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APPROVAL PACKAGE FOR:

APPLICATION NUMBER
21-344

Clinical Pharmacology and Biopharmaceutics Review

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS NDA REVIEW

NDA number, type: NDA 21-344, 1S

Brand name: FASLODEX® Injection

Generic name: fulvestrant

Type of dosage form and strength(s): pre-filled syringes, 2.5 ml (125 mg) and 5 ml (250 mg)

Indication(s): FASLODEX is indicated for the

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Applicant name: AstraZeneca Pharmaceuticals, LP

Submission (letter date): initial (March 28, 2001)
BB (November 13, 2001)
C (December 31, 2001)
BB (January 30, 2002)

OCPB and ORM Division names: Division of Pharmaceutical Evaluation 1 and Division of Oncologic Drug Products

OCPB Reviewer(s) and Team Leader names: Gene Williams, Ph.D. and N.A.M. Rahman, Ph.D.

Type of Submission: New Drug Application (NME)

I. Executive Summary

A. Recommendations

The Clinical Pharmacology and Biopharmaceutics portion of this NDA is acceptable. No new risk management recommendations have resulted from this review.

B. Phase 4 commitments

A single Phase 4 commitments is recommended. We recommend that the Applicant perform a study of the effect of ketoconazole on fulvestrant pharmacokinetics. For ease, to allow for fewer patients (the IV route has less inter-individual variability than the IM route) and to increase safety during performance of the study, we recommend that this study be conducted using the intravenous formulation of fulvestrant.

II. Table of Contents

Glossary

^{14}C – radioactive carbon (molecular weight = 14)
AUC – area under the concentration versus time curve
Cl -- clearance
Cmax – maximum concentration
Cmin – minimum concetration: the concentration just prior to the next dose
Ctrough – minimum concetration: the concentration just prior to the next dose
CV – coefficient of variation
CYP – cytrochrome P450
ER – estrogen receptor
FSH – follicle stimulating hormone
Gmean – geometric mean
HDL – high-density lipoprotein
IM – intra-muscular
in vitro – not in humans or animals
in vivo – in humans or animals
IV – intra-venous
kg – kilogram
L – liter
LA – long-acting
LDL – low-density lipoprotein
LH – lutenizing hormone
mg – milligram
min – minute(s)
ml – milliliter
NDA – New Drug Application
NME – new molecular entity (a molecule not previously approved as a human drug)
OCPB – Office of Clinical Pharmacology and Biopharmaceutics
P450 – cytochrome P450
PD – pharmcodynamic(s); a measure of drug effect
PgR – progesterone receptor
Phase 4 – the post-approval stage of drug development
PK – pharmacokinetic(s)
PK/PD – relating concentration (PK) to effect (PD)
POSTHOC – an analysis option within the NONMEM software program
Q – once every
SA – short-acting
Tmax – time at which maximum concentration (Cmax) is reached or was measured
V – volume of distribution
Vd – volume of distribution of the central compartment
VLDL – very-low-density lipoprotein
Vss – steady-state volume of distribution
μM – micromolar or micromoles

III. Summary of Clinical Pharmacology and Biopharmaceutics Findings

In vivo and *in vitro* data support the following conclusions:

- In clinical use, drug exposure is controlled by the properties of the LA IM injection
    - the ratio of Cmax to Ctrough for a 5 ml IM injection and a 28-day inter-dose interval is approximately 2.5.
    - On a Q 28-day regimen, levels approach approximate steady-state after 3 doses.
    - the pharmacokinetics of fulvestrant 250 mg were shown to be similar when administered as either a single 5-ml or as two 2.5-ml injections.
    - no clear relationship has been established between efficacy measurements (time to progression, objective response) and pharmacokinetic parameters such as Cmax, Cmin, AUC, and clearance.
- The general pharmacokinetics are:
    - fulvestrant is rapidly distributed following administration by IV infusion, with plasma concentrations decreasing rapidly in a multiexponential fashion. Estimates of mean terminal elimination half-lives range from approximately 14.0 to 18.5 hours.
    - fulvestrant is rapidly cleared (>10 ml/min/kg) and renal elimination is low (i.e. <1%).
    - fulvestrant is extensively metabolized.
- No meaningful differences in the pharmacokinetics are apparent between male and either pre- or postmenopausal female subjects following administration of a single IV dose, nor between male and postmenopausal female subjects following IM administration (irrespective of age).
- Fulvestrant has been shown to be highly bound (99%) to plasma proteins (predominantly lipoproteins) and to have a large steady-state volume of distribution (approximately 3 to 5 L/kg), which suggests that the distribution of the compound is largely extravascular.
- Preclinical studies with human cytochrome P450 isoenzymes and results from clinical pharmacokinetic trials involving the co-administration of fulvestrant with midazolam or rifampin suggest that

- therapeutic doses of fulvestrant have no inhibitory effects on cytochrome P450 enzymes

- the clinical pharmacokinetics of fulvestrant are unlikely to be affected by cytochrome P450 inducers.

- There was no apparent effect caused by renal insufficiency or mild hepatic impairment on the pharmacokinetics of fulvestrant. Although data is not available, clearance may be reduced in patients with moderate or severe hepatic impairment.

- No differences were seen in fulvestrant clearance among Black, Hispanic, native Japanese, or White subjects.

- The 17-keto and sulfone metabolites of fulvestrant found in human plasma, and formed in the rat and the dog (but not in the plasma in these species), show no estrogenic activity. Only the 17-keto compound demonstrates a level of antiestrogenic activity of the same order of magnitude as fulvestrant and its activity is 4.5-fold lower than that of the parent compound.

- A variety of pharmacodynamic endpoints were studied. Generally, the results support that fulvestrant is an estrogen receptor antagonist that acts primarily peripherally.

The Table below lists the studies that were present in the NDA. Except for the pharmacokinetic assessments performed in the initial in-patient studies, all of these studies contributed to this NDA review.

	present	absent
Bioavailability and Bioequivalence Studies		
Mass Balance Study	x	
BA Studies		
Absolute BA	x	
Relative BA		x
BE Studies		x
Food-Drug Interaction		x
Dissolution Tests (*In Vitro-In Vivo* Comparison Studies)		x
Studies Using Human Biomaterials		
Plasma Protein Binding Studies	x	
Metabolism Studies Using Hepatocytes, Microsomes, etc.	x	
In Vitro Drug Interaction Studies	x	
Human Pharmacokinetics Studies		
PK, and Initial Safety and Tolerability in Healthy		
Volunteers		
Single Dose		x
Multiple Dose		x
PK, and Initial Safety and Tolerability in Patient		
Volunteers		
Single Dose	x	
Multiple Dose	x	
Dose Proportionality		
Single Dose	x	
Multiple Dose		x
PK in Population Subsets to Evaluate Effects of Intrinsic		
Factors		
Ethnicity		x
Gender		x
Pediatrics		x
Geriatrics		x
Renal Impairment		x
Hepatic Impairment		x
PK to Evaluate Effects of Extrinsic Factors		
Drug-Drug Interaction: Effects on Primary Drug	x	
Drug-Drug Interaction: Effects of Primary Drug	x	
Population PK studies	x	
PK/PD studies in Volunteers	x	
PK/PD studies in patients	x	
Other		
Genotype/Phenotype Studies	x	

IV. Question-Based Review

A. General Attributes

1. What are the highlights of the chemistry and physical-chemical properties of the drug substance, and the formulation of the drug product?

Fulvestrant is 7-alpha-[9-(4,4,5,5,5-pentafluoropentylsulphinyl) nonyl]estra-1,3,5-(10)-triene-3,17-beta-diol.

Fulvestrant is a white powder with a molecular weight of [redacted] The solution for injection is a clear, colorless to yellow, viscous liquid. Each injection contains as inactive ingredients: alcohol, benzyl alcohol, benzyl benzoate as co-solvents, and castor oil as a co-solvent and release rate modifier.

What are the proposed mechanism of drug action and therapeutic indications?

Fulvestrant is an antiestrogenic agent that binds estrogen receptor (ER) in a competitive manner. Preclinical studies show that fulvestrant is a reversible inhibitor of the growth of estrogen-sensitive human breast cancer cells and of tamoxifen-resistant breast cancer cells *in vitro*. In studies with immature female rats, fulvestrant blocks the uterotrophic action of estradiol and the estrogenic (partial agonist) effect of tamoxifen.

FASLODEX is indicated for the [redacted]

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What is the proposed dosage and route of administration?

FASLODEX is supplied in sterile single patient pre-filled syringes containing 50-mg/ml fulvestrant either as a single 5 ml or two concurrent 2.5 ml injections to deliver the required monthly dose. FASLODEX is administered as an intramuscular injection of 250 mg once monthly.

2. What efficacy and safety information (e.g., biomarkers, surrogate endpoints, and clinical endpoints) contribute to the assessment of clinical pharmacology and biopharmaceutics study data (e.g., if disparate efficacy measurements or adverse event reports can be attributed to intrinsic or extrinsic factors that alter drug exposure/response relationships in patients)?

No independent variables (covariates) have been identified by either the Applicant or the Clinical Division that appear to explain efficacy or safety.

B. General Clinical Pharmacology

1. What is the basis for selecting the response endpoints, i.e., clinical or surrogate endpoints, or biomarkers (also called pharmacodynamics, PD) and how are they measured in clinical pharmacology and clinical studies?

The primary efficacy variable upon which approval is based is time to response rate as quantified by radiographic imaging. This response is selected based upon the expectation that it will predict clinical benefit. Other measured responses were chosen based upon fulvestrant's mechanism of action (the drug is an anti-estrogen and estrogen, or lack of it, results in tumor, reproductive tract, endocrine and bone responses).

The clinical pharmacodynamic program comprised included data on the effects of fulvestrant on tumor markers (Trials 0002 and 0018), the female reproductive tract (including endometrial growth) (Trials 0003, 0019, and 0036), endocrinology (Trials 0003, 0004, 0019, 0020, and 0021), and bone resorption (Trial 0019). The endpoints are identified below. None of these endpoints form the basis of approval.

Category	Measurement	Result	Contribution to PD portion of insert
Tumor marker	Ki67 labeling index and/or the apoptotic index (AI) in breast tumor tissue	Ki67 labeling decreased, AI not changed	no
Tumor marker	ER and PgR indices in breast tumor tissue	ER & PgR decreased	yes
Tumor marker	levels of the protein pS2	pS2 appears decreased but statistics not performed	no
Female reproductive tract -- Endometrium	change in endometrial thickness (drug- or disease- induced)	reduction in change of thickness	yes
Female reproductive tract -- Hypothalamic-pituitary-ovarian axis	ovarian volume and/or the presence of ovarian follicles after fulvestrant dosing to premenopausal women.	no effect	no
Female reproductive tract -- Vaginal cytology	1. Karyopyknotic Index, 2. Maturation Value: both measure estrogenization of the vaginal tract	no apparent effect but statistics not performed	no
Female reproductive tract -- Fibroid volume	uterine fibroid volume	no effect	no

Category	Measurement	Result	Contribution to PD portion of insert
Endocrinology	levels of estradiol, progesterone, FSH, LH, Sex hormone-binding globulin (SHBG)	statistics not performed	yes
Bone resorption in premenopausal women	assays of cross-linked N-telopeptide and free deoxypyridinoline	not powered for non-inferiority to placebo, but does show less resorption than with goserelin	no

2. Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships? (if yes, refer to IV. F, Analytical Section; if no, describe the reasons)

Fulvestrant exists as a mixture of 2 diastereoisomers which are epimeric at the sulfur atom in the side chain. In finalizing the drug substance specification, these 2 diastereoisomers were named fulvestrant sulfoxide A and fulvestrant sulfoxide B. These isomers are present in the ratio (A:B) of approximately 45:55.

Preclinical studies have shown no difference between the pharmacokinetic profiles of sulfoxide A and sulfoxide B, and the 2 diastereoisomers were shown to be equally pharmacologically potent in preclinical in vitro models. It was not therefore anticipated that there would be any differences in the pharmacokinetic profiles in man, and the main bioanalytical method measured the diastereoisomers as a mixture for pharmacokinetic analysis. However, to validate this approach, a specific chiral method was used to monitor the concentrations of the 2 diastereoisomers in 3 clinical trials (Trials 0018, 0021 and 0026). The results of Trial 18 are most relevant as the route of administration (IM) and formulation (clinical) was that which is to-be-marketed.

After IM administration of 50, 125, and 250 mg LA fulvestrant in Trial 0018, the data for the 28 days following the injection from 3 patients at each dose level indicated that the 2 diastereoisomers were present in similar proportions at all time points, i.e., with concentration ratios of approximately 50:50. These data suggest that the disposition processes following IV and IM administration are achiral and support the use of total fulvestrant measurements for pharmacokinetic analysis.

In vitro protein binding measurements, using fresh plasma samples from human, obtained with the equilibrium dialysis technique and using [^{14}C]-fulvestrant, provided limited information because of the very low aqueous solubility of fulvestrant. However, as at very high concentrations (10 mg/ml) fulvestrant was highly bound (at least 99%) to plasma proteins.

The *ex vivo* binding distribution of fulvestrant and its metabolites was determined in human plasma obtained at 12, 24, and 96 hours after a single IM dose of [^{14}C]-fulvestrant (samples collected from Trial 0029). [redacted] was used to separate plasma albumin, alpha-acid glycoprotein, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), and chylomicrons. Results showed that lipoproteins appeared to be major binding components in human plasma: LDL 29%, VLDL 27%, HDL 17% at 12 hours. Given the much larger proportion of HDL in plasma, this indicated that HDL was of lesser importance in the binding of fulvestrant or its metabolites. The role of sex hormone-binding globulin in fulvestrant binding, if any, could not be determined because of the extreme instability of reference material in the test system.

No studies were conducted on drug-drug competitive protein binding interactions. No mutual displacement interactions appear to have been reported for other hydrophobic drugs binding to lipoproteins, such as cyclosporin and amphotericin B (Wasan and Cassidy 1998).

Following IV administration of ^{14}C-fulvestrant, 51% (n = 8, range = 37 – 64%) of the AUC of plasma ^{14}C was accounted for by parent drug. Restated, 49% of the circulating radioactivity was in moieties other than fulvestrant. The identity of this radioactivity was not determined. Chromatograms of feces showed more than a dozen peaks with no single component contributing more than approximately 10% of the total area under the sum of the chromatographic areas.

Since less than 1% of the dosed radioactivity had been recovered in the urine after 7 days of collection, metabolite profiling was not carried out on urine samples.

In Trial 0036, plasma levels of the 17-ketone and sulfone metabolites were determined in 3 volunteers after single injections of LA IM fulvestrant 125 or 250 mg. The results showed that plasma concentrations of both metabolites were low in comparison with the parent drug; all samples analyzed were close to or below the LOQ ([redacted]ng/ml and 1 ng/ml for the 17-ketone and sulfone metabolites, respectively).
Similarly, in Trial 0021 after single and multiple injections of LA IM fulvestrant, the plasma concentrations of the 17-ketone and sulfone metabolites of fulvestrant were low and the majority of the samples analyzed fell below the LOQ. Low concentrations of the 17-ketone (typically 2 to 3 ng/ml) were found in some samples after multiple dosing. These results are consistent with the single-dose information generated in Trial 0036 and confirm that the circulating levels of these metabolites do not appear to alter after repeated administration of fulvestrant.

Metabolites resulting from conversions at the 3- and 17-positions of the steroid nucleus to form ketone(s) or sulfate have been synthesized and tested for estrogenic and antiestrogenic activity. None showed any estrogenic activity and only the 17-keto compound demonstrated a level of antiestrogenic activity of the same order of magnitude as fulvestrant: its activity was 4.5-fold less than that of the parent compound. Based on these data, the Applicant concluded that the metabolites of the steroidal part of the fulvestrant molecule are unlikely to contribute in a significant manner to drug activity.

The effect of metabolism of the 17 B-hydroxy to 17-keto, conjugation of the 3 B- and 17 B-hydroxy groups and oxidation of the side chain sulphoxide to sulphone, was tested in the immature rat uterotropic/antiuterotropic assay. The putative sulphone metabolite had no estrogenic activity but had antiestrogenic activity comparable with that of the parent drug. None of the putative metabolites at the steroid 3- and 17-positions had any estrogenic and only the 17-keto compound demonstrated a level of antiestrogenic activity of the same order of magnitude as ICI 182,780, approximately 4.5-fold less than that of the parent drug. The Applicant concluded that oxidation of the side-chain sulphoxide to sulphone could contribute to drug activity, and that the metabolites of the steroidal part of the ICI 182,780 molecule are unlikely to contribute in a significant manner to drug activity.

3. What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy and safety?
a) based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

In studies using different dose levels, maximum plasma concentrations (Cmax) and exposure (AUC(0-28 d)) appeared to increase in a dose-related manner. Formal statistical analyses (ANCOVA) were performed on the AUC summary data from Trial 0018 to assess dose proportionality. This result of this analysis of covariance (ANCOVA) indicated that, across the dose range of 50 – 250 mg, exposure was approximately proportional to dose, i.e., the proportionality coefficient was not statistically different from 1 (at the 5% level).

APPLICANT'S TABLES

Table 8 **Geometric mean (CV%) of $AUC_{(0-28\ d)}$ values at different doses of I.A im fulvestrant in postmenopausal patients (Trial 0018)**

Dose (mg)	$AUC_{(0-28\ d)}$ (ng.d/ml)
50 (n=15)	32.9 (43.3)
125 (n=16)	69.1 (51.0)
250 (n=20)	116 (25.0)

Table 9 **Dose proportionality analysis of AUC (Trial 0018)**

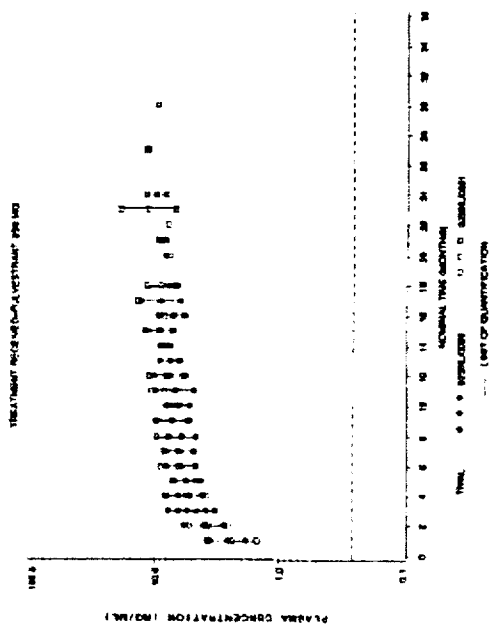
Parameter	Proportionality coefficient estimate	95% CI	p-value
$AUC_{(0-28\ d)}$	0.8833	0.7385, 1.0281	0.1112

b) do PK parameters change with time following chronic dosing?

Based upon trough plasma concentrations (see Figure below), accumulation occurs with Q 28 days dosing, but accumulation-independent PK parameters (clearance, volume of distribution) appear not to change.

APPLICANT'S FIGURE

Figure 9 Geometric mean (SD) trough fulvestrant concentrations (Trials 0021 and 0020)



c) how long is the time to the onset and offset of the pharmacological response or clinical endpoint?

Presumably onset of estrogen receptor binding occurs very shortly after drug administration. The primary clinical efficacy endpoint is response rate (tumor shrinkage) – not a time-related measure.

d) are the dose and dosing regimen consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

The 250 mg dose appears appropriate. Higher doses cannot be used due to volume restrictions (the current dose is a 5 ml IM injection). Lower doses are unlikely to be sufficiently efficacious as the 250 mg dose failed superiority analyses and is being

approved on the basis of non-inferiority. Further, a 125 mg dose was studied early in Phase 3 but was eliminated because efficacy results were not sufficiently promising.

4. How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

a) what are the basic PK parameters?

Following IV administration of 10 mg to healthy postmenopausal women (trial 0038, n=6) the following values were obtained (mean (standard deviation)):

half-life (h)	Vss (L/kg)	Cl (ml/min/kg)
14.4 (3.1)	4.1 (1.6)	11.1 (1.7)

No study directly compared pharmacokinetics between healthy postmenopausal women and postmenopausal breast cancer patients. However, across study comparisons can be made between studies using the to-be-marketed long-acting intramuscular formulation in healthy post-menopausal women (2 studies, one in Japanese women) and in breast cancer patients (6 studies). These data show no definitive difference between these populations:

APPLICANT'S TABLE

Table 7 Pharmacokinetic parameters following a single 250-mg dose of I.A im fulvestrant in healthy postmenopausal volunteers, postmenopausal breast cancer patients, and premenopausal patients with benign gynecologic disease

Trial	$AUC_{(0\text{-}28\ d)}$ (ng.d/ml) Gmean (CV%)	C_{max} (ng/ml) Gmean (CV%)	C_{min} (ng/ml) Gmean (CV%)	t_{max} (days) Median
Postmenopausal volunteers				
0036 (n=10)	176 (34.5)	11.4 (44.8)	2.6 (25.2)	6.0
O-15-11 (n=5)[a]	168 (63.0)	10.6 (4.3)	2.7 (1.1)	7.0
Postmenopausal breast cancer patients				
0004 (n=15)	140 (15.4)	10.5 (1.7)	3.1 (1.2)	7.0
0018 (n=22)	116 (25.0)[e]	7.4 (28.0)	2.1 (28.1)[e]	7.0
0020 (n=16)[b]	148 (45.3)[g]	8.2 (63.8)	2.6 (33.4)[h]	7.0[i]
0021 (n=4)[c]	88.4 (47.3)	4.8 (68.1)	1.9 (23.7)	8.8
0039, 1 x 5 ml (n=20)	107 (69.6)	6.0 (83.2)	2.3 (57.6)	7.0
0039, 2 x 2.5 ml (n=18)	105 (59.3)	6.2 (67.3)[f]	2.1 (41.0)	7.0[f]
Premenopausal benign gynecology patients				
0019 (n=7)[d]	148 (60.1)[j]	8.1 (4.4)	3.3 (4.2)	6.9

[a] For Trial O-15-11 (conducted in Japan), mean and SD presented.
[b] Trial 0020 used the 1 x 5-ml dosing regimen.
[c] Trial 0021 used the 2 x 2.5-ml dosing regimen.
[d] Patients with uterine fibroids.
[e] n=20.
[f] n=17.
[g] n=13.
[h] n=14.
[i] n=15.
[j] n=5.
CV Coefficient of variation.

b) is this a high extraction ratio or a low extraction ratio drug?

No experiments to directly assess clearance across an individual organ were performed. Based upon clearance approximating hepatic blood flow, and low concentration following oral administration (possibly attributable to low oral bioavailability possibly

due to high first pass effect), it can be speculated that fulvestrant is a high extraction drug.

c) does mass balance study suggest renal or hepatic the major route of elimination?

Following IV dosing of ^{14}C, less than 1% of the ^{14}C was recovered in urine. Approximately 80% of the ^{14}C dose was recovered in feces, of which approximately 8% was fulvestrant. It appears that metabolism is the primary route of elimination and that feces is the primary roue of excretion of drug-derived material.

5. What is the inter- and intra-subject variability of PK parameters in volunteers and patients, and what are the major causes of variability?

Intra-subject variability has not been formally assessed. Inter-subject variability is high, as evidenced by the table below which is excerpted from Table 6 (Section 4.2) of the Applicant's Study Report for Trial 0039.

EXCERPTED from APPLICANT'S TABLE

Treatment group	AUC(0-t. 28d)	Cmax	Tmax	Cmin
	(ng d/ml)	(ng/ml)	(d)	(ng/ml)
	Gmean (CV %)	Gmean (CV %)	Median (range)	Gmean (CV %)
2 x 2.5 ml injections, n = 17[a]	105.5 (59.3)	6.17 (67.3)	6.98 (3.0 to 9.1)	2.13 (41.0)

AUC(0-t, 28d) -- Area under the plasma concentration-time curve from time zero to 28 days after injection.
Cmax -- Maximum plasma concentration.
Cmin -- Plasma concentration at 28 days after dosing.
Tmax -- Time to maximum plasma concentration.
[a]n = 18 for Cmax and Tmax.

Attempts to attribute intersubject variability to patients characteristics will be discussed below in the sections "**C. Intrinsic Factors**" and "**D. Extrinsic Factors**."

C. Intrinsic Factors

1. What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) influence exposure and/or response and what is the impact of any differences in exposure on the pharmacodynamics?

The dependency of fulvestrant pharmacokinetics on the effects of various disease states and demographic factors was investigated by collectively analyzing data from the Phase III efficacy trials (Trials 0021 and 0020). Data from a total of 294 subjects after single and multiple doses were analyzed (73 administered 125 mg and 221 administered 250 mg

fulvestrant). Relationships presented below were generated from NONMEM using Bayesian based methodology (POSTHOC). Although the parameter shown in the Figures below is clearance, similar results were obtained for modeled Cmax, Cmin, single dose AUC and steady-state AUC.

Hepatic and renal impairment

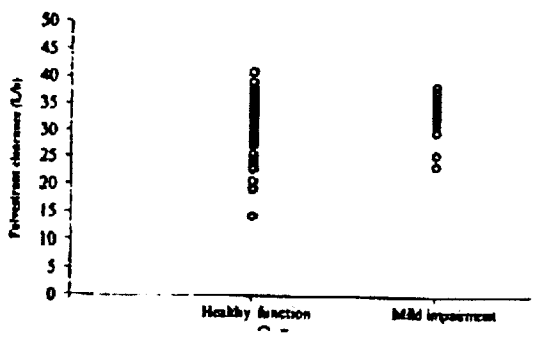
The Applicant sought, and received from FDA, prior commitment that lack of a study in patients with severe hepatic impairment would not be a filing issue. The Applicant's rationale for not performing such a study is that IV drug administration is accomplished via apheresis and that the majority of cirrhotic patients do not meet the apheresis requirements for hematologic factors and negative infectious disease (e.g., hepatitis C).

There were several patients with hepatic or renal impairment at entry to Trials 0021 or 0020. For the purposes of this analysis, mild hepatic impairment was defined as an alanine aminotransferase concentration (at any visit) greater than the upper limit of the normal reference range (ULN) but less than twice the ULN, or if any 2 of the following 3 parameters were between 1- and 2-times the ULN: aspartate aminotransferase, alkaline phosphatase, or total bilirubin. Two hundred sixty-one patients were classified as having normal liver function while 24 had mild impairment. Categorical renal impairment was not defined but fulvestrant kinetics were assessable relative to creatinine clearance in 280 patients.

There was no clear relationship between fulvestrant clearance and hepatic impairment (see Figure, excerpted from the Applicant's Pharmacokinetics Summary, below). The kinetics of the LA IM formulation are controlled by absorption and hence this lack of effect is not surprising.

APPLICANT'S FIGURE

Figure 11 Scatter plot of individual clearance as a function of liver function (categorical assessment) (Trials 0021 and 0020, n=288)

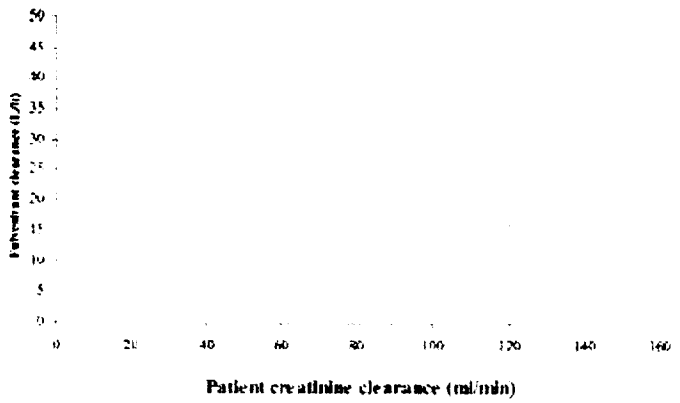


Although there were several patients with low creatinine clearance (<30 ml/min), there was no clear relationship between this parameter and fulvestrant clearance (see Figure,

excerpted from the Applicant's Pharmacokinetics Summary, below). This is consistent with the fact that fulvestrant is eliminated almost entirely by metabolism/biliary excretion. This suggested that clearance was relatively stable in these groups and may be due to the slow release of the compound from the injection site and the capacity of an impaired organ to metabolize in excess of this rate of release.

APPLICANT'S FIGURE

Figure 12 Scatter plot of individual clearance as a function of creatinine clearance (Trials 0021 and 0020, n=294)



Age

As most of the clinical pharmacokinetic data were obtained in postmenopausal female volunteers or patients, a separate trial to evaluate the pharmacokinetics of fulvestrant in the elderly was not conducted. To examine the relationship between fulvestrant concentrations and age, data obtained from the Phase III efficacy trials (Trials 0021 and 0020) were evaluated using a population pharmacokinetic model of data from 294 patients with ages ranging from 33 to 89 years. No clear relationships could be identified between fulvestrant clearance and age (see Figure, excerpted from the Applicant's Pharmacokinetics Summary, below).

APPLICANT'S FIGURE

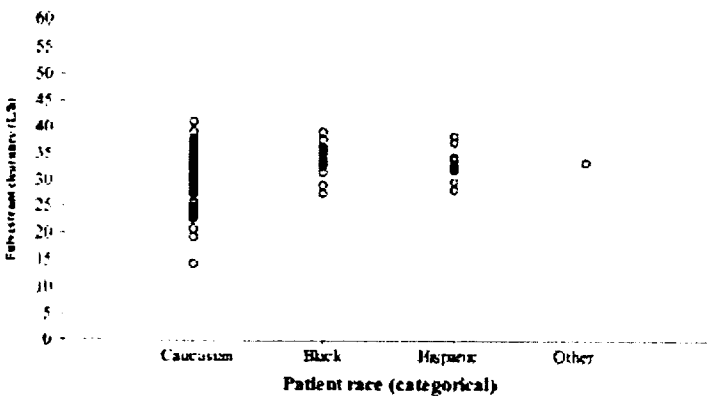
Figure 13 Scatter plot of individual clearance as a function of age (Trials 0021 and 0020, n=294)

Ethnicity

The patient population in Trials 0021 and 0020 included 257 white, 23 black, 13 Hispanic, and 1 Asian subject. The Figure below (excerpted from the Applicant's Pharmacokinetics Summary, below) illustrates that there was no relationship between fulvestrant clearance and race.

APPLICANT'S FIGURE

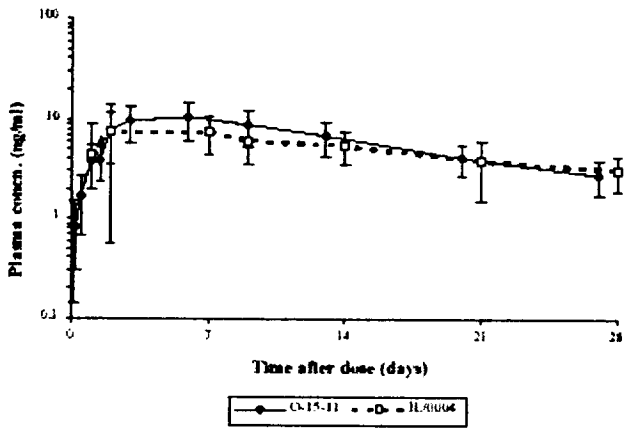
Figure 14 Scatter plot of individual clearance as a function of race (Trials 0021 and 0020, n=294)



In addition to the demographic analysis from Trials 0021 and 0020, pharmacokinetics for 5 female Japanese at the 250-mg dose (Trial O-15-11) were similar to those obtained from Trial 0004, which was conducted in a predominantly white population in the United Kingdom (18 white and 1 black). The mean plasma fulvestrant profiles for Japanese and UK data are illustrated in the Figure below.

APPLICANT'S FIGURE

Figure 15 Comparison of the mean±SD plasma concentration profiles for fulvestrant LA 250 mg in Trials 0-15-11 (n=5) and 0004 (n=15)



Gender

It should be noted that the Indication is gender specific: "...treatment of postmenopausal women...".

The pharmacokinetics of fulvestrant following IV injection were characterized in healthy men and women in Trials 0012 (postmenopausal women only) and 0038 (both pre-and postmenopausal women). Comparison of the data shows little difference between men and postmenopausal women in terms of the pharmacokinetics of fulvestrant. Statistical comparison (ANOVA) of the effect of gender indicated that AUC_{0-8} values obtained for men and postmenopausal women were almost identical, giving a ratio close to 1 (see Table below). There does appear to be little difference in AUC_{0-8} values between men and premenopausal women. However, the sample sizes are small.

APPLICANT'S TABLE

Table 20 Statistical analysis of fulvestrant exposure in men and pre- and postmenopausal women after a single 10-mg iv dose (Trial 0038)

Comparison	$AUC_{0-\infty}$ (ng.h/ml) GLSmean		Estimate of treatment ratio	90% confidence interval
	Men	Women		
Men vs. postmenopausal women (n=8)	231	241*	0.96	0.84 to 1.10
Men vs. premenopausal women (n=8)	231	192	1.20	1.06 to 1.36

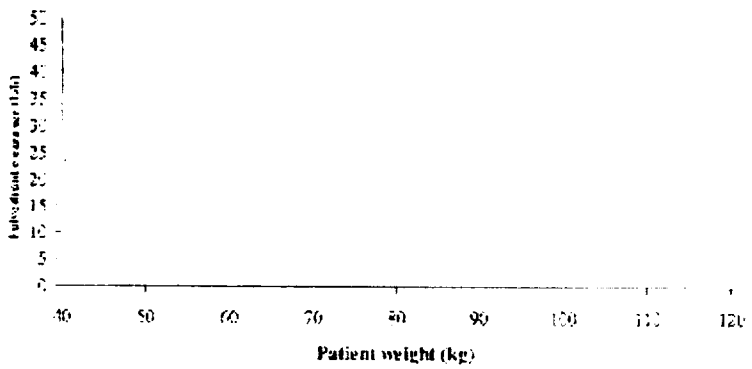
*n=6.

Body weight

No relationship was identified between fulvestrant clearance and body weight in 285 patients ranging from 40 to 127 kg (Figure below).

APPLICANT'S FIGURE

Figure 16 Scatter plot of individual clearance as a function of body weight (Trials 0021 and 0020, n=294)



2. **Based upon what is known about exposure-response relationships and their variability, and the groups studied (volunteers vs. patients); what dosage regimen adjustments, if any, are recommended for each of these subgroups (examples shown below)? If dosage regimen adjustments are not based upon exposure-response relationships, describe the alternative basis for the recommendation.**

No dosage adjustments are recommended. Consistent with the indication (post-menopausal) no pregnancy and lactation use information is in the application.

D. Extrinsic Factors

1. **What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence exposure and/or response and what is the impact of any differences in exposure on pharmacodynamics?**

With the exception of drugs, which will be discussed below, no extrinsic factors were studied.

2. **Based upon what is known about exposure-response relationships and their variability, what dosage regimen adjustments, if any, do you recommend for each of these factors? If dosage regimen adjustments across factors are not based on the exposure-response relationships, describe the basis for the recommendation.**

No dosage adjustments for extrinsic factors are recommended.

3. Drug-Drug Interactions

a) is there an *in vitro* basis to suspect in vivo drug-drug interactions?

Yes. In human liver microsomes fulvestrant appears to be metabolized by CYP 3A4.

b) is the drug a substrate of CYP enzymes?

To investigate which human hepatic cytochrome P450 isoenzymes were involved in the metabolism of [^{14}C]-fulvestrant, the compound (20 mg/ml) was incubated with hepatic microsomal protein (2 mg/ml) in the presence of NADPH. The effects of the selective chemical inhibitors on the metabolism of [^{14}C]-fulvestrant are summarized below. Although the quantitative effects on sulfone and ketone formation are shown, the formation of other unidentified minor metabolites was also markedly reduced by ketoconazole, a selective chemical inhibitor of CYP 3A4. Furafylline, sulfaphenazole, omeprazole, and quinidine, selective chemical inhibitors of CYP 1A2, 2C9, 2C19, and 2D6, respectively, had no obvious inhibitory effect on [^{14}C]-fulvestrant metabolism. There was no consistent effect on ketone formation, which may be catalyzed by steroid keto-reductase, a non-cytochrome-P450 enzyme.

APPLICANT'S TABLE

Table 23 Effect of P450-selective chemical inhibitors on [^{14}C]-fulvestrant metabolism by human liver microsomes

P450	Selective chemical inhibitor	Inhibitor concentration (μM)	Percentage of total drug related material in sample		
			Sulfone	Fulvestrant	Ketone
	Control	0	31.5	31	1
1A2	Furafylline	1	28.5	29.5	3
		5	27.5	28	4.5
		25	30.5	25.5	0.5
2C9	Sulfaphenazole	1[a]	31	30	1
		5[a]	48	41	0
		25[a]	34	28	2
2C19	Omeprazole	6[a]	27	37	2
		12[a]	10	28	2.5
		20[a]	26	28	3
2D6	Quinidine	0.05[a]	24	25	5
		0.1[a]	25	32	4
		1[a]	25	27	4
3A4	Ketoconazole	0.04	28.5	29.5	4
		0.1	27.5	31.5	3.5
		1	15.5	62.5	5

[a] Values are single results.

c) is the drug an inhibitor and/or an inducer of CYP enzymes?

In *in vitro* studies, human hepatic microsomal protein was incubated with selected CYP substrates in the presence of a range of concentrations of fulvestrant (up to 2.0 mg/ml; Study KMX045). Although concentration-related inhibition of some of the enzymes (CYP 1A2, 2C9, and 3A4) was apparent, these effects were minimal, with less than 20%

inhibition occurring at the highest concentration of fulvestrant tested. Fulvestrant would therefore not be expected to cause clinically significant drug interactions through inhibition of cytochrome P450-mediated metabolism of co-administered agents. The effects of fulvestrant on human hepatic cytochrome P450 isoenzymes are summarized below.

APPLICANT'S TABLE

Table 22 Effect of fulvestrant on P450 marker substrate activities (Study KMX045)

Fulvestrant concentration (µg/ml)	Percentage of control enzyme activity				
	Phenacetin O-deethylase (CYP 1A2)	Tolbutamide 4'-hydroxylase (CYP 2C9)	S-mephenytoin 4-hydroxylase (CYP 2C19)	Dextromethorphan O-demethylase (CYP 2D6)	Testosterone 6β-hydroxylase (CYP 3A4)
0.05	104.8	101.2	100.3	99.9	102.5
0.1	100.9	102.7	101.7	86.8	99.6
0.5	104.3	97.1	101.2	100.9	96.7
1.0	97.9	89.3	99.4	100.8	93.7
1.5	96.5	84.2	98.3	97.2	89.1
2	86.3	80.9	95.0	97.1	89.0

d) is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes?

This has not been studied.

e) are there other metabolic/transporter pathways that may be important?

The Applicant emphasizes that the primary elimination pathway is sulfation. With regard to metabolic drug interactions, sulfation is important because, if it is the principle pathway, metabolic interactions with CYP 450 inhibitors would not be expected.

[^{14}C]-fulvestrant was metabolized slowly by human hepatocytes with 41% and 69% of the parent compound remaining after 3 hours in the 2 human liver samples examined. By co-chromatography, the principal component produced by human hepatocytes (up to 25%) corresponded to a metabolite observed in the dog shown to be formed by sulfation of fulvestrant at the 3-hydroxyl group, this metabolite also appeared to be present in the rat sample. The Applicant concludes that in man, fulvestrant is likely to be cleared by a number of metabolic routes but with sulfation representing a principal pathway.

f) does the label specify co-administration of another drug (e.g., combination therapy in oncology) and, if so, has the interaction potential between these drugs been evaluated?

The label does not specify co-administration.

g) what other co-medications are likely to be administered to the target patient population?

Opioid analgesics might be prescribed, particularly for patients with bone metastases.

h) are there any in vivo drug-drug interaction studies that indicate the exposure alone and/or exposure-response relationships are different when drugs are co-administered?

Two drug interaction studies were performed.

Trial 0031 assessed the effect of fulvestrant (short-acting IM formulation) on the kinetics of midazolam. Midazolam is a cytochrome P450 substrate and is an established marker for CYP 3A4 activity, and as such is routinely used to assess CYP enzyme inhibition in clinical trials. The potential for subtherapeutic exposure in patients due to induction has been evaluated in Trial 0024 in which the effects of rifampin, a potent and relatively non-specific inducer of CYP enzymes, on the pharmacokinetics of fulvestrant were assessed.

Trial 0031 was a crossover trial involving male volunteers, randomly allocated to 1 of 2 groups. The first group received a single 36-mg dose of SA IM fulvestrant followed by a 7.5-mg oral dose of midazolam, and then, after a washout phase of 3 weeks, received a second 7.5-mg dose of midazolam. The second group received a 7.5-mg dose of midazolam followed by a washout period of 3 weeks, and then received a 36-mg dose of fulvestrant followed by a second 7.5-mg dose of midazolam.

The 36-mg dose of fulvestrant chosen for this trial maintained plasma concentrations of fulvestrant within the target range between 24 and 48 hours after dosing. These concentrations were similar to those observed with the intended clinical dose and formulation (250 mg of LA IM fulvestrant) in Trial 0018 (7.39 ng/ml at 7 days declining to 2.13 ng/ml at 28 days).Therefore the fulvestrant injection was given 24 hours before the midazolam dose to enable fulvestrant levels to reach a plateau.

As can be seen in the Applicant's Table 24 (re-produced below) fulvestrant increased midazolam AUC by 11% but decreased Cmax by 25%. Thus, consistent with the *in vitro* data, it appears that fulvestrant has little or no effect on metabolism of co-administered CYP 3A4 substrates.

APPLICANT'S TABLES

Table 24 Effect of SA im fulvestrant on the pharmacokinetic parameters of midazolam in healthy male volunteers (Trial 0031, n=7)

Trial treatment	AUC (ng.h/ml) Gmean (CV%)	C_{max} (ng/ml) Gmean (CV%)	t_{max} (h) Median (range)	$t_{1/2}$ (h) Mean (SD)
Midazolam (7.5 mg)	124 (55.0)	51.6 (52.4)	1.00 (0.48 to 1.50)	5.14 (0.90)
Midazolam (7.5 mg) plus fulvestrant (36 mg)	133 (26.7)	38.4 (42.5)	1.00 (0.50 to 1.52)	5.02 (0.77)

Table 25 Analysis results of effect of SA im fulvestrant on AUC for midazolam in healthy male volunteers (Trial 0031, n=7)

Comparison	AUC GLSmean (ng.d/ml)		Treatment effect (ratio)	90% confidence interval
	Midazolam plus fulvestrant	Midazolam		
Midazolam (7.5 mg) plus fulvestrant (36 mg) vs. midazolam (7.5 mg)	136	123	1.11	0.83 to 1.47

Trial 0024 was an open, crossover trial involving male volunteers, randomly allocated to 1 of 2 groups. In the first group, volunteers were given 600 mg rifampin (as 2 x 300-mg capsules), which is the standard therapeutic dose, for 7 days, on the sixth day of which they were also given a single 10-mg iv dose of fulvestrant; following a washout period of 3 weeks, these volunteers were given a second 10-mg dose of fulvestrant. In the second group, volunteers were given a 10-mg iv dose of fulvestrant followed by a washout period of 3 weeks; they were then given 600-mg daily doses of rifampin for 7 days, on the sixth day of which they were also given a10-mg iv dose of fulvestrant.
Fulvestrant was administered as a 10-mg iv dose to ensure that therapeutic plasma concentrations (or above) would be achieved over a sufficient period of time to enable any effects on its pharmacokinetics to be measured. Fulvestrant was given on the sixth day of rifampin dosing.

The pharmacokinetic parameters of fulvestrant, in the presence and absence of rifampin are presented in the Applicant's Table 26 (re-produced below).

APPLICANT'S TABLE

Table 26 Effect of rifampin on pharmacokinetic parameters of iv fulvestrant in healthy male volunteers (Trial 0024)

Trial treatment	$AUC_{(0-t)}$ (ng.h/ml) Gmean (CV%)	C_{last} (ng/ml) Gmean (CV%)
Fulvestrant (10 mg) (n=5)	216 (11.7)	147 (21.6)
Fulvestrant (10 mg) plus rifampin (600 mg) (n=6)	209 (32.1)	146 (17.1)

Gmean rifampin concentrations achieved in this trial were similar to the Cmax levels reported by Kenny and Strates (Ref 1981). Clear evidence of CYP 3A4 induction was confirmed by increases in the urinary excretion of 6 B-hydroxycortisol, which is recognized as a sensitive and specific endogenous marker for increased hydroxylation of

cortisol reflecting the extent of CYP 3A4 induction (Ref Ged et al. 1989). To eliminate any confounding effects due to daily and/or inter-individual variations in cortisol levels, concentrations of free urinary cortisol were also measured and the results were presented as the ratio of 6 B-hydroxycortisol/free cortisol. These results were consistent with published findings that administration of rifampin causes an approximate 3-fold increase in the ratio of 6 B-hydroxycortisol/free cortisol (Ref Ged et al. 1989) and that maximal induction is attained after 4 to 5 doses of rifampin (Ref Borcherding et al. 1992). AUC(0-t) and Cinf were very similar to those seen in previous studies with IV fulvestrant (Trial 0038).

Although suggestive, the rifampin study does not allow for a firm conclusion that co-medication of a strong 3A4 inhibitor with fulvestrant will not alter fulvestrant concentrations. For this reason we recommend a Phase 4 commitment to perform a study of the effect of ketoconazole on fulvestrant pharmacokinetics. For ease, to allow for fewer patients (the IV route has less inter-individual variability than the IM route) and to increase safety during performance of the study, we recommend that this study be conducted using the intravenous formulation of fulvestrant.

i) is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any?

No pharmacodynamic drug interactions have been described.

j) are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions or protein binding?

The possibility of uncharacterized active metabolites cannot be ruled out (see Section IV.B.2. beginning on p. 8 of this review). Protein binding drug interactions have not been performed but the Applicant argues that other drugs highly bound to lipoproteins do not undergo displacement interactions.

4. What issues related to dose, dosing regimens or administration are unresolved, and represent significant omissions?

There are no "significant omissions."

E. General Biopharmaceutics

A single formulation was used in the efficacy and safety trials. The drug is administered as an IM injection only. *In vitro* release rate testing is not required for this parenterally administered drug. For the above reasons there are no biopharmaceutics issues.

It should be mentioned that the two phase 3 efficacy and safety trials performed used different regimens. Study 20 was performed in Europe and used 1X5 ml injection every 28 days. Study 21 was performed in North America and used 2X2.5 ml injection every 28

days. As the figures below demonstrate, while not bioequivalent using 80 – 125 criteria, the two regimens are very similar.

APPLICANT'S TABLES

Table 6 Summary of pharmacokinetic parameters

Treatment group	$AUC_{(0 \text{ to } 28d)}$ (ng.d/ml) Gmean (CV %)	C_{max} (ng/ml) Gmean (CV %)	t_{max} (d) Median (range)	C_{min} (ng/ml) Gmean (CV %)
1 x 5 ml injection (n = 20)	106.8 (69.7)	6.01 (83.2)	7.00 (1.2 to 11.0)	2.32 (57.6)
2 x 2.5 ml injections (n = 17[a])	105.5 (59.3)	6.17 (67.3)	6.98 (3.0 to 9.1)	2.13 (41.0)

$AUC_{(0 \text{ to } 28d)}$: Area under the plasma concentration-time curve from time zero to 28 days after injection.
C_{max} Maximum plasma concentration.
C_{min} Plasma concentration at 28 days after dosing.
t_{max} Time to maximum plasma concentration.
[a] n=18 for C_{max} and t_{max}.

Table 11 Statistical comparison of $AUC_{(0\text{-}28\ d)}$ between a single and divided dose of 250 mg LA im fulvestrant (Trial 0039)

Parameter	GLSmean 1 x 5-ml injection (n=20)	GLSmean 2 x 2.5-ml injection (n=18)[a]	Treatment effect (ratio of GLSmeans)	95% CI	p-value
$AUC_{(0\text{-}28\ d)}$ (ng.d/ml)	106.8	105.5	1.0125	0.6803, 1.5070	0.9497

[a] Data available from 17 patients.
GLSmean Geometric least squares mean.

F. Analytical Section

1. How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?

See Section IV.B.2, p.8.

2. Which metabolites have been selected for analysis and why?

Parent fulvestrant was, for nearly all studies, the only moiety quantitated.

3. For all moieties measured, is free, bound or total measured? What is the basis for that decision, if any, and is it appropriate?

Total drug was measured in all studies. The basis for this decision is likely convenience: fulvestrant is highly protein bound, and there is no data to support that free is a constant fraction of total. Based on what is known, and the principle that free drug will best correlate with activity, measuring free would be recommended. The lack of correlations between concentrations and effects may be a consequence of a lack of correlation between what was measured (total drug) and what was responsible for activity (free drug).

4. What bioanalytical methods are used to assess concentrations?

An overview of the analytical methods is presented below. A more detailed description of the analytical methods used, and their accuracy and precision, for each study which contributes to this review, is included as Appendix E. The analytical methods for each of the studies included in this review has been judged adequate by the Reviewer.

Trial	Method	Analyte	Date of analysis	Appendix E (p. #)
12		Fulvestrant	Dec 1998 to Feb 1999	140
18		Fulvestrant	Feb 1998 to Aug 1999	149
		Diastereomers	May 1999	157
19		Fulvestrant	Sep 1998 to May 1999	160
20		Fulvestrant	Jul 1998 to Jan 2000	170
21		Fulvestrant	Feb 1998 to Jul 2000	178
		Diastereomers	Jul 1998 to Jun 2000	192
		Metabolites	July 2000	196
24		Fulvestrant	Jul 1998 to Jan 2000	199
		Rifampin	Aug to Sep 1999	207
		Cortisol & 6B-OH-cortisol	.	211
26		Fulvestrant	Nov to Dec 1997	216
29		Fulvestrant	Jun to Jul 1999	222
31		Midazolam	Jun to Jul 1999	231
		Fulvestrant	July 1999	236
36		Fulvestrant	Jun to Jul 1999	241
38		Fulvestrant	Dec 1999 to Jan 2000	248
39		Fulvestrant	Dec 1999 to Jan 2000	256
O-15-11		Fulvestrant	Mar to Jul 1998	264
		Diastereomers	Jun to Jul 1998	271

Concentrations of racemic fulvestrant in human plasma were determined using a method based upon [redacted] followed by [redacted] HPLC-[redacted] This method was originally developed by the [redacted] The method has undergone minor changes since Revision 1. The assay and subsequent modifications and cross-validation are described below.

- [redacted] test method [redacted]- Revision 1

The effective date for this method was 4 January 1996.

ICI 182,780 is

Following [redacted] equipped with a high-performance liquid chromatography (HPLC) [redacted] are measured. Quantitation is performed using [redacted]

- Revision 2

During initial sample analysis, there was some evidence of an interfering metabolite co-eluting at a similar retention to fulvestrant in some ex vivo samples. A longer HPLC was used to separate the 2 components and this required
The effective date for this method was 14 February 1996.

- Revision 3

A of the method was performed in July 1998 to assess the ICI 182,780 as an internal standard. The effective date for this method was 6 October 1997.

Human plasma samples were analyzed for ICI 182,780 racemate concentrations according to using a previously validated method (ICI 182,780 and the internal standard were Following equipped with an HPLC The product ion of ICI 182,780 were of the internal standard. Quantitation of the ICI 182,780 racemate was performed using a

- Revision 4

The only change to the method was moving the low QC sample to ng/ml in line with changes to the QC policy. The effective date for this method was 28 August 1998.

- Revision 5

The calibration range of the method was extended at the upper limit of quantification from ng/ml to ng/ml in line with policy changes to assay extrapolation. A of the method was performed and this work is presented in . The effective date for this method was 22 January 2000.

- [redacted] versus AstraZeneca cross-validation

Cross-validation experiments were conducted between [redacted] and AstraZeneca, UK, in September 1997 to establish the assay for use in DMPK, Alderley (formerly Drug Kinetics Group, Zeneca Pharmaceuticals, UK).

[redacted] were exchanged and assayed by the 2 laboratories. Four replicates of each concentration were analyzed on 4 separate occasions at each laboratory and a total of 16 results per sample were obtained.

- AstraZeneca [redacted]

The method first established at AstraZeneca was described in [redacted]
[redacted] The extraction and chromatography conditions were closely based upon [redacted]
[redacted] This method had an effective date of 18 July 1997.

The performance of the analytical method was monitored using [redacted]

V. Detailed labeling recommendations

Are recommending labeling changes are given below. The Applicant's proposed labeling appears in the left column and our revised version appears in the right column.

APPEARS THIS WAY
ON ORIGINAL

29 pages redacted from this section of the approval package consisted of draft labeling

Appendix C. Consult Review (including Pharmacometric Reviews)

Appendix D. Cover Sheet and OCPB Filing/Review Form

APPENDIX E

Analysis of ICI 182,780 Individual Diastereomer Concentrations in Human Plasma

E1 Sample Receipt

A group of samples were selected at specific time points in each dose group and analysed for the individual diastereomers of ICI 182,780, ZM208,926 and ZM208,927. This analysis was performed in three runs carried out between June 1st and July 27th 1998. There were no failed runs and no samples required dilution into the working range of the assay.

E2 Assay Method

E3 Method Validation

The method was originally developed by the [redacted] in December 1995. Information pertaining to method validation in human plasma can be found in study number 182,780 KPV060 (Full GLP validation of a normal phase HPLC [redacted] method for the determination of individual ICI 182,780 diastereomer concentrations in human plasma).

Short-term stability experiments were performed at concentrations of [redacted] ng/ml ICI 182,780 and from this ZM208,926 and ZM208,927 were found to be stable when stored in injection solvent at room temperature for up to [redacted] hours, in human plasma at room temperature for up to [redacted] hours and when subjected to [redacted] freeze thaw. Recovery of the assay was good, exceeding 90% for both diastereomers at all concentrations tested.

There was no evidence of matrix interference found in six different lots of blank human plasma or in any of the following over-the-counter drugs; ibuprofen, salicylic acid, acetominophen, caffeine, phenylpropanolamine, chlorpheniramine, diphenydramine, dextromethorphan, cimetidine, pseudoephedrine and bromphiramine.

E4 Assay Performance

The performance of the analytical method was monitored using quality control (QC) samples which were spiked at concentrations of [redacted] ng/ml ICI 182,780 into control human plasma, yielding concentrations of 1.43, 7.17 and 19.1 ng/ml for the ZM208,926 diastereomer and 1.57, 7.83 and 20.9 ng/ml for ZM208,927 diastereomer. These were sub-aliquotted and stored at approximately -20°C prior to analysis. A minimum of two QC samples from each level (low, medium and high) were analysed in each batch of sample analysis. The run was considered valid if at least one QC at each level and two thirds overall were within ± 20% of their theoretical values.

A summary of typical assay performance taken from the method validation study (182,780 KPV060) conducted at [redacted] is presented in Table E1. This study indicated an assay coefficient of variation of between [redacted] % and accuracy of between [redacted] %. A summary of the assay performance generated during this study is presented in Table E2 together with the individual QC data in Table E3. These results demonstrate good assay performance over the three analytical runs performed in the study.

A typical calibration series is presented in Figure E1 together with representative chromatograms obtained during this trial for calibration standards (blank, LOQ, top standard), QC samples and unknown samples obtained at various time points after drug administration which are presented in Figures E2 to E10. Please note that chromatograms for ZM208,926 and ZM208,927 are presented without internal standard.

Table E1 ICI 182,780 Japanese Phase I Study. Assay performance during method validation at [redacted] (study number 182,780 KPV060)

Nominal concn. (ng/ml)	Mean concn. (ng/ml)	Intra-batch CV(%)	Inter-batch CV(%)	Total CV (%)	Accuracy (%)	n
			ZM208,926			
1.40	1.46					11
7.02	7.18		[redacted]			11
18.7	18.5					11
			ZM208,927			
1.60	1.66					11
7.98	8.05		[redacted]			11
21.3	20.4					11

Table E2 ICI 182,780 Japanese Phase I Study. Assay performance of ICI 182,780 during Japanese Phase I Study

Nominal concn. (ng/ml)	Mean concn. (ng/ml)	Intra-batch CV(%)	Inter-batch CV(%)	Total CV (%)	Accuracy (%)	n
			ZM208,926			
1.43	1.41					6
7.17	7.32		[redacted]			7
19.1	18.9					6
			ZM208,927			
1.57	1.65					7
7.83	7.87		[redacted]			7
20.9	20.5					6

NC Not Calculated
CV Coefficient of Variation

Table E3 ICI 182,780 Japanese Phase I Study. Individual QC data generated during analysis of individual diastereomers

	ZM208,926		
Analysis date	QCA (1.43 ng/ml)	QCB (7.17 ng/ml)	QCC (19.1 ng/ml)
06.01.98			
07.16.98			
07.02.98			
	ZM208,927		
	QCA (1.57 ng/ml)	QCB (7.83 ng/ml)	QCC (20.9 ng/ml)
06.01.98			
07.01.98			
07.27.98			

NR No result

* Not included in calculations

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/s/

Gene Williams
2/12/02 01:17:50 PM
BIOPHARMACEUTICS

Atiqur Rahman
2/12/02 02:46:23 PM
BIOPHARMACEUTICS