing (Phase 2). Irregular cycle = interval between estrous was <4d or >5d, or estrous was recorded on more than 2 consecutive days.

The most common change in cyclicity was an increase in cycle length.

Mating and fertility data of ZD 1033 and control dams

Parameter	Control	LD	MD	HD
Phase 1[a]				
# pregnant at d13 necropsy w/live embryos	22	21	20	4 ($p<0.001$)
/resorptions only			1	
/not pregnant		1	1	18
Copulation index (%)	100	100	100	100
Fertility index (%)	100	95.5	95.5	18.2
Phase 2[b]				
# pregnant at scheduled necropsy w/live embryos	19			16
/not pregnant	3			6
Copulation index (%)	100			100
Fertility index (%)	86.4			72.7

[a] ♀mated following 2w of dosing; 22♀ mated
[b] ♀ mated following 3w of dosing and 5w of recovery (control and HD only); 22♀ mated

HD females of the Phase 1 study exhibited infertile mating; the fertility index was significantly reduced compared to controls and LD and MD administered ZD 1033. HD females dosed for 3w in the Phase 2 study, and allowed to recover for 5w exhibited recovery of fertility (~55%); the fertility index was not significantly reduced compared to controls (~14%).

13

Uterine examination summary

Parameter	Control	LD	MD	HD
Phase 1				
# live fetuses/litter	15/22	15/21	11/20	2/4
# implants/dam	16/22	16/21	12/20	2/4
#corpora lutea/dam	17/22	18/22	16/22	16/22
Preimplantation loss/dam	1/22	1/22	5/22	13/22
Phase 2				
# live fetuses/litter	15/19			16/16
# implants/dam	16/19			16/16
#corpora lutea/dam	17/22			17/22
Preimplantation loss/dam	1/19			0/16
Postimplantation loss/dam	1/19			0/16

HD females in the Phase 1 study exhibited reduced numbers of live fetuses/litter and reduced number of implants/dam with an increased pre-implantation loss of ova or fetus. The effect was observed to a lesser extent at the LD and MD. These findings were not observed following Phase 2 dosing and recovery.

Reproductive and developmental toxicology summary (current NDA supplement):
Fertility and subsequent numbers of live births were significantly reduced in dams administered 1mg/kg/day ZD 1033 for 2w; the fertility index was depressed 82% compared to a 5% depression in dams administered 0.002 and 0.02mg/kg/day. Reduced number of implants/dam and an increased pre-implantation loss of ova or fetus were also observed at the HD; the effect was observed to a lesser extent at the LD and MD. HD females dosed for 3w and allowed to recover for 5w exhibited recovery of fertility; the fertility index was not significantly reduced compared to controls.

Reproductive and developmental toxicology conclusions (NDA):
Following oral administration of 0.1mg/kg/day in rats and rabbits, ZD 1033 was found to cross the placenta. Administered during the period of organogenesis at doses of 0.1 and 0.02mg/kg/day to rats and rabbits, ZD 1033 increaed pregnancy loss (increased pre- and/or post-implantation loss, increased resorption, and decreased numbers of live fetuses); effects were dose related in rats. Placental weights were significantly increased in rats at doses of 0.1mg/kg/day or more.

Evidence of fetotoxicity, including delayed fetal development (ie., incomplete ossification and depressed fetal body weights), was observed in rats administered 1mg/kg/day; there was no evidence of teratogenicity up to 1mg/kg/day. In rabbits, ZD 1033 caused pregnancy failure at doses equal to or greater than 1mg/kg/day; there was no evidence of teratogenicity in rabbits administered 0.2mg/kg/day.

Labeling recommendations:
*Teratogenicity and fetotoxicity findings indicated under **WARNINGS** section of label unchanged.*

PRECAUTIONS
Impairment of Fertility:

14

Pregnancy: Pregnancy Category *unchanged*

Nursing Mothers: *unchanged*

VIII. SPECIAL TOXICOLOGY STUDIES: NONE

VIII. DETAILED CONCLUSIONS AND RECOMMENDATIONS (CURRENT NDA SUPPLEMENT):

Conclusions:
When rats were pretreated with 25mg/kg (150mg/m^2) ZD 1033 for 2 weeks prior to administration of ZD 1033 (25mg/kg) in combination with tamoxifen (20mg/kg, 120mg/m^2) for 2 weeks, the number of DNA adducts was reduced by >50% compared to adducting by tamoxifen alone. These findings were attributed to enzyme induction of ZD 1033 and the resultant reduction in the number of tamoxifen metabolites in the liver. The thyroid tumors exhibited in male rats administered 25mg/kg ZD 1033 in the 2-year carcinogenicity study may be mechanistically related to an increased clearance of T_4 resulting from enzyme induction, conclusions were based on an assessment of thyroid function in rats administered 25mg/kg (150mg/m^2) ZD 1033 for 30 days. In addition, the increased incidence of liver tumors observed in 25mg/kg/day female rats in the 2-year carcinogenicity study may be the result of a non-genotoxic process of liver induction; conclusions were based on an assessment of hepatocyte proliferation in female rats administered 5 (30mg/m^2) and 25mg/kg (150mg/m^2) ZD 1033 for 7 and 28 days, and include an assessment of enzymatic induction following ZD 1033 administration.

Fertility and subsequent numbers of live births were significantly reduced in dams administered 1mg/kg/day ZD 1033 for 2w; the fertility index was depressed 82% compared to a 5% depression in dams administered 0.002 and 0.02mg/kg/day. Reduced number of implants/dam and an increased pre-implantation loss of ova or fetus were also observed at the HD; the effect was observed to a lesser extent at the LD and MD. HD females dosed for 3w and allowed to recover for 5w exhibited recovery of fertility; the fertility index was not significantly reduced compared to controls.

General Toxicology Issues:
The additional preclinical toxicology studies submitted with the sNDA contributed to the complete ZD 1033 database, but were not required studies with the exception of study TKR/2749. This study was recommended by the division to determine the potential of the ZD 1033/tamoxifen combination to alter the level of DNA adducting in comparison to adducting with tamoxifen alone. Results of the fertility study in rodents are incorporated into the label under **PRECAUTIONS** as indicated below. The **Impairment of Fertility** section of the label has been changed from the proposed label submitted by the sponsor.

Recommendations: The sNDA for ZD 1033 is approvable from a Pharmacology/Toxicology perspective with the label changes as described below.

Labeling with basis for findings:
*Carcinogenicity and mutagenicity findings indicated under **PRECAUTIONS** Carcinogenesis and Mutagenesis sections of label unchanged.*
*Teratogenicity and fetotoxicity findings indicated under **WARNINGS** section of label unchanged.*

15

PRECAUTIONS
Impairment of Fertility:

DRAFT

Pregnancy: Pregnancy Category *unchanged*

Nursing Mothers: *unchanged*

X. APPENDIX/ATTACHMENTS:

Addendum to review: none

Other relevant materials: none

Any compliance issues: none

16

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

/s/

Margaret Brower
8/9/02 12:30:28 PM
PHARMACOLOGIST

John Leighton
8/9/02 12:45:42 PM
PHARMACOLOGIST