CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

202379Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Zytiga (abiraterone acetate)

Date: April 19, 2011
To: File for NDA 202379
From: John K. Leighton, PhD, DABT
Associate Director for Pharmacology/Toxicology
Office of Oncology Drug Products

I have examined pharmacology/toxicology supporting review and labeling for abiraterone acetate provided by Dr. Aziz and the Team Leader memorandum provided by Dr. Dorsam. I concur with their conclusions that Zytiga may be approved for the proposed indication and that no additional nonclinical studies are needed.

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/s/

JOHN K LEIGHTON
04/19/2011

MEMORANDUM

Date: April 19, 2011
From: Robert T. Dorsam, Ph.D.
Acting Pharmacology/Toxicology Team Leader
Division of Drug Oncology Products, HFD-150
To: File for NDA 202379
Abiraterone Acetate
Re: Approvability of Pharmacology and Toxicology

Non-clinical studies characterizing the pharmacology and toxicology of abiraterone acetate in support of NDA 202379 for the treatment of patients with metastatic castration resistant prostate cancer were reviewed by Toxicologist Robeena M. Aziz MPH, Ph.D. These studies investigated the pharmacology, safety pharmacology, pharmacokinetics and toxicology of abiraterone in non-clinical species using the oral route to support the oral administration of abiraterone acetate in males with advanced prostate cancer. Published literature from both the sponsor and others has also been reviewed in support of this application.

The pharmacology studies for abiraterone acetate indicate that this compound dose dependently and irreversibly inhibits CYP17, which has 17-hydroxylase activity and C17, 20 lyase activity. *In vivo* data demonstrate that abiraterone acetate decreased testosterone levels and produced secondary effects on androgen-sensitive male and female reproductive organs. These data support the pharmacologic classification of abiraterone acetate as a CYP17 inhibitor which is reflected in the drug label.

The general toxicology of abiraterone acetate was assessed in the rat and monkey. Target organs identified in these studies include the liver, adrenal glands, eye (26-week rat study) as well as androgen-sensitive organs in the male and female reproductive systems. Notably, suppression of testosterone was only present in the high dose (2000 mg/kg/day) males in the 13-week study on Week 13, however significantly reduced testosterone levels were observed in all test-article-treated males on week 3 and 39 of the 39-week study in monkeys. Drug exposure in these studies approximates the clinical exposure and appears to have adequately characterized the nonclinical toxicities associated with abiraterone acetate and the secondary effects of androgen depletion. ECG assessments in the toxicology study and safety pharmacology studies did not identify cardiac functional abnormalities associated with the administration of abiraterone acetate. Pulmonary and gastric functions were not significantly impaired in the studies provided in support of this application.

The genetic toxicity of abiraterone acetate, its metabolite abiraterone, and several impurities were adequately assessed by *in vitro* and *in vivo* studies and I concur with Dr. Aziz's assessment that these studies do not identify a genotoxic potential for abiraterone acetate. Given the short life expectancy of the indicated patient population, assessments of carcinogenicity were not performed. If the sponsor proposes to extend their indication to a less refractory population that has a longer life expectancy, a need for

carcinogenicity studies should be revisited. In addition, the effects of abiraterone acetate on embryofetal development and toxicity were not assessed in NDA 202379 because the proposed indication is for patients with (b) (4) metastatic prostate cancer. While this is acceptable given the current indication, assessments of embryofetal development and toxicity may need to be addressed if the sponsor plans to gain marketing approval for abiraterone acetate in other patient populations.

Recommendations: I concur with Dr. Aziz's conclusion that the submitted pharmacology and toxicology studies support the approval of abiraterone acetate for the treatment of (b) (4) metastatic prostate cancer. Additional assessments of carcinogenicity or reproductive toxicity may be necessary to support alternate indications.

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/s/

ROBERT T DORSAM
04/19/2011

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

NDA NUMBER:	**202, 379**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**12/20/2010**
PRODUCT:	**Abiraterone acetate (ZYTIGA)**
INTENDED CLINICAL POPULATION:	**Metastatic prostate cancer**
SPONSOR:	**Centocor Ortho Biotech, Inc.**
DOCUMENTS REVIEWED:	**Electronic Submission**
REVIEW DIVISION:	**Oncology Drug Products**
PHARM/TOX REVIEWER:	**Robeena Aziz, MPH, PhD**
PHARM/TOX SUPERVISOR:	**Robert Dorsam, PhD (Acting)**
DIVISION DIRECTOR:	**Robert Justice, MD, MS**
PROJECT MANAGER:	**Amy Tilley**

Date of review submission to Document Archiving, Reporting, and Regulatory Tracking System (DARRTS): **4/19/2011**

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability
Approvable. The non-clinical studies with abiraterone acetate support the safety of its use in metastatic prostate cancer.

B. Recommendation for nonclinical studies
No additional non-clinical studies are required for abiraterone acetate.

C. Recommendations on labeling
The recommendations to the sponsor's proposed labeling are given, with a detailed report regarding the rationale for the recommended changes, in a subsequent review.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The nonclinical findings have shown the target sites of toxicity with abiraterone acetate (CB7630 or JNJ-589485-AAA) to be the liver, adrenals, male and female reproductive organs, male mammary gland (26 and 39-week rat and monkey studies), pituitary (rats only), and eye (26-week rat study). Many of these toxicities are seen clinically and appear to be direct effects of the androgen depletion resulting from the pharmacology of abiraterone acetate.

Two different formulations of abiraterone acetate as well as abiraterone were shown not to be mutagenic or clastogenic in the *in vitro* assays. Abiraterone acetate and abiraterone was not clastogenic (induction of micronuclei) in the *in vivo* rat micronucleus assay at the highest dose tested, 2000 mg/kg (≈12,000 mg/m^2).

Due to the patient population, embryo fetal development studies were not conducted and are not necessary to support the safety of abiraterone acetate for the proposed metastatic cancer indication in males. However, based on studies in rats (13- and 26-week studies) and monkeys (13- and 39-week studies), male and female reproductive organs were a target organ of toxicity therefore administration of this drug may impair reproduction.

B. Pharmacologic activity

Abiraterone is the active metabolite of abiraterone acetate *in vivo*. The major pharmacological activity of abiraterone is to selectively inhibit the enzyme 17α-hydroxylase/C17,20-lyase or P45017α (CYP17). CYP17 is expressed in testicular, adrenal and prostatic tumor tissues and is required for androgen biosynthesis. CYP17 catalyzes two sequential reactions that are rate limiting to

the biosynthesis of androgens: 1) the conversion of pregnenolone and progesterone to their 17α-hydroxy derivatives by 17α-hydroxylase activity and 2) the subsequent formation of dehydroepiandrosterone (DHEA) and androstenedione, respectively, by C17, 20 lyase activity. DHEA and androstenedione are androgens and are precursors of testosterone. Inhibition of CYP17 by abiraterone can also result in increased mineralocorticoid production by the adrenals.

The inhibitory effect of abiraterone on human and rat CYP17 activity has been reported in published literature. Some variations in the data were reported due to be related to experimental conditions used by different laboratories. Overall, the *in vitro* activity of abiraterone is consistent with the irreversible inhibition of CYP17. Abiraterone acetate also inhibited CYP17 activity, however, to a lesser extent compared to abiraterone.

The *in vivo* effects of abiraterone acetate on the circulating hormone levels and organ weights were investigated in mice and rats. Abiraterone acetate decreased the growth of androgen dependent organs (prostate, seminal vesicles, adrenals, and testes) and suppressed plasma testosterone levels at 39.2 mg/kg/day (both mice and rats) and 196 mg/kg/day (mice only) when given i.p. daily for 14 days (>90%). When given orally to rats at 50 mg/kg/day and for a shorter time frame (daily x 3 days), abiraterone acetate was less effective at decreasing testosterone levels (40%).

The two major circulating human metabolites of abiraterone acetate, abiraterone sulphate and *N*-oxide abiraterone sulphate also inhibited CYP17 in rat testes, however, the inhibition was considered weak in comparison to abiraterone in human adrenocortical carcinoma cell lines (IC_{50}= 3.6 and 26.2 μM, respectively). The two compounds also inhibited androstenedione (IC_{50}= 0.85 and 1.3 μM, respectively) and testosterone formation (IC_{50}= 0.73 and 2.9 μM, respectively) and to a lesser extent cortisol formation (IC_{50}= 2.8 and 6.2 μM, respectively). Both metabolites had no effect on aldosterone formation. In contrast, abiraterone concentration-dependently blocked aldosterone formation with an IC_{50} of 2.7 μM.

The recommended pharmacological classification of abiraterone acetate is a CYP17 inhibitor. This classification captures the inhibitory activity of abiraterone towards CYP17 which has both 17-α hydroxylase and C17, 20 lyase activities, and also differentiates it from other androgen-lowering therapeutics that have a designated pharmacologic classification that is specific to the enzyme which it targets.

C. Nonclinical safety issues relevant to clinical use

The nonclinical safety issues observed in the toxicology program with abiraterone acetate included toxicities in the liver, adrenals, male and female reproductive system, male mammary glands (26-and 39-week rat and monkey studies), pituitary (rats only), and eye (26-week rat study only). Most toxicities are related

to the related to interference of abiraterone acetate with the steroid metabolism due to selective CYP17 inhibition.

Abiraterone acetate had no remarkable findings on the CNS when administered male rats as a single oral dose up to 400 mg/kg/day (2400 mg/m^2). Abiraterone had no physiologically relevant effect on the membrane K+ current (Ikr) in hERG-transfected HEK293 cells up to a concentration of 27 µM. The IC_{50} for abiraterone acetate was 12.2 µM. As abiraterone acetate is rapidly converted *in vivo* to abiraterone, the hERG inhibition by abiraterone acetate is considered of limited physiological relevance. ECG waveforms obtained for monkeys were, however, within normal limits and there was no significant change in QTc when dosed up to 2,000 mg/kg/day (24000 mg/m^2). These studies suggest that abiraterone acetate does not have an acute adverse effect on cardiovascular function.

Radio-labeled abiraterone (JNJ-589485) is highly bound (≥99.8%) to proteins in plasma across multiple species (mouse, rat, rabbit, monkey and man) with no relevant species differences occurring. Furthermore, in human plasma, abiraterone was primarily or exclusively bound (≥89%) to both human serum albumin (HAS) and human α1-acid glycoprotein (α1-AGP) at a concentration of 1750 ng/mL.

In vitro studies using human hepatocytes that were incubated with abiraterone acetate showed a slight inhibition of CYP3A4 activity and no effect on CYP2C9. In studies with human microsomes, abiraterone acetate and abiraterone strongly inhibited CYP1A2 and CYP2D6 while CYP2C9 and CYP3A4/5 were moderately inhibited. Both abiraterone acetate and abiraterone did not inhibit CYP2A6 activity. Abiraterone acetate strongly inhibited CYP2C19 and moderately inhibited CYP2E1 while abiraterone did not inhibit CYP2E1 but moderately inhibited CYP2C19.

In vitro data have showed that CYP3A4 is involved in the formation of many *in vitro* Phase 1 metabolites of abiraterone. Hydrolysis of abiraterone acetate to abiraterone is not CYP-mediated but the result of esterase activity. SULT2A1 is involved in the formation of abiraterone sulphate, a major human *in vitro* and *in vivo* metabolite of abiraterone acetate while UGT1A4 and to some extent UGT1A3 are involved in the formation of Phase 2 glucuronidated metabolites.

In rats, the excretion of radio-labeled abiraterone in urine was limited (< 2 % of the administered dose), and almost all radio-labeled abiraterone (89.9-93.4% by 168 h post-dose) was recovered in the feces. There was no difference in routes and rates of excretion between male and female rats. In bile duct-cannulated rats, 18.7 % of the administered radio-labeled abiraterone was excreted in bile over 120 h. For all excretion routes, almost all radio-labeled abiraterone was excreted within 24 h. In man, the majority of radiolabeled material excreted occurred predominantly (87.9%) in the feces, in which the main compound was

unchanged abiraterone acetate. About 5 % of administered radio-labeled abiraterone was recovered from the urine.

In both 13-and 39-week monkey studies, the liver was one of the major organs affected when abiraterone acetate was given for a longer duration (39 weeks). At 13-weeks, bile duct hyperplasia was evident at all dose levels in both males and females ranging from minimal changes at 250 mg/kg/day (3000 mg/m^2) to slight to moderate changes at ≥750 mg/kg/day (≥9000 mg/m^2). At 39-weeks, bile duct/oval cell hyperplasia was seen at all dose levels without a clear dose-related effect and males were affected to a greater extent compared to females (due to gender differences in exposure). The bile duct hyperplasia was slightly less in severity compared to the 13-week study. After 4 weeks of recovery, a lower incidence and severity of this lesion was observed. At both 13-and 39-weeks, increased liver weights were observed at ≥250 mg/kg/day (≥3000 mg/m^2) and were partially to fully reversible after recovery. The increase in liver weights from both studies were directly associated with histological correlation of bile duct/oval cell hyperplasia. Abiraterone acetate resulted in a consistent but not dose-related increase in serum ALP. This increase ranged up to 5-fold at 500 mg/kg/day (6000 mg/m^2) by Week 26 but decreased to 2-fold by Week 39. In 13-week studies, ALP levels were also elevated at 750 mg/kg/day (9000 mg/m^2 and in females only). However, this increase was not as significant. Total bilirubin also increased 6-fold by 13-weeks at 2000 mg/kg/day (24000 mg/m^2) and 2-fold by 39-weeks at 1000 mg/kg/day (12000 mg/m^2). However, at 13-weeks, bile duct hyperplasia was seen at lower dose levels than serum ALP increases.

In 39-week studies with monkeys, the male and female reproductive systems were another target of abiraterone acetate treatment. In males, microscopic findings were found in the testes (atrophy of the spermatogenic epithelial lining of testicular tubules and interstitial cell hyperplasia), epididymides (aspermia or hypospermia), seminal vesicles (increased mineralization), and prostate (epithelial atrophy) at ≥250 mg/kg/day (≥3000 mg/m^2). Many of these were related to changes in hormones including decreases in testosterone levels. In females, histological findings were noted in the vagina (epithelial atrophy), uterus (endometrial pseudodecidual change) and ovaries (follicular cysts). The effects in the ovaries can be related to decreased estrogen while the changes in the uterus correlate with the abiraterone acetate-induced increase in serum progesterone. Mucosal atrophy in the vagina was related to a decrease in DHEA. Atrophy of male and female reproductive organs were also observed in rats starting at ≥50 mg/kg/day (≥300 mg/m^2) at both 13-and 39-weeks.

In the mammary gland of male monkeys, epithelial hyperplasia with fibrosis and edema was seen at all dose levels (≥250 mg/kg/day or ≥3000 mg/m^2) up to 13 weeks of treatment. Male mammary gland atrophy was seen in rat studies at ≥50 mg/kg/day (≥300 mg/m^2) for the same duration. This finding was coupled with increases in progesterone at ≥250 mg/kg/day (≥3000 mg/m^2). In longer term studies in rats and monkeys (26-and 39-week), no changes in the male mammary gland occurred despite elevated serum progesterone levels.

In monkeys, a consistent decrease in serum cortisol, associated with increased adrenocorticotropic hormone (ACTH) levels, was seen at both 13- and 39-weeks. This is consistent with the pharmacological activity of abiraterone acetate. Increases in ACTH hormone concentrations corresponded with brown discolorations of the adrenal cortex, increased adrenal weights, and microscopic evidence of adrenocortical hypertrophy in both males and females at all dose levels (≥250 mg/kg/day; ≥3000 mg/m^2).

In the 26-week rat study, a dose-related incidence of cortical cataracts occurred in males at ≥50 mg/kg/day (≥300 mg/m^2) and females at ≥150 mg/kg (≥900 mg/m^2). There was a greater incidence of cataracts in males compared to females at the high dose of 400 mg/kg/day (2400 mg/m^2). The increased incidence may be attributed to elevated abiraterone exposure in males. Histological evaluation of the lens revealed lens fiber swelling, which appeared to precede the formation of cataracts. No abnormal ocular findings were observed in monkeys at both 13- and 39-weeks.

Both abiraterone and abiraterone acetate were not mutagenic or clastogenic in the *in vitro* assays. Abiraterone and abiraterone acetate was not clastogenic (induction of micronuclei) in the *in vivo* rat micronucleus assay at the highest dose tested (2000 mg/kg; 12,000 mg/m^2).

Impurities of abiraterone acetate (b) (4) (b) (4) (b) (4) were present at low concentrations in one or more of the drug substance batches tested in toxicology studies. Both *in vitro* assays (Ames and the chromosomal aberration assay in mammalian cells) showed no evidence of mutagenic activity when abiraterone acetate was spiked with the 5 impurities at nominal levels (to give a total of (b) (4) impurities – See Table below). When *a*biraterone acetate spiked with 5 impurities was administered to rats at 40 and 400 mg/kg/day daily for 1 month (oral gavage), no toxicologically relevant differences between groups dosed with five impurities and the groups without impurities for all examined parameters.

Due to the structure of these impurities (b) (4) (b) (4) (b) (4) impurities had a structural alert for genotoxicity. The (b) (4) (b) (4) (b) (4) ((b) (4) was present at (b) (4) (b) (4) in one of the drug substance batches used in the 13-week rat and 13-week monkey study and at (b) (4) (b) (4) in one of the batches used in the 26-week rat and 39-week monkey study. Both (b) (4) impurities (b) (4) were not mutagenic *in vitro* (Ames) while the (b) (4) (b) (4) (b) (4)) did not produce any mutagenicity alerts in DEREK. The (b) (4) (b) (4) (b) (4) was also not clastogenic in an *in vitro* chromosome aberration assay. In a 28-day repeat dose toxicity study in rats, the toxicity profile of abiraterone acetate was similar in absence and presence of 5 spiked impurities which included the (b) (4) impurity. The (b) (4) (b) (4) ((b) (4) was mutagenic when tested *in vitro*. However, the level of these impurities in the drug substance is lower (b) (4) than the acceptable daily

intake of genotoxic impurities defined in the draft Guidance for Industry: Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches for products administered for ≥12 months (1.5 µg/day) and therefore appears to be at acceptable levels in the drug product.

There were no reproductive toxicology studies submitted with abiraterone acetate. However, studies with rats and monkeys show that most reproductive organs were affected. An effect on fertility and embryo fetal development was not assessed, however, an effect cannot be ruled out. Based on the proposed patient population, mechanism of action and toxicology data provided in this application, pregnancy category X is recommended.

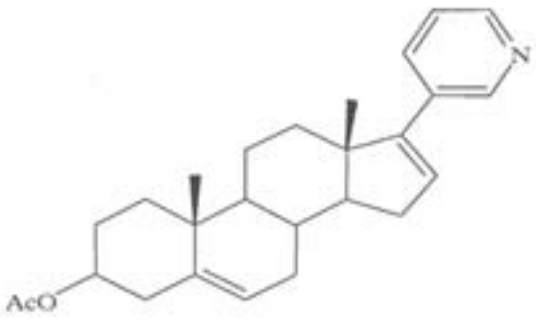
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 202, 379
Review number: 1
Sequence number/date/type of submission: 000/Dec. 20, 2010/Electronic
Information to sponsor: Yes () No (X)
Sponsor and/or agent: Centocor Ortho Biotech, Inc
Manufacturer for drug substance: Patheon, Inc.
Ontario, L5N 7K9, Canada
Reviewer name: Robeena M. Aziz, MPH, PhD
Division name: Division of Drug Oncology Products
Review completion date: April 1, 2011

Drug:

Trade name: ZYTIGA
Generic name: abiraterone acetate (prodrug); abiraterone (drug)
Code name: CB7630; JNJ-212082-AAA
Chemical name: 3β-Acetoxy-17-(3-pyridyl) androsta-5, 16-diene
CAS name: 154229-18-2
Molecular formula/molecular weight: $C_{26}H_{33}NO_2$/391.5 g/mol
Structure:



Relevant INDs/NDAs/DMFs: IND 71, 023

Drug class: CYP17 inhibitor

Intended clinical population: Metastatic (b) (4) (metastatic castration-resistant prostate cancer [mCRPC]) in patients who have received prior chemotherapy containing a (b) (4)

Clinical Formulation: Abiraterone acetate 250-mg tablets have been developed as an orally administered, immediate-release uncoated tablet. The tablet is white to off-white oval shaped debossed with 'AA250'.

Table 1: Composition of Abiraterone Acetate 250-mg Tablet (Formulation JNJ-212082-AAA-G-002)

Component	Reference to Quality Standard[a]	Function	mg/tablet
Abiraterone Acetate	Company Standard	Active	250.00
Lactose Monohydrate	NF/Ph. Eur.		(b) (4)
Microcrystalline Cellulose	NF/Ph. Eur.		
Croscarmellose Sodium	NF/Ph. Eur.		
Povidone (b) (4)	USP/Ph. Eur.		
Sodium Lauryl Sulfate	NF/Ph. Eur.		
Colloidal Silicon Dioxide	NF/Ph. Eur.		
Magnesium Stearate	NF/Ph. Eur.		
(b) (4)	USP/Ph. Eur.		
Total Tablet Weight:			715.0

[a] Where multiple compendia are listed, the compendium applied is specific to the applicable region of the submission.

(b) (4)

NA = Not applicable

[Table excerpted from sponsor]

Route of administration: Oral

Starting dose: 1000 mg/day

Frequency: Daily

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

Pharmacology

Primary Pharmacodynamics:

Study No.	Title
N/A	Novel steroidal inhibitors of human cytochrome P45017, (l7α-hydroxylase-Cl7,20-lyase): potential agents for the treatment of prostatic cancer
N/A	The 16, 17-double bond is needed for irreversible inhibition of human cytochrome P45017-α by abiraterone (17-(3-pyridyl)androsta-5,16-dien-3*β*-ol) and related steroidal inhibitors
N/A	Effects of novel 17α-hydroxylase/C17, 20-lyase (P450, CYP17) inhibitors on androgen biosynthesis *in vitro and in vivo*
N/A	Novel steroidal pyrimidyl inhibitors of P450 17 (l7α-hydroxylase-Cl7,20-lyase)
N/A	Pharmacology of novel steroidal pyrimidyl inhibitors of P450 17 (17α-hydroxylase/C17-20 lyase)
N/A	*In vitro and in vivo* models for the evaluation of potent inhibitors of male rat l7α-hydroxylase-Cl7,20-lyase
8655156	*In Vitro* Pharmacology: Steroids Nuclear Receptor Binding Assay. Study of Four Compounds
8655175	*In Vitro* Pharmacology: Human PR Receptor Binding and Functional Assays Study of JNJ-589485-AAA and JNJ-48171188-AAA
2010ORB001	Rat CYP 17 inhibition by JNJ-48171188-AAA, JNJ-49029578-AAA and JNJ-589485-AAA against ketoconzole. Steroid formation in NCl-H295R cells upon exposure to JNJ-48171188-AAA, JNJ-49029578-AAA and JNJ-589485-AAA against ketoconzole

Pharmacodynamic drug interactions:

Study No.	Title
400378	*In Vitro* Evaluation of CB7630 as an Inducer/Suppressor of CYP1A2, 2C9 and 3A4 Expression in Primary Cultured of Human Hepatocytes
400379	*In Vitro* Evaluation of CB7598 and CB7630 as Potential Inhibitors of Human Cytochrome P450 Enzymes
FK7476	Study on the possible induction and/or inhibition of hepatic drug metabolising enzymes by JNJ-212082 in male and female Sprague Dawley rats after oral administration by gavage for 1 month at doses of 0, 40 and 400 mg/kg body weight/day (TOX9587)

Safety Pharmacology

Study No.	Title
TOX9587	1-month Repeated Dose Oral Toxicity Study of JNJ-212082-AAA in the Rat with integrated Irwin observations (impurity qualification).
8210847	Respiratory and Safety Pharmacology Evaluation Using Head-Out Plethysmography of CB7630 following oral gavage administration to rats
1632-1	An acute gastric irritation study in the male mouse, a single dose

Study No.	Title
	toxicity study in the male rat, a multiple dose toxicity study in the male rat and toxicokinetic study in the male rat.
692409	A Pharmacological Assessment of the Effect of CB7630 on the Cardiovascular System of the Cynomolgus Monkey Using Telemetry
071018	Effects of Abiraterone and Abiraterone Acetate on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells

Pharmacokinetics

Absorption:

Study No.	Title
8202265	Determination of the Apparent Permeability of Abiraterone and Abiraterone Acetate through Caco-2 Cell Monolayers and Evaluation as P-glycoprotein Substrates and Inhibitors
(b) (4)-001	Pharmacokinetics of abiraterone in the mouse
05-501648	Pharmacokinetic modeling of CB7630 and its metabolites CB7598 following oral and intravenous administration of various formulations of CB7630 to cynomolgus monkeys

Distribution:

Study No.	Title
FK7603	The binding of ^{14}C-abiraterone to proteins in plasma from mouse, rat, rabbit, monkey and man and to human serum albumin (HSA) and α1-acid glycoprotein (α1-AGP).
FK7448	The protein binding of ^{14}C-JNJ-589485 in human pre-dose plasma of subjects of a phase 1 single dose open-label pharmacokinetic study (COU-AA-11).

Metabolism:

Study No.	Title
FK7532	Structural characterization of metabolites of abiraterone acetate in selected monkey plasma samples form (b) (4) Study No. 7777-103
BA1732	Comparison of the exposure of selected metabolites of JNJ-212082 (abiraterone acetate) between human and animal species used for safety evaluation of JNJ-212082

Toxicology

Study No.	Title
7777-101	13-week oral gavage toxicity and toxicokinetic study with CB7630 in monkeys with a 4-week recovery period
7777-103	39-week oral gavage toxicity and toxicokinetic study with CB7630 in monkeys with a 4-week recovery period

Special Toxicology

Impurity Studies:

Study No.	Title
TOX9589	JNJ-212082-AAA spiked with (b) (4) impurities: Bacterial Reverse

Study No.	Title
	Mutation Test
8200783	Bacterial reverse mutation assay with a confirmatory assay
TOX9597	*In Vitro* Bacterial Reverse Mutation Test with (b) (4) in *Salmonella typhimurium*
TOX9598	*In Vitro* Bacterial Reverse Mutation Test with (b) (4) in *Salmonella typhimurium*
TOX9599	*In Vitro* Bacterial Reverse Mutation Test with (b) (4) in *Salmonella typhimurium*
TOX9725	*In Vitro* Bacterial Reverse Mutation Test with (b) (4) in *Salmonella typhimurium*
TOX9602	*In Vitro* Mammalian Chromosome Aberration Test with JNJ-212082-AAA spiked with (b) (4) impurities in Human Lymphocytes
TOX9587	1-month Repeated Dose Oral Toxicity Study of JNJ-212082-AAA in the Rat with integrated Irwin observations (impurity qualification).

Studies previously reviewed within IND 71, 023:
Pharmacokinetics

Study No.	Title
400380	Metabolite profiling of abiraterone acetate in cryopreserved hepatocytes from humans
8214362	Identification of metabolites of abiraterone acetate in selected human plasma samples
8282443	Pharmacokinetics, distribution, metabolism and excretion of radioactivity following oral administration of 14C- abiraterone acetate to rats
8202266	*In vitro* protein binding of 14C-abiraterone to plasma proteins from rat, monkey, and human and to human serum albumin (HAS) and ά1- acid glycoprotein (AAG)
FK7389	*In vitro* metabolism of 14C-JNJ-212082 (CB7630) in liver microsomes and hepatocytes of male and female mouse, male and female rat, male dog, female rabbit, male monkey and man – Preliminary report

Toxicology

Study No.	Title
7777-100	13-week oral gavage toxicity and toxicokinetic study with CB7630 in rats with a 4-week recovery period
7777-105	26-week oral gavage toxicity and toxicokinetic study with CB7630 in rats with a 4-week recovery period

Genetic Toxicology

Study No.	Title
CTBR 960666	CB7630 (Lot No.562-51-3): Bacterial Mutation Test
CTBR 960736	CB7630 (Lot No.CML-121/05-CR1): Bacterial Mutation Test
CTBR 960744	CB-7598: Bacterial Mutation Test
CTBR 960667	CB7630 (Lot No.562-51-3): Chromosome Aberration Test

Study No.	Title
CTBR 960737	CB7630 (Lot No.CML-121/05-CR1): Chromosome Aberration Test
CTBR 960745	CB-7598: Chromosome Aberration Test
CTBR 960747	CB7630: Rat Micronucleus Test- initial CML process
CTBR 960747	CB7630: Rat Micronucleus Test-current CML process
CTBR 960747	CB-7598: Rat Micronucleus Test

Studies not reviewed within this submission:
Pharmacokinetics
Analytical method validation:

Study No.	Title
TNJS05-056A	Quantitative determination of CB7630 and CB7598 in rat plasma by LC/MS/MS
COU-2008-008	Validation of an electrospray LC/MS/MS Assay for quantitation of abiraterone acetate and abiraterone in K2EDTA rat plasma
COU-2008-010	Long-term frozen storage stability of abiraterone acetate in K2EDTA rat plasma preserved with sodium fluoride at –20°C and –80°C
BA1602	Validation (full) of an LC-MS/MS method for the determination of JNJ-589485 and JNJ-212082 in rat EDTA NaF plasma.
TNJS05-057	Quantitative determination of CB7630 and CB7598 in monkey plasma by LC/MS/MS
COU-2008-009	Validation of an electrospray LC/MS/MS Assay for quantitation of abiraterone acetate and abiraterone in K2EDTA Cynomolgus monkey plasma
7777-107	Validation of a method for the determination abiraterone acetate and abiraterone in monkey plasma by HPLC with MS/MS Detection
COU-2008-011	Long-term frozen storage stability of abiraterone and abiraterone acetate in K2EDTA cynomolgus monkey plasma preserved with sodium fluoride at –20°C and –80°C
FK7475	Evaluation of the (possible) impact of an isobaric metabolite on the abiraterone exposure.

Toxicology

Study No.	Title
1632-1	An acute gastric irritation study in the male mouse, a single dose toxicity study in the male rat, a multiple dose toxicity study in the male rat and a toxicokinetic study in the male rat
TOX9586	Single Dose Oral Toxicity Study of JNJ-212082-AAA in the Mouse followed by a 15-Day Repeated Dose Oral Toxicity Study (Tolerability Study)
7565	CB7630: A 28-Day Repeat Dose Oral Toxicity Study with a 28-Day Recovery Period in Male Sprague-Dawley Rats
1818-001	CB7630 28-Day Oral (Gavage) Repeated Dose Toxicity Study in the Cynomolgus Monkey with a 4-Week Recovery Phase

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Pharmacology studies have been conducted with abiraterone, abiraterone acetate (CB 7630) and metabolites of abiraterone. Abiraterone acetate is a pro-drug for abiraterone meaning that abiraterone acetate is rapidly converted to abiraterone *in vivo* by esterases (See Figure 1 below). Abiraterone is the circulating active metabolite that irreversibly inhibits cytochrome P450 17α (17α-hydroxylase/C17-20 lyase or CYP17) an enzyme crucial in the production of androgens in the testes and adrenal glands. P450 17α catalyzes two reactions: (1) 17α hydroxylation of C21 steroids and (2) cleavage of the C17,20 bond of C21 steroids (See Figure 1 below). The 17α hydroxylation activity is a required step in cortisol biosynthesis, whereas the C17, 20 bond side chain cleavage is essential for subsequent biosynthesis of androgens. P450 17α is expressed in testicular and adrenal tissues and catalyzes the conversion of pregnenolone or progesterone into dehydroepiandrosterone (DHEA) or androstenedione, respectively, two precursors of testosterone.

Figure 2 shows the mechanism of action of abiraterone in adrenal steroidogenesis via reducing testosterone levels by specifically inhibiting P450 17α (17α-hydroxylase/C17,20-lyase or CYP17).

Figure 1: Chemical Structure of Abiraterone Acetate ($C_{26}H_{33}NO_2$) and Abiraterone

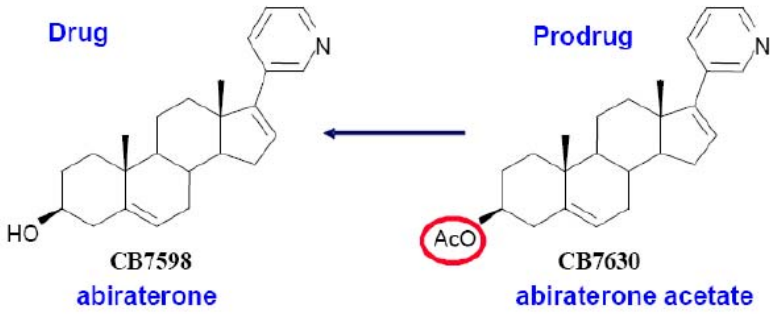
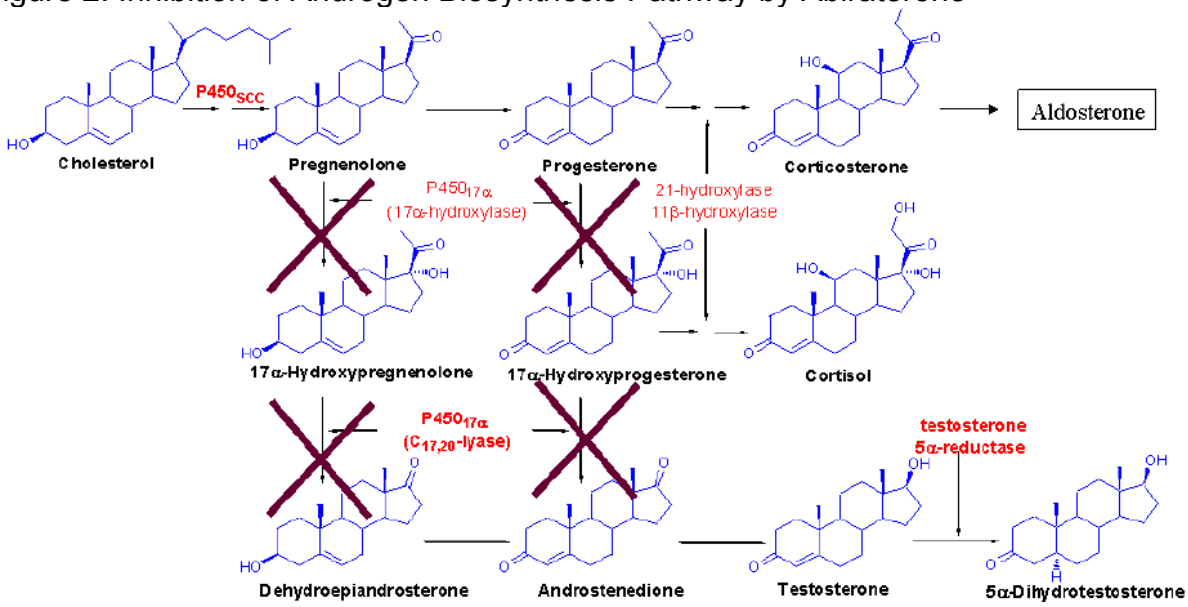


Figure 2: Inhibition of Androgen Biosynthesis Pathway by Abiraterone



[Figure excerpted from sponsor]

Abiraterone acetate was selected for use in non-clinical studies due to its ease in development compared to abiraterone. Most of the available pharmacology data on the pro-drug, abiraterone acetate, and its active metabolite, abiraterone, were derived from published literature articles. However, the sponsor included three additional studies characterizing the pharmacological activity of abiraterone and its metabolites on nuclear receptors and cell lines for prostate cancer (NCI-H295R). Throughout the pharmacology review, specifications will be made on whether abiraterone and/or abiraterone acetate were used.

The inhibitory effect of abiraterone on human and rat P450 17α (or CYP17) microsomes was examined by several investigators. In human testicular microsomes, Jarman *et al*. (J. Med. Chem., 1998) and Potter at el. (J. Med. Chem., 1995) showed that the concentration of abiraterone needed to irreversibly inhibit 50% of CYP17 activity (IC_{50}) was approximately 4 nM. In a separate study, Haider et al. (Arch. Pharm. Med. Chem, 2001) determined the IC_{50} of abiraterone to be 73 nM.

In rat testicular microsomes, Haider et al. (Arch. Pharm, Med. Chem, 2001) also showed abiraterone inhibiting P450 17α (or CYP17) with an IC_{50} of 220 nM; however, Duc *et al*. (Duc and et. J.of Steroid Biochem. And Molecular Biology, 2003), estimated the IC_{50} to be 5.8 nM. These values are summarized in Table 1 below.

Table 1: Inhibition of CYP17 in Human or Rat Testicular Microsomes

Reference	IC50 (nM) Human	IC50 (nM) Rat
Jarman et al., 1998[4]	4	-
Haidar et al., 2003[5]	73	220
Duc et al., 2003[7]	-	5.8

IC_{50} = concentration of inhibitor to give 50% inhibition.

[Table excerpted from sponsor]

Haidar *et al*. (Haider et al., J. of Steroid Biochem. And Molecular Biology, 2003) also demonstrated that the pro-drug, abiraterone acetate, inhibited human and rat P450 17α (or CYP17) but was less active than abiraterone with respective IC_{50}'s of 110 nM and 1600 nM, respectively.

Differences in IC_{50} values by each laboratory were likely caused by the different experimental conditions developed by individual laboratories to measure enzyme activities. For example, Duc et al. froze the samples before analyzing them with a radio-immune assay while Haidar et al. analyzed fresh samples by using a high-performance liquid chromatography (HPLC). Despite variations of absolute values from one laboratory to another, these observations consistently show that abiraterone inhibited P450 17α (or CYP17).

The *in vivo* effects of abiraterone acetate on circulating hormone levels and androgen target-organ weights were evaluated at 7.8, 39.2, and 196 mg/kg/day in mice and 39.2 and 50 mg/kg/day in rats. In mice, abiraterone acetate reduced organ weights of several androgen-sensitive organs (prostate, seminal vesicles, adrenals, and testes) in a dose-dependent manner when given daily for 14 days. Plasma testosterone levels showed greater than a 90% decrease while levels of luteinizing hormone (LH) increased at 39.2 and 196 mg/kg/day (+212 and 325% at 39.2 and 196 mg/kg/day, respectively). LH increases were expected since this was an endocrine response due to androgen depletion.

Data in rats were similar to that in mice. Rats had decreased ventral prostate (54-84%), complete prostate (54-84%), seminal vesicles (37-81%), and testes weights (17-36%) after 14-days of treatment. Furthermore, plasma testosterone levels decreased up to 95% compared to control when given daily for 14 days at 39.2 mg/kg/day. When given daily for 3 days at 50 mg/kg/day, abiraterone acetate significantly increased LH levels (+378%) but had a modest effect on plasma testosterone levels.

Metabolites of abiraterone acetate including abiraterone (JNJ-589485), abiraterone sulphate (JNJ-48171188) and *N*-oxide abiraterone sulphate (JNJ-49029578) were inactive when tested for nuclear receptor binding (glucocorticoid receptor binding, estrogen receptor-α binding, estrogen receptor-β binding and androgen receptor) at 1.0 μM. Abiraterone and abiraterone sulphate inhibited the human progesterone receptor at 52% and 69%, respectively. *N*-oxide abiraterone sulphate was inactive at the human progesterone receptor (8% inhibition of binding). The ketone analog of abiraterone was effective at inhibiting both

progesterone and androgen receptors, however, the data is of little relevance since this analog is not a metabolite.

Follow up studies were performed to determine if the metabolites altered progesterone activity when evaluated in receptor binding and cellular functional binding assay when tested at concentrations ranging from 0.01 to 5.0 μM. Both agonist and antagonist activities were examined. Abiraterone was more active than abiraterone sulfate at inhibiting the progesterone agonist (promegestone) in the receptor binding assay (abiraterone IC_{50}=230 nM and abiraterone sulfate IC_{50}=400 nM). However, both compounds were inactive at inhibiting progesterone in the functional cell based assay. Therefore, the progesterone receptor binding activity observed with isolated receptors was not sufficient to translate into a cellular response.

The two major circulating human metabolites of abiraterone acetate, abiraterone sulphate and *N*-oxide abiraterone sulphate also inhibited CYP17 in rat testes, however, the inhibition was considered weak in comparison to abiraterone in human adrenocortical carcinoma cell lines (IC_{50}= 3.6 and 26.2 μM, respectively). The two compounds also inhibited androstenedione (IC_{50}= 0.85 and 1.3 μM, respectively) and testosterone formation (IC_{50}= 0.73 and 2.9 μM, respectively) and to a lesser extent cortisol formation (IC_{50}= 2.8 and 6.2 μM, respectively). Both metabolites had no effect on aldosterone formation. In contrast, abiraterone concentration-dependently blocked aldosterone formation with an IC_{50} of 2.7 μM.

Abiraterone and abiraterone sulphate showed weak binding activity (IC_{50} = 0.23 and 0.4 μM, respectively) to the progesterone receptor (PR). This was 100 times less active than the control ligand and studies in a cellular progesterone reporter assay revealed no agonist or antagonist activity.

No studies were performed investigating the secondary pharmacodynamics of abiraterone acetate due to its relative inhibition of CYP17 and its organ specific toxicity observed in non-clinical studies. The effects for abiraterone acetate appear to be related to androgen deprivation.

In conclusion, *in vitro* data show that abiraterone, the circulating active metabolite, selectively and irreversibly inhibits P450 17α (or CYP17), a key enzyme in androgen biosynthesis. In both mice and rats, abiraterone acetate decreased the growth of androgen dependent organs (prostate, seminal vesicles, adrenals, and testes) and suppressed plasma testosterone levels at 39.2 (both mice and rats) and 196 mg/kg/day (mice only) when given daily for 14 days. However, it has a modest effect at decreasing testosterone levels when given as a single dose. Abiraterone sulphate and *N*-oxide abiraterone sulphate exhibited weak pharmacological activity (CYP17 inhibition) compared to abiraterone in human adrenocortical carcinoma cell lines.

Mechanism of action:

Potter et al. *J. Med. Chem*., 1995: Novel steroidal inhibitors of human cytochrome P45017 (l7α-hydroxylase/C l7,20-lyase): potential agents for the treatment of prostatic cancer

Report #:	J. Med. Chem, 38, 2463-2471, 1995
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	n/a
GLP Compliance:	No
QA Report:	n/a
Drug, lot #, and % purity:	n/a

This article reported the synthesis and activity of abiraterone (Compound 2 in Table 1) and abiraterone acetate (Compound 3 in Table 1). Abiraterone acetate is the pro-drug for abiraterone. When tested in human testicular microsomes, abiraterone had an IC_{50} of 2.9 nM and 4 nM at inhibiting enzymatic C17, 20-lyase and 17α-hydroxylase activity, respectively (See Table 1).

Abiraterone, however, did not inhibit aromatase and 5α-reductase at similar and/or higher concentrations. Abiraterone acetate was less active compared to abiraterone with IC_{50}'s of 17 nM and 18 nM, respectively. Similar to abiraterone, abiraterone acetate did not show any activity at inhibiting aromatase and 5α-reductase. Compounds 5, 9-11, 14, 15, 18, 21-22, 26-28, and ketoconazole are other steroidal compounds having 17-(3-pyridyl) substituent together with a 16,17-double bond similar to that found in abiraterone. These compounds are also active and/or non-active inhibitors of l7α-hydroxylase C17/20-lyase in human testicular microsomes. Table 1 shows the IC_{50}s of these compounds for comparison.

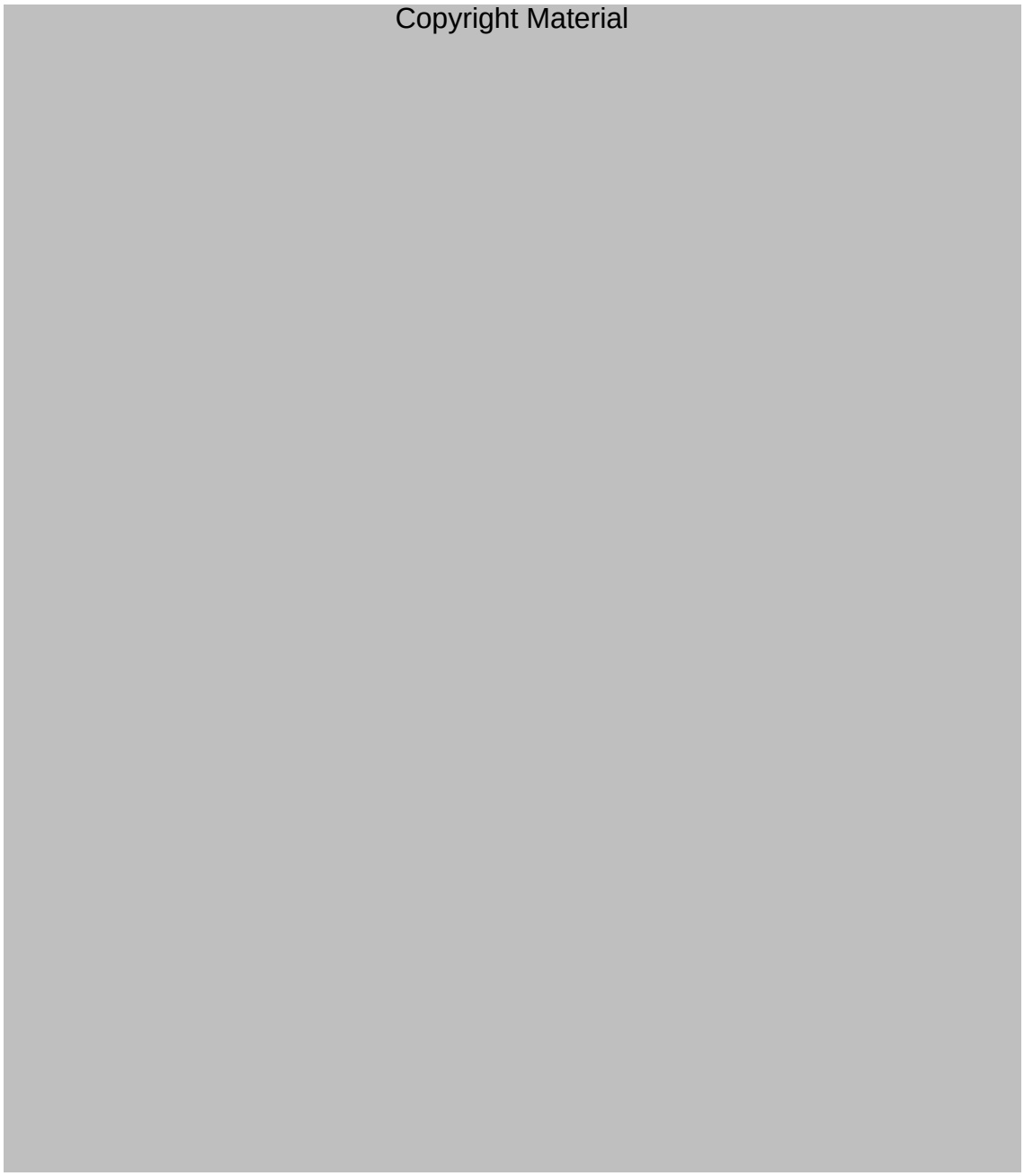
Jarman et al. *J. Med. Chem*. 1998: The 16, 17-double bond is needed for irreversible inhibition of human cytochrome P45017-α by abiraterone (17-(3-pyridyl) androsta-5,16-dien-3*β*-ol) and related steroidal inhibitors

Report #:	*J. Med. Chem.* 41, 5375-5381, 1998
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	n/a
GLP Compliance:	No
QA Report:	n/a
Drug, lot #, and % purity:	n/a

This article reported the *in vitro* inhibitory activity of abiraterone and its analogues on human cytochrome CYP17. In addition, the author emphasized the importance of the 16,17-double bond to the molecular structure of abiraterone and its contribution to the irreversible inhibition of CYP17.

In vitro, abiraterone inhibited CYP17 with an IC_{50} of 4 nM in human testicular microsomes (See Table 1). After 24 hours of dialysis, no recovery of the CYP17 activity was observed indicating irreversible inhibition. Additional experiments using abiraterone analogues (Compound 19, 25, and 26: See Figure 1 and Table 1) showed the necessity of the 16, 17-double bond for irreversible binding of these pyridyl steroids to cytochrome P45017α. However, oxidation to an epoxide

(Compound 6: See Figure 1) is not involved since epoxide showed moderate activity at inhibiting CYP17 with an IC_{50} of 260 nM.



Haider et al. *J. of Steroid Biochem. And Molecular Biology,* 2003: Effects of novel 17α-hydroxylase/C17, 20-lyase (P450, CYP17) inhibitors on androgen biosynthesis *in vitro and in vivo*

Report #:	J. of Steroid Biochem. and Molecular Biology, 84, 555-562, 2003
Conducting Laboratory and Location	(b) (4)
Date of Study Initiation:	n/a
GLP Compliance:	No
QA Report:	n/a
Drug, lot #, and % purity:	n/a

This article examined the effect of abiraterone and abiraterone acetate (and other compounds but not mentioned in detail for this review) on androgen biosynthesis *in vitro* and *in vivo*.

For the *in vitro* section of this study, microsomal fractions containing human or rat CYP17 were prepared from human or rat testes. In addition, Escherichia coli-co-expressing human CYP17 and NADPH-P450-reductase was also added. The inhibitory activity on these fractions were evaluated and the IC_{50} values were calculated.

Table 2: Inhibition of CYP17 in Human and Rat Testicular Microsomes and in E.Coli Expressing Human CYP17 and NADPH-P450 Reductase

Compound	IC_{50} (nM) Human	IC_{50} (nM) Rat	IC_{50} (nM) E. coli
Abiraterone	73	220	54
Abiraterone acetate	110	1600	>2500

IC_{50} = concentration of inhibitor to give 50% inhibition.

[Table excerpted from sponsor]

Table 2 shows that abiraterone was a stronger inhibitor than its prodrug (abiraterone acetate) at inhibiting CYP17 activity in human, rat and *E. coli* test systems with IC_{50}s of 73, 220, and 54 nM, respectively. Furthermore, reversibility of the inhibitory effect was evaluated. After a pre-incubation of abiraterone with the CYP17, the unbound inhibitor was removed with charcoal, and enzyme activity was determined after various time intervals (up to 320 minutes). Results showed that there was no recovery of CYP17 activity indicating irreversible inhibition (See Figure 3 below).

For the *in vivo* section of this study, adult male Sprague-Dawley rats were administered abiraterone acetate (CB7630) at 39.2 mg/kg/day (0.1 mmol/kg/day) daily for 14 days intraperitoneally. The animals were sacrificed at the end of treatment, blood was collected and tissues were removed. Abiraterone acetate (CB7630) decreased ventral prostate (54-84%), complete prostate (54-84%), seminal vesicles (37-81%), and testes weights (17-36%) after 14 days of treatment (See Figure 4). Furthermore, abiraterone acetate decreased testosterone levels from 2.2 ng/ml (control) to 0.1 ng/ml (See Table 3).

Haider et al. ***Arch. Pharm. Med. Chem, 2001:*** Novel steroidal pyrimidyl inhibitors of P450 17 (17α-hydroxylase/C17-20 lyase)

Report #:	Arch. Pharm. Pharm. Med. Chem, 2001
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	n/a
GLP Compliance:	No

QA Report: n/a
Drug, lot #, and % purity: n/a

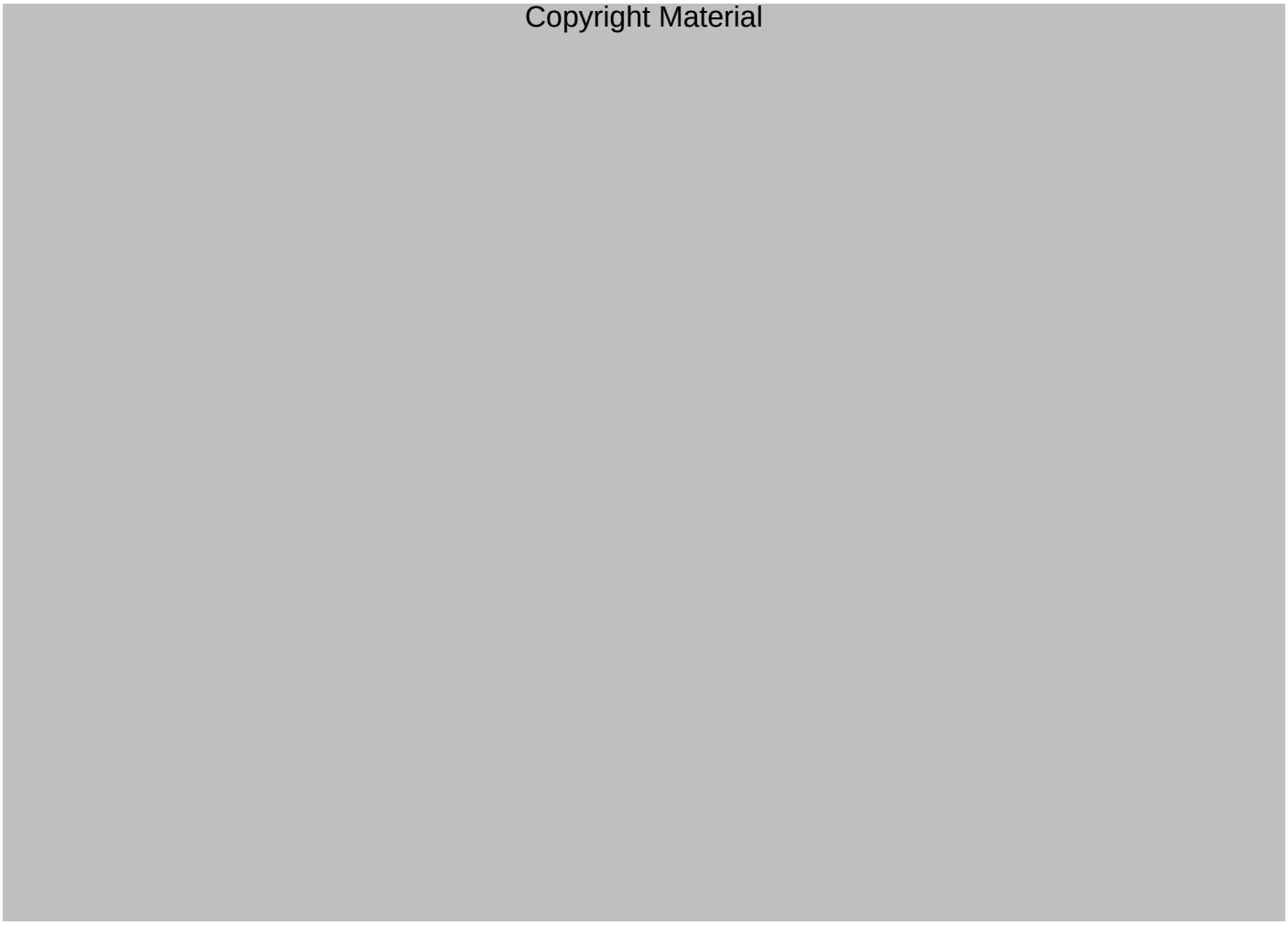
Androgens are growth factors for approximately 80% of prostate cancer and other diseases such as benign prostatic hyperplasia (BPH). P450 17 (17α-hydroxylase/C17-20 lyase) is a key enzyme in androgen biosynthesis. Inhibition of this enzyme leads to tumor regression. Ketoconazole, an antifungal agent, is an inhibitor of androgen biosynthesis; however, it has not been widely used due to its side effects. This article examined other inhibitors (Figure 2) of P450 17 (17α-hydroxylase/C17-20 lyase) including abiraterone (Figure 1) in human testicular microsomes.

As shown in Table 1, compounds 1 and 2 are approximately two to three times more active than abiraterone at inhibiting P450 17 in human testicular microsomes. The progesterone derivative (compound 3) also exceeds the activity of abiraterone.

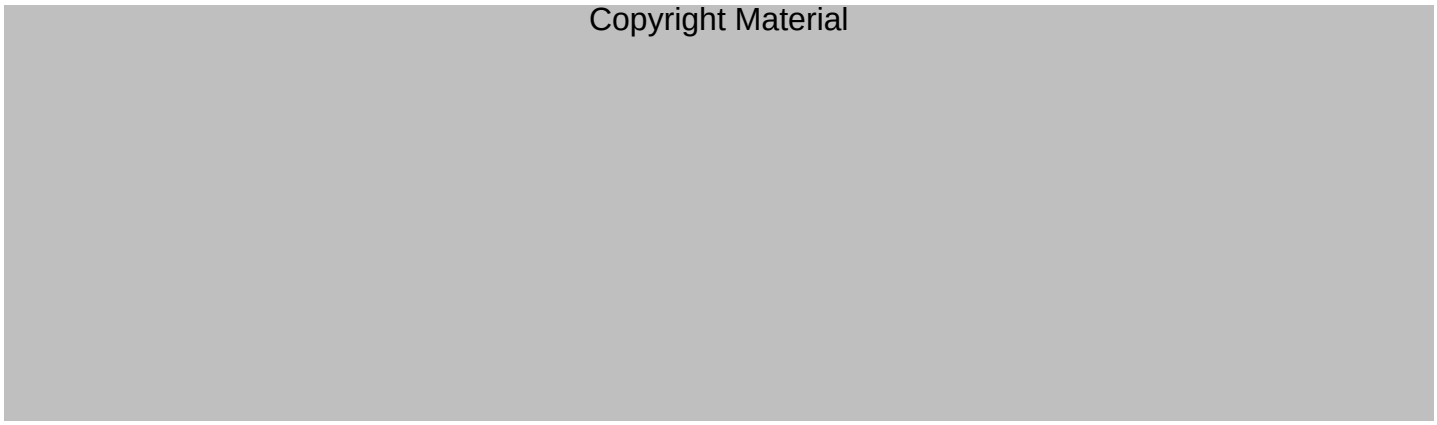
Barrie et al., *J. Steroid Molec. Biol., 1994:* Pharmacology of novel steroidal inhibitors of cytochrome P450 17α(17α-hydroxylase/C17-20 lyase)

Report #:	J. Steroid Molec. Biol, 50, 267-273, 1994
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	n/a
GLP Compliance:	No
QA Report:	n/a
Drug, lot #, and % purity:	n/a

In this study, abiraterone acetate (dissolved in 5% benzyl alcohol and 95% safflower oil) was administered by intraperitoneal injection to adult male WHT mice for 14 consecutive days at 0 (vehicle), 7.8, 39.2, and 196 mg/kg/day (23, 118, and 588 mg/m^2/day). On Day 15, animals were anesthetized, and blood was collected for testosterone (T) and lutenizing hormone (LH) levels and selected organs were collected and weighed. Others groups of mice were sacrificed at 1, 2, or 4 weeks after initiation of treatment, and selected organs were weighed. No mortality or apparent signs of toxicity were observed in any animals. As shown in Table 3, castration decreased the weight of ventral prostate, seminal vesicles, and kidneys 2 weeks after surgery. Treatment with abiraterone acetate also caused a reduction of several androgen-sensitive organs (prostate, seminal vesicles, adrenals, and testes) in a dose-dependent manner (Figure 2). As observed in castrated animals, reduction of seminal vesicle weights by treatment with abiraterone acetate was greater than that of the ventral prostate weights.



When testing plasma testosterone (T) and lutenizing hormone (LH) levels, abiraterone acetate significantly decreased testosterone levels to 92 and 96% at 39.2 and 196 mg/kg/day, respectively (See Table 4 below). LH levels increased at 39.2 and 196 mg/kg/day (+212 and 325% at 39.2 and 196 mg/kg/day, respectively). LH was expected to increase since this was an endocrine response and does not appear to increase testosterone levels. These data suggest that abiraterone acetate treatment induced a significant and sustained reduction in testosterone levels in mice when given over 14 days.



Duc et al, *J. Steroid Biochem and Mol. Bio*., 2003*:* *In vitro and in vivo* models for the evaluation of potent inhibitors of male rat l7α-hydroxylase Cl7/20-lyase

Report #:	J. Steroid Molec. Biol, 84, 537-542, 2003
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	n/a
GLP Compliance:	No
QA Report:	n/a
Drug, lot #, and % purity:	n/a

C17,20-lyase is a key enzyme in the biosynthesis of androgens by both testes and adrenals. Complete inhibition of this enzyme would lead to an alternate route of androgen suppression for the treatment of prostatic cancers. The first part of this study was to compare the *in vitro* inhibitory activity of steroidal (abiraterone) and non-steroidal compounds (YM55208, TX1196-11S-enantiomer, TX977 racemate, TX1197R-enantiomer, and bifonazole) on C17/20 lyase isolated from rat testes microsomes. Abiraterone and YM55208 served as the steroidal and non-steroidal references, respectively. The second part of the study examined the *in vivo* inhibitory effects of these steroidal and non-steroidal compounds on plasma testosterone (T) and luteininzing hormone (LH) levels in the testes, adrenals, seminal vesicles and prostate weights when given to adult male rats orally daily for 3 days at a dose of 50 mg/kg/day.

In vitro:
Results of the i*n vitro* portion of this study showed that the decreasing sequence of inhibitory activity for C17,20-lyase activity were as follows: abiraterone >YM55208 >TX1196-11S-enantiomer> TX977 (racemate product)>TX1197R-enantiomer>bifonazole (See Table 1). TX977 and its S-enantiomer were as active as abiraterone and YM55208, the steroidal and non-steroidal references. Bifonazole and the R-enantiomer were less active at inhibiting C17/20 lyase activity.

In vivo:
Results of the *in vivo* portion showed abiraterone acetate inhibited prostate (VP) and seminal vesicle (SV) weights by 14 and 37%, respectively, (See Figures 2A and 2B). There was no effect on adrenal weights (minor decrease of 7%). YM55208 was more active compared to abiraterone at reducing prostate and seminal vesicle weights (decreases of 37 and 48%, respectively). However, it showed an increase in adrenal weights (17%). TX977 and its S-enantiomer showed similar reductions in prostate (-24 to -22%) and seminal vesicle weights (-42 and -41%) but an increase in adrenal weights (11%). R-enantiomer caused a reduction in prostate weights (-24%), however, it was a weak inhibitor at reducing seminal vesicle weights (-2%). None of the compounds tested had an effect on testes weights.

Abiraterone acetate significantly increased LH levels (+378%) while TX977,S-and R enantiomer increased LH levels by 240, 255, 225%, respectively (See Figure 2C). The non-steroidal reference, YM55208, showed the most activity at reducing testosterone levels while abiraterone acetate had a modest effect (approximately 40%).

Studies on metabolites:

8655156: *In Vitro* Pharmacology: Steroids Nuclear Receptor Binding Assay. Study of Four Compounds

Report #:	8655156
Conducting Laboratory and Location	(b) (4)
Date of Study Initiation:	6/29/2010
GLP Compliance:	Yes
QA Report:	Yes (), No (X)
Drug, lot #, and % purity:	Drugs: JNJ589485, JNJ46252908,

JNJ48171188, and JNJ49029578.
Lot numbers: 1635-67-1, 34472249, 35616973, BJVE-08-072-2, respectively.

Methods

Purpose: To investigate the effects of abiraterone (JNJ589485), ketone analog of abiraterone (JNJ46252908), abiraterone sulphate (JNJ48171188), and N-oxide abiraterone sulphate (JNJ49029578) in the following *in vitro* nuclear receptor binding assays (See Sponsor Table 1 below) at a single concentration of 1 uM.

- Glucocorticoid receptor (GR)
- Estrogen-α (ER-α)
- Estrogen-β (ER-β)
- Progesterone (PR)
- Androgen (AR)

Study Design:

- Results showing an inhibition higher than 50% are considered to represent significant effects of the test compound. 50% is the most common cut-off value for further investigation (determination of IC50 or EC50 values from concentration-response curves)
- Results showing an inhibition between 25% and 50% are indicative of weak to moderate effects
- Results showing an inhibition lower than 25% are not considered significant and mostly attributable to variability of the signal around the control level

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Table 1. *In Vitro* Pharmacology: Binding Assays

Assay	Origin	Ligand	Conc.	Kd	Non Specific	Incubation	Method of Detection	Bibl.
Nuclear receptors								
GR *(h)* (agonist radioligand)	IM-9 cells (cytosol)	[^{3}H]dexamethasone	1.5 nM	1.5 nM	triamcinolone (10 µM)	6 h 4°C	Scintillation counting	283
ERα *(h)* (agonist fluoligand)	human recombinant (Sf9 cells)	fluormone^{TM}ES2	1 nM	4 nM	17-β-estradiol (10 µM)	120 min 22°C	Fluorescence polarisation	304
ERβ *(h)* (agonist fluoligand)	human recombinant (Hi5 cells)	fluormone^{TM}ES2	1 nM	4 nM	17-β-estradiol (10 µM)	120 min 22°C	Fluorescence polarisation	304
PR *(h)* (agonist radioligand)	T47D cells (cytosol)	[^{3}H]progesterone	0.5 nM	2 nM	promegestone (1 µM)	20 h 4°C	Scintillation counting	930
AR *(h)* (agonist radioligand)	LNCaP cells (cytosol)	[^{3}H]methyltrienolone	1 nM	0.8 nM	mibolerone (1 µM)	24 h 4°C	Scintillation counting	498

[Table excerpted from sponsor]

Results

- All metabolites were inactive when tested for glucocorticoid receptor binding, estrogen receptor-α binding, estrogen receptor- β binding and androgen receptor binding.

- Abiraterone (JNJ589485) and abiraterone sulphate (JNJ48171188) showed a 52% and 69% inhibition, respectively, at binding the [3H]-progesterone to the human progesterone receptor.
- *N*-oxide abiraterone sulphate (JNJ49029578) was essentially inactive with 8% inhibition of binding.
- Inhibition of progesterone and androgen receptor binding (85% and 83% inhibition binding, respectively) was observed with the ketone analog of abiraterone (JNJ-46252908).
- Results are shown in sponsor Table 2 below.

Table 2: Results of metabolites of abiraterone on steroid nuclear receptor binding assays.

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Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
GR *(h)* (agonist radioligand)			
8655156-1	JNJ589485	1.0E-06	5
8655156-2	JNJ46252908	1.0E-06	36
8655156-3	JNJ48171188	1.0E-06	1
8655156-4	JNJ49029578	1.0E-06	4
ERα *(h)* (agonist fluoligand)			
8655156-1	JNJ589485	1.0E-06	-1
8655156-2	JNJ46252908	1.0E-06	0
8655156-3	JNJ48171188	1.0E-06	2
8655156-4	JNJ49029578	1.0E-06	-7
ERβ *(h)* (agonist fluoligand)			
8655156-1	JNJ589485	1.0E-06	14
8655156-2	JNJ46252908	1.0E-06	12
8655156-3	JNJ48171188	1.0E-06	7
8655156-4	JNJ49029578	1.0E-06	-6
PR *(h)* (agonist radioligand)			
8655156-1	JNJ589485	1.0E-06	51
8655156-2	JNJ46252908	1.0E-06	85
8655156-3	JNJ48171188	1.0E-06	69
8655156-4	JNJ49029578	1.0E-06	8
AR *(h)* (agonist radioligand)			
8655156-1	JNJ589485	1.0E-06	13
8655156-2	JNJ46252908	1.0E-06	83
8655156-3	JNJ48171188	1.0E-06	-3
8655156-4	JNJ49029578	1.0E-06	-12

[Table excerpted from sponsor]

8655175: *In Vitro* Pharmacology: Human PR Receptor Binding and Functional Assays Study of JNJ-589485-AAA and JNJ-48171188-AAA

Report #: 8655175

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: 6/29/2010

GLP Compliance: Yes

QA Report: Yes (), No (X)

Drug, lot #, and % purity: Drugs: JNJ589485-AAA, JNJ48171188-AAA.
Lot numbers: 1635-67-1 and 35616973

Methods

Purpose: To investigate the effects of abiraterone (JNJ589485-AAA), abiraterone sulphate (JNJ48171188-AAA) in an *in vitro*

progesterone receptor binding and progesterone cellular functional assay (See Sponsor Table 1 below) at concentrations ranging from 0.01 to 5 µM.

Study Design:
- Results showing an inhibition higher than 50% are considered to represent significant effects of the test compound. 50% is the most common cut-off value for further investigation (determination of IC_{50} or EC_{50} values from concentration-response curves)
- Results showing an inhibition between 25% and 50% are indicative of weak to moderate effects
- Results showing an inhibition lower than 25% are not considered significant and mostly attributable to variability of the signal around the control level

Table 1

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A.1.1. In Vitro Pharmacology: Binding Assay

Assay	Origin	Ligand	Conc.	Kd	Non Specific	Incubation	Method of Detection	Bibl.
Nuclear receptors								
PR *(h)* (agonist radioligand)	T47D cells (cytosol)	[^{3}H]progesterone	0.5 nM	2 nM	promegestone (1 µM)	20 h 4°C	Scintillation counting	930

A.1.2. In Vitro Pharmacology: Cellular Functional Assays

Assay	Origin	Stimulus	Incubation	Reaction Product	Method of Detection	Bibl.
Nuclear receptors						
PR *(h)* (agonist effect)	human recombinant	none (10 µM progesterone for control)	22°C	coactivator recruitment	AlphaScreen	1048
PR *(h)* (antagonist effect)	human recombinant	progesterone (100 nM)	22°C	coactivator recruitment	AlphaScreen	1048

[Table excerpted from sponsor]

Results
- In the binding assay, abiraterone was more active than abiraterone sulphate with IC_{50} of 230 and 400 nM, respectively when tested at concentrations ranging from 0.01 to 5.0 µM (See Table 3.1). The control ligand promegestone had an IC_{50} of 500 nM.
- In the cellular functional assay, both compounds were inactive when tested at concentrations ranging from 0.01 to 5.0 µM (See Table 3.2). The control ligand promogestone had an IC_{50} of 280 nM.
- These data suggest binding of abiraterone to the progresterone receptor may not translate to *in vivo* activity.

3.1. *In Vitro* Pharmacology: Binding Assay

3.1.1. IC_{50} Determination: Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	IC_{50} (M)	K_I (M)	n_H
PR *(h)* (agonist radioligand)				
8655175-1	JNJ-589485-AAA	2.3E-07	1.8E-07	*0.8*
8655175-2	JNJ-48171188-AAA	4.0E-07	3.2E-07	*0.8*

3.2. *In Vitro* Pharmacology: Cellular Functional Assays

3.2.1. EC50 Determination: Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.	Flags
PR *(h)* (agonist effect)		
8655175-1	JNJ-589485-AAA	N.C.
8655175-2	JNJ-48171188-AAA	N.C.

N.C. EC50 value not calculable. Concentration-response curve shows less than 25% effect at the highest tested concentration.

[Tables excerpted from sponsor]

2010ORB001: Rat CYP 17 inhibition by JNJ-48171188-AAA, JNJ-49029578-AAA and JNJ-589485-AAA against ketoconzole. Steroid formation in NCI-H295R cells upon exposure to JNJ-48171188-AAA, JNJ-49029578-AAA and JNJ-589485-AAA against ketoconzole.

Report #:	2010ORB001
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	9/15/2010
GLP Compliance:	Yes
QA Report:	Yes (), No (X)
Drug, lot #, and % purity:	JNJ-49029578-AAA, JNJ-48171188-AAA and JNJ-589485-AAA, 100%

Methods

Purpose (2 parts):

Rat CYP17 inhibition assays

- *In vitro* assays were performed to assess the activity of metabolites of abiraterone (JNJ-49029578-AAA, JNJ-48171188-AAA and JNJ-589485-AAA) as inhibitors of CYP17 isolated from rat testes against the reference compound, ketoconazole.

Steroid biosynthesis selectivity assay

- The potential influence of metabolites of abiraterone (JNJ-49029578-AAA, JNJ-48171188-AAA and JNJ-589485-AAA) and a reference compound, ketoconazole, on steroidogenesis *in vitro* was assessed using the

adrenocortical cell line NCI-H295R.

Study Design: **Rat CYP17 inhibition assays**:

- The rat CYP17-containing testes (protein preparation) were obtained from adult male Sprague-Dawley rats.
- Metabolites of abiraterone: JNJ-48171188-AAA, JNJ-49029578-AAA and JNJ-589485-AAA, provided by the sponsor
- Reaction mixtures were pre-incubated for 5 min at 32°C in assay buffer consisting of phosphate buffer (0.05 M, pH 7.4). An aliquot of the particle-free supernatant was subjected to LC-MS/MS.
- Results were displayed as peak area ratio (area of the analyte peak is divided by the area of the internal standard peak).

Steroid biosynthesis selectivity assay

- NCI-H295R cells were obtained from ATCC (catalog No. CRL 2128) and maintained in DMEM/Ham's F12 medium
- Metabolites of abiraterone: JNJ-48171188-AAA, JNJ-49029578-AAA and JNJ-589485-AAA
- Metabolites were tested at 10 µM, 3.12 µM, 1 µM, 0.312 µM, 0.1 µM, 0.0312 µM, 0.01 µM and 0.00312 µM.
- The reference compound, ketoconazole, was tested at 10 µM, 2 µM, 0.2 µM, 0.02 µM, 0.002 µM, and 0.0002 µM.
- Quantification of androstenedione, testosterone, cortisol, and aldosterone was assessed by enzyme immunoassay.

Results

Rat CYP17 inhibition assay:

- Abiraterone sulphate (JNJ-48171188-AAA) showed a greater inhibition compared to the *N*-oxide abiraterone sulphate (JNJ-49029578) metabolite at inhibiting P450 17α in rat testes with an IC_{50} =3.6µM compared to 26.2 µM (See Sponsor's Figures 6 and 8).

Table 6: Rat CYP17 inhibition (in vitro IC_{50} determination) of JNJ-48171188-AAA

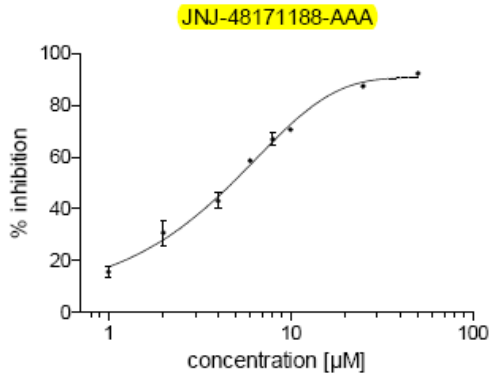
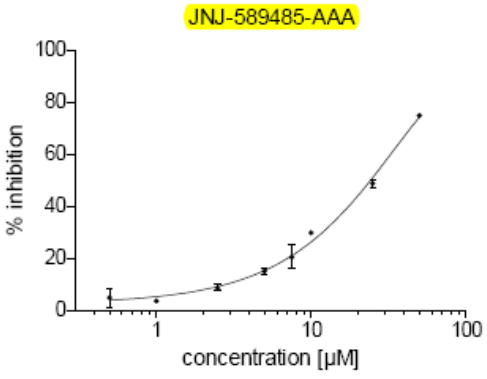
Compound	Concentration [µM]	*Mean inhibition [%] n=2*
JNJ-48171188-AAA	50	92.4
	25	87.5
	10	70.6
	8	66.9
	6	58.7
	4	43.1
	2	30.8
	1	15.7
Ketoconazole	60	50.3
	30	34.8
IC_{50} = 3.6 µM[8]	JNJ-48171188-AAA 	

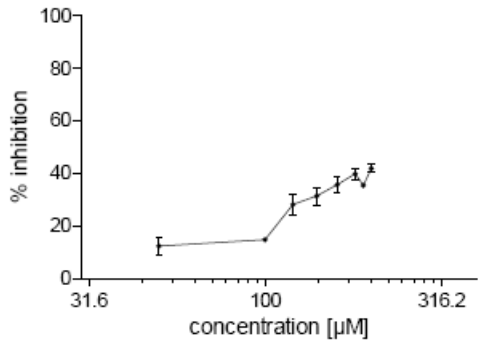
Table 8: Rat CYP17 inhibition (in vitro IC_{50} determination) of JNJ-589485-AAA

Compound	Concentration [µM]	*Mean inhibition [%] n=2*
JNJ-589485-AAA	50	74.8
	25	48.7
	10	29.8
	7.5	20.4
	5.0	15.0
	2.5	8.6
	1.0	3.6
	0.5	4.9
Ketoconazole	60	46.1
	30	29.8
IC_{50} = 26.2 µM[11]	JNJ-589485-AAA 	

[Figure excerpted from sponsor]

- Determination for an IC50 value for the ketone analog of abiraterone (JNJ46252908) was not applicable due to % values being <50% at the highest concentration tested [See sponsor's table 7 below].

Table 7: Rat CYP17 inhibition (in vitro IC_{50} determination) of JNJ-49029578-AAA

Compound	Concentration [µM]	*Mean inhibition [%] n=2*
JNJ-49029578-AAA	200	41.9
	190	35.4
	180	39.7
	160	35.6
	140	31.3
	120	28.2
	100	14.8
	50	12.4
Ketoconazole	60	54.0
	30	33.4
IC_{50} = > 200 µM[10]	JNJ-49029578-AAA 	

[Figure excerpted from sponsor]

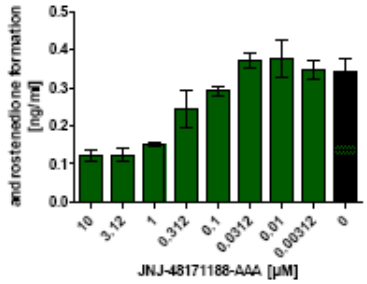
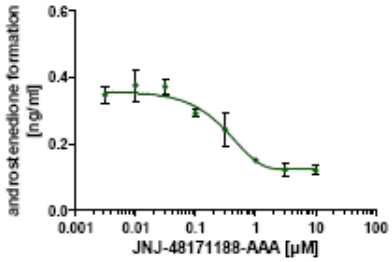
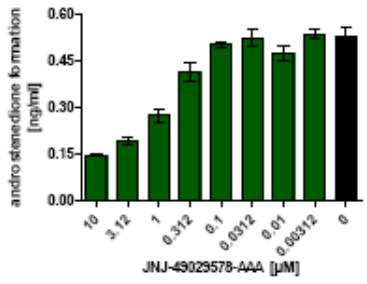
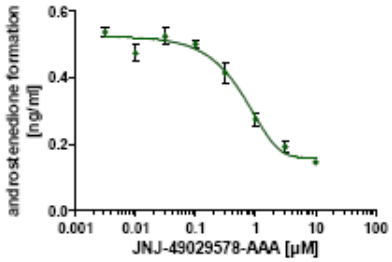
- The reference compound ketoconazole had an IC50 of 54.6 µM. [See sponsor's table 9 below].

Steroid biosynthesis selectivity assay

Effect of metabolites on androstenedione formation:

- Metabolite abiraterone sulphate (JNJ-48171188-AAA) was more active than the N-oxide abiraterone sulphate (JNJ-49029578-AAA) at decreasing androstenedione formation in NCI-H295R cells with an IC_{50} of 0.85 µM and 1.3 µM, respectively. [See sponsor's table 10 below].
- For abiraterone (JNJ-589485-AAA), an IC_{50} value could not be calculated since this compound did not lead to a concentration-dependent decrease of androstenedione formation.
- The reference compound ketoconazole had an IC_{50} value of 0.13 µM.

Table 10: Concentration-response relation and % inhibition of androstenedione formation in presence of test items

	Test item concentration [µM]	Androstenedion formation [ng/ml]		% Inhibition		IC_{50} value
		mean	± SD	mean	± SD	
JNJ-48171188-AAA	10.0	0.121	0.012	64.8	4.3	IC_{50}:0.85 µM[13]
	3.12	0.124	0.015	64.0	5.4	
	1.00	0.152	0.003	55.9	1.0	
	0.312	0.244	0.040	29.2	14.2	
	0.100	0.292	0.011	15.2	3.8	
	0.0312	0.372	0.017	-8.1	6.1	
	0.0100	0.376	0.039	-9.2	13.7	
	0.00312	0.348	0.020	-1.1	7.3	
	0 (0.5% DMSO)	0.345	0.030			
						
JNJ-49029578-AAA	10.0	0.152	0.008	71.1	1.8	IC_{50}: 1.3 µM[13]
	3.12	0.193	0.012	63.4	2.8	
	1.00	0.275	0.017	47.8	3.9	
	0.312	0.415	0.024	21.4	5.6	
	0.100	0.502	0.009	5.0	2.1	
	0.0312	0.524	0.021	0.7	5.0	
	0.0100	0.474	0.019	10.2	4.5	
	0.00312	0.537	0.013	-1.8	3.1	
	0 (0.5% DMSO)	0.528	0.030			
						

[Figure excerpted from sponsor]

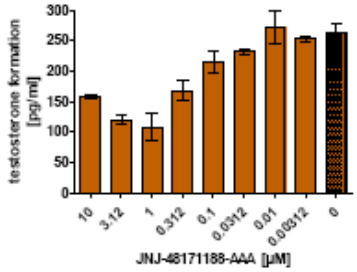
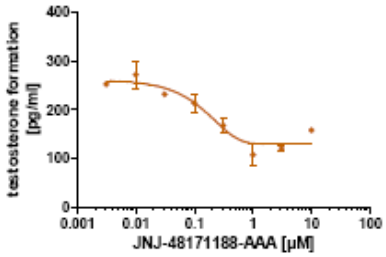
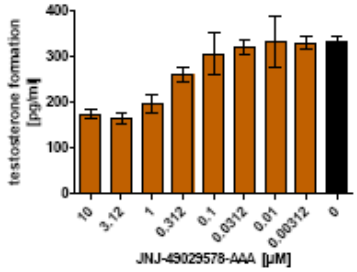
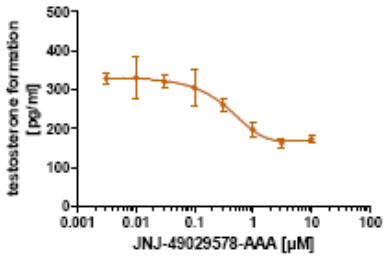
Effect of test items on testosterone formation:

- Metabolite abiraterone sulphate (JNJ-48171188-AAA) was more active than the N-oxide abiraterone sulphate (JNJ-49029578-AAA) at decreasing testosterone levels in NCI-H295R cells with an IC_{50} of 0.73 µM and 2.9 µM, respectively. [See sponsor's table 11 below].
- As described for androstenedione formation, abiraterone (JNJ-589485-AAA) is also an inhibitor of testosterone biosynthesis, but an IC_{50} value could not be

calculated since this compound did not lead to a concentration-dependent decrease of testosterone formation.

- The reference compound ketoconazole had the lowest IC_{50} value of 0.087 µM.

Table 11: Concentration-response relation and % inhibition of testosterone formation in presence of test items (mean ± SD)

	Test item concentration [µM]	Testosterone formation [pg/ml]		% Inhibition		IC_{50} value
		mean	± SD	mean	± SD	
JNJ-48171188-AAA	10.0	157.9	3.1	39.6	1.5	IC_{50}: 0.73 µM[14]
	3.12	120.0	5.6	54.1	2.6	
	1.00	107.2	18.5	59.0	8.6	
	0.312	167.6	13.0	35.9	6.1	
	0.100	213.8	14.9	18.3	7.0	
	0.0312	231.4	2.7	11.6	1.3	
	0.0100	272.0	22.7	-4.0	10.6	
	0.00312	252.5	4.6	3.5	2.2	
	0 (0.5% DMSO)	261.6	15.0			
						
JNJ-49029578-AAA	10.0	173.2	7.5	47.8	2.8	IC_{50}: 2.9 µM[14]
	3.12	163.1	9.5	50.9	3.5	
	1.00	196.9	15.9	40.7	5.9	
	0.312	261.2	13.2	21.3	4.9	
	0.100	305.0	37.9	8.1	14.0	
	0.0312	320.4	13.9	3.5	5.1	
	0.0100	331.1	45.2	0.2	16.7	
	0.00312	329.0	10.6	0.8	3.9	
	0 (0.5% DMSO)	331.9	11.0			
						

[Figure excerpted from sponsor]

Effect of test items on cortisol formation:

- Metabolite abiraterone sulphate (JNJ-48171188-AAA) was more active than the N-oxide abiraterone sulphate (JNJ-49029578-AAA) at inhibiting the formation of cortisol in NCI-H295R cells with an IC50 of 2.8 µM and 6.2 µM, respectively. [See sponsor's table 12 below].

- Abiraterone sulphate (JNJ-48171188-AAA) was a weaker inhibitor of cortisol biosynthesis compared to the formation of androgens (IC50 value of 2.8 µM for cortisol verses 0.85 µM for androstenedione [See sponsor's table 12 below].
- For the N-oxide abiraterone sulphate (JNJ-49029578-AAA), the IC50 value was 6.2 µM thus this compound is assumed to have a higher activity for inhibition of androgen synthesis than for glucocorticoid synthesis. [See sponsor's table 12 below].
- Abiraterone (JNJ-589485-AAA) was also an inhibitor of cortisol biosynthesis with the lowest IC50 value (0.003 µM). [See sponsor's table 12 below].
- The reference compound ketoconazole had an IC50 of 0.11 µM, which was approximately in the same range as the IC50 values determined for the inhibition of androgen formation, i.e. showing almost no selectivity of inhibition.

Table 12: Concentration-response relation and % inhibition of cortisol formation in presence of test items

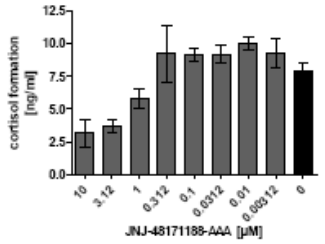
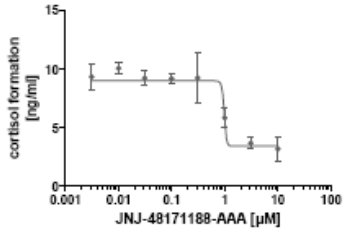
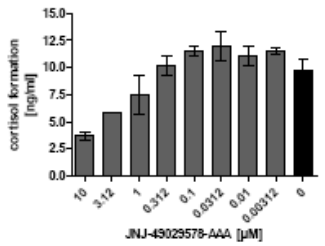
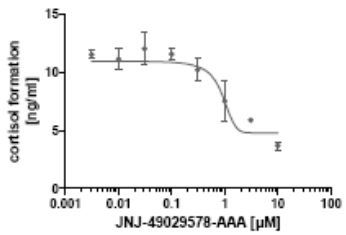
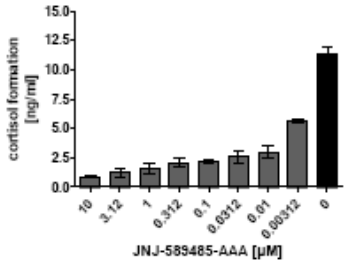
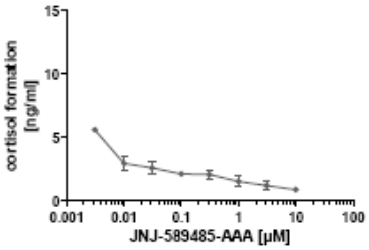
	Test item concentration [µM]	Cortisol formation [ng/ml]		% Inhibition		IC_{50} value
		mean	± SD	mean	± SD	
JNJ-48171188-AAA	10.0	3.16	0.85	60.3	13.1	IC_{50}: 2.8 µM[15]
	3.12	3.68	0.40	53.9	6.2	
	1.00	5.83	0.65	26.9	9.9	
	0.312	9.26	1.79	-16.1	27.5	
	0.100	9.20	0.37	-15.4	5.7	
	0.0312	9.24	0.54	-15.9	8.3	
	0.0100	10.07	0.39	-26.4	6.0	
	0.00312	9.33	0.94	-17.1	14.5	
	0 (0.5% DMSO)	7.97	0.51			
						
JNJ-49029578-AAA	10.0	3.68	0.28	62.4	3.5	IC_{50}: 6.2 µM[14]
	3.12	5.87	0.003	40.0	0.0	
	1.00	7.50	1.46	23.3	18.3	
	0.312	10.16	0.77	-3.9	9.7	
	0.100	11.51	0.37	-17.7	4.7	
	0.0312	11.98	1.12	-22.5	14.0	
	0.0100	11.11	0.76	-13.5	9.6	
	0.00312	11.50	0.27	-17.5	3.3	
	0 (0.5% DMSO)	9.78	0.96			
						

Table 12 (continued)

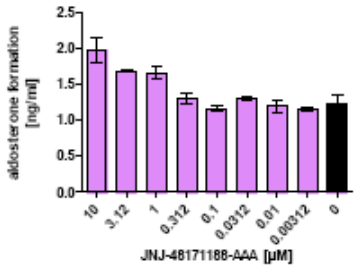
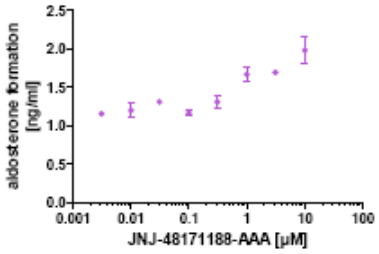
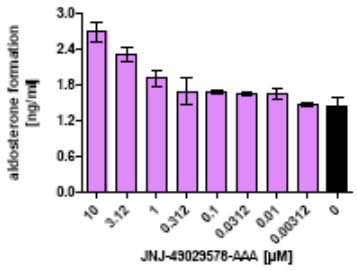
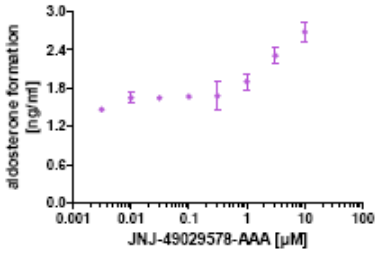
	Test item concentration [µM]	Cortisol formation [ng/ml]		% Inhibition		IC_{50} value
		mean	± SD	mean	± SD	
JNJ-589485-AAA	10.0	0.90	0.08	92.1	0.9	IC_{50}: 0.003 µM[17]
	3.12	1.22	0.26	89.2	2.8	
	1.00	1.54	0.35	86.4	3.8	
	0.312	2.09	0.24	81.5	2.6	
	0.100	2.14	0.02	81.1	1.3	
	0.0312	2.35	0.29	77.0	4.7	
	0.0100	3.20	0.39	73.7	5.0	
	0.00312	5.68	0.01	50.4	1.0	
	0 (0.5% DMSO)	11.31	0.61			
						

[Figure excerpted from sponsor]

Effect of test items on aldosterone formation:

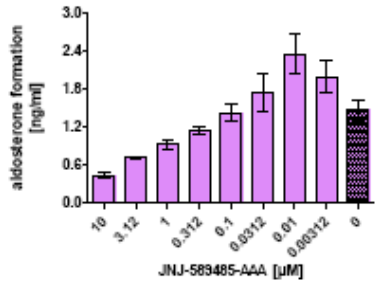
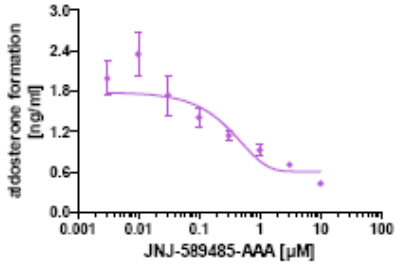
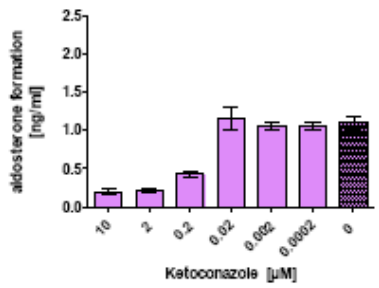
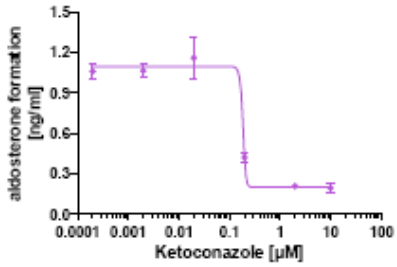
- No IC50 values were determined for abiraterone sulphate (JNJ-48171188-AAA) and N-oxide abiraterone sulphate (JNJ-49029578-AAA) at concentrations from 10.0 to 0.312 and 10.0 to 0.01 µM since aldosterone levels were increased compared to the concentrations measured in solvent-treated control. [See sponsor's table 13 below].
- In contrast, abiraterone (JNJ-589485-AAA) concentration-dependently blocked aldosterone formation. The resulting IC50 was 2.7 µM. [See sponsor's table 13 below].
- The reference compound ketoconazole had an IC50 0.17 µM indicating a concentration-dependent inhibition of aldosterone formation.

Table 13: Concentration-response relation and % inhibition of aldosterone formation

	Test item concentration [µM]	Aldosterone formation [ng/ml]		% Inhibition		IC_{50} value
		mean	± SD	mean	± SD	
JNJ-48171188-AAA	10.0	1.98	0.14	-58.4	13.8	IC_{50}: n/a
	3.12	1.70	0.02	-35.4	1.8	
	1.00	1.67	0.08	-33.2	7.5	
	0.312	1.31	0.06	-4.6	5.6	
	0.100	1.17	0.03	6.8	3.3	
	0.0312	1.31	0.01	-4.7	1.2	
	0.0100	1.20	0.08	4.2	7.7	
	0.00312	1.16	0.02	7.7	1.8	
	0 (0.5% DMSO)	1.25	0.11			
						
JNJ-49029578-AAA	10.0	2.67	0.13	-80.7	10.4	IC_{50}: n/a
	3.12	2.30	0.11	-55.8	8.9	
	1.00	1.90	0.10	-28.5	8.6	
	0.312	1.68	0.18	-13.3	14.9	
	0.100	1.67	0.02	-12.6	1.8	
	0.0312	1.65	0.03	-11.3	2.2	
	0.0100	1.64	0.07	-10.9	6.1	
	0.00312	1.46	0.03	1.1	2.6	
	0 (0.5% DMSO)	1.48	0.09			
						

[Figure excerpted from sponsor]

Table 13 (continued)

	Test item concentration [µM]	Cortisol formation [ng/ml] mean	± SD	% Inhibition mean	± SD	IC_{50} value
JNJ-589485-AAA	10.0	0.43	0.03	71.1	2.6	IC_{50}: 2.7 µM[18]
	3.12	0.71	0.02	52.8	1.4	
	1.00	0.93	0.06	38.5	5.1	
	0.312	1.14	0.06	24.1	4.6	
	0.100	1.42	0.12	6.0	8.8	
	0.0312	1.87	0.20	-15.6	19.6	
	0.0100	2.48	0.25	-56.6	21.5	
	0.00312	1.86	0.12	-32.4	16.9	
	0 (0.5% DMSO)	1.51	0.16			
						
Ketoconazole	10.0	0.20	0.03	82.3	3.2	IC_{50}: 0.17 µM[18]
	2.0	0.21	0.02	81.0	1.8	
	0.2	0.42	0.03	61.8	3.6	
	0.02	1.16	0.13	-4.7	14.3	
	0.002	1.07	0.03	3.8	4.7	
	0.0002	1.06	0.06	4.3	5.4	
	0 (0.5% DMSO)	1.11	0.09			
						

[Figure excerpted from sponsor]

Conclusion:

Rat CYP17 inhibition assays:

- Abiraterone sulphate (JNJ-48171188-AAA) showed a greater inhibition compared to the *N*-oxide abiraterone sulphate (JNJ-49029578) metabolite at inhibiting P450 17α in rat testes with an IC50 =3.6µM compared to 26.2µM.

Steroid biosynthesis selectivity assay

- Both abiraterone sulphate (JNJ-48171188-AAA) and N-oxide abiraterone sulphate (JNJ-49029578-AAA) showed moderate activity at inhibiting the adrenocortical tumor cell line NCI-H295R androgen biosynthesis system at the tested concentrations. For these compounds, the IC50 values for

androstenedione and testosterone formation were found to be in the same order of magnitude.

- Abiraterone (JNJ-589485-AAA) also appeared also to be an inhibitor of androgen biosynthesis but an IC_{50} could not be calculated since this compound did not lead to a concentration-dependent decrease of either androstenedione and/or testosterone.
- Abiraterone sulphate (JNJ-48171188-AAA) and N-oxide abiraterone sulphate (JNJ-49029578-AAA) were also inhibitors, to a lower extend, of cortisol formation. It might be speculated that the inhibition of the androgen biosynthesis pathway could lead to an accumulation of its substrates progesterone and pregnenolone. As a consequence, the degradation of these steroids could be performed via the mineralocorticoid pathways leading to higher concentrations of the aldosterone as the final product.
- In contrast, abiraterone (JNJ-589485-AAA) concentration-dependently blocked aldosterone formation with an IC_{50} of 2.7 µM.

2.6.2.3 Secondary pharmacodynamics – No studies submitted

2.6.2.4 Safety Pharmacology

Safety pharmacology studies were performed to investigate the effect of abiraterone acetate (CB7630) on cardiovascular, behavioral or neurological, gastric and pulmonary function. No overt effects of single dose treatment with the drug were seen on pulmonary or neurologic function in rats. Furthermore, no signs of gastric irritation were seen in male mice 14 days after treatment with a single oral dose (800 mg/kg). The sponsor included a cardiovascular study in cynomolgus monkeys as well as an *in vitro* hERG assay.

In the *in vitro* study, both abiraterone (CB7598) and abiraterone acetate (CB7630) were tested up to a maximum concentration of 27 µM. Abiraterone inhibited the hERG potassium current at 10 and 27 µM by 2% and 6%, respectively. The inhibition does not have physiological relevance since the IC_{50} for the inhibitory effect of abiraterone on hERG potassium current was less than 50% at a maximum dose of 27 µM. Abiraterone acetate inhibited hERG K+ current up to 84% at 27 µM with an IC_{50} of 12.2 µM. Since abiraterone acetate is rapidly converted *in vivo* to abiraterone and abiraterone acetate concentrations were below the lower limit of quantification (LLOQ), the hERG inhibition of abiraterone acetate is considered of limited clinical relevance.

In the *in vivo* oral dose monkey study, the sponsor reported test article-related clinical signs following dose administration. Soft, pale, white feces with mucoid material was noted in 1/4 monkeys at the high dose of 2000 mg/kg. At 250 and 750 mg/kg, pale feces was observed in all four monkeys the day following dose administration. No other significant changes were noted. Overall, these studies suggest that abiraterone acetate does not have an effect on cardiovascular function.

Neurological effects:

TOX9587: 1-month Repeated Dose Oral Toxicity Study of JNJ-212082-AAA in the Rat with integrated Irwin observations (impurity qualification).

Key Study Findings:
- No signs of neurologic, autonomic abnormalities, or general toxicity.
- At 40 mg/kg: slight ↓ in alertness, ↓ pinna reflex was observed.
- At 400 mg/kg: slight ↓ in alertness, ↓ pinna reflex, ↓ touch escape was observed (24 hours).
- The observed behavioral changes noticed at 40 or 400 mg/kg are considered of minor clinical relevance.
- Abiraterone plasma Cmax at 400 mg/kg was 3,670 ng/mL.

Report #:	TOX9587
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	March 4, 2010
GLP Compliance:	Yes
QA Report:	Yes (X), No ()
Drug, lot #, and % purity:	JNJ-212082-AAA or CB7630, CMLG-045/10-CR4, 100%
Doses:	0, 40, and 400 mg/kg
Species/strain:	Sprague Dawley Rat/ Crl:CD (SD)IGS
Number/sex/group or time point:	5 males/group
Route; formulation; volume; and infusion rate:	Oral gavage once for 28 days 0.5% w/v Methocel A4M (Methylcellulose) + 0.1% w/v Tween 80 10 mL/kg/day
Age:	Approximately 7 weeks
Weight:	234.4-280.5 g
Parameters:	• Behavior, • Skeletal muscle tone, • Reflexes, • Body temperatures • Overt autonomic and neurological effects
Methods	Functional Observational Battery measurements taken predosing, and at approximately at 1, 2, 4, 6, and 24 hours on Day 7, 13, and 22

Pulmonary effects:

8210847: Respiratory and Safety Pharmacology Evaluation Using Head-Out Plethysmography of CB7630 following oral gavage administration to rats

Key Study Findings:

- No mortality in the study.
- Lower tidal volume was observed in rats at 750 mg/kg (4500 mg/m^2).

Report #:	8210847
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation	May 26, 2009
GLP Compliance	Yes
QA Report	Yes (X), No ()
Drug, lot #, and % purity	CB7630; 1274-43-2, purity information not provided
Doses:	0, 100, 750, 2000 mg/kg (0, 600, 4500, and 12000 mg/m^2)
Species/strain:	Rat/ Crl:CD(SD)
Number/sex/group or time point:	8 males/group
Route; formulation; volume; and infusion rate:	Oral Gavage single dose; [0.5% (w/v) Methocel A4M, 0.1% (w/v) Tween 80, and 0.9% (w/v) sodium chloride in reverse osmosis water 10 mL/kg
Age:	13 weeks
Weight:	365-437 g
Parameters:	Respiratory rate, tidal volume, and minute volume

Table excerpted from sponsor:

Group Designation	No. of Animals[b] Male	Dose Level (mg/kg)	Dose Concentration (mg/mL)
1 (Control)[a]	8	0	0
2 (Low)	8	100	10
3 (Mid)	8	750	75
4 (High)	8	2000	200

a Animals in Group 1 received vehicle control article only.
b All animals were dosed at a volume of 10.0 mL/kg.

Results and Conclusion:

- No mortalities were observed.
- The only clinical sign was labored breathing in 1 M at 750 mg/kg and 2000 mg/kg. The labored breathing took place after being placed in the plethysmography chamber on both Day 1 and 2 of the dosing phase. This observation was not dose related since respiration returned to normal once animals were adjusted in the chambers.
- The pulmonary ventilation of male rats treated with a single dose of CB7630 or the vehicle control was monitored pre-dosing (to obtain baseline), through the times of Cmax (0-6 hours post dose), and at 24 hours post dosing.

- No significant differences were seen in tidal volume or respiration rate (See sponsor's Figures 2 and 3).
- A decrease in tidal volume (-16%) was observed at 750 mg/kg (45000 mg/kg). This finding is not likely to be clinically relevant as changes in tidal volume did not occur in a dose-dependent manner (See sponsor's Figure 1 below).

Figure 1
Tidal Volume

(Mean ± S.E.M.)

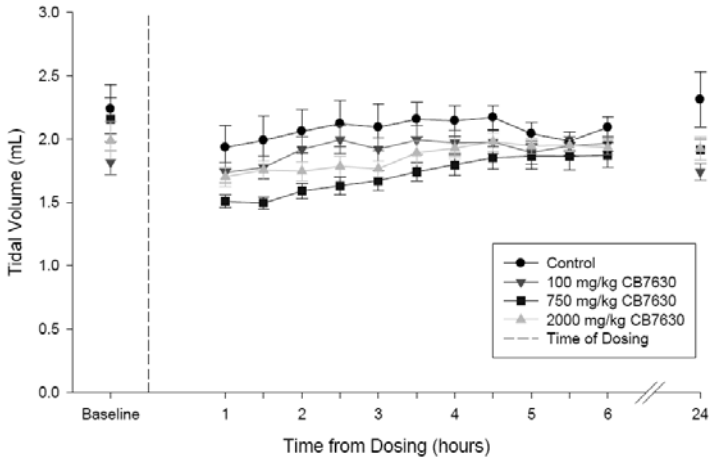
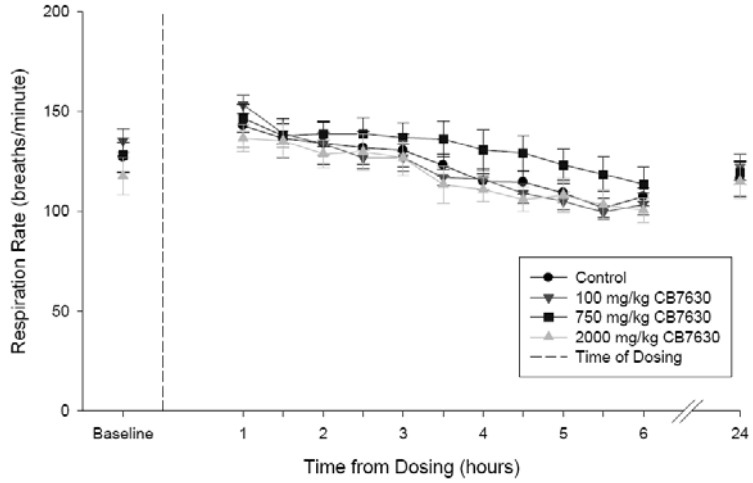
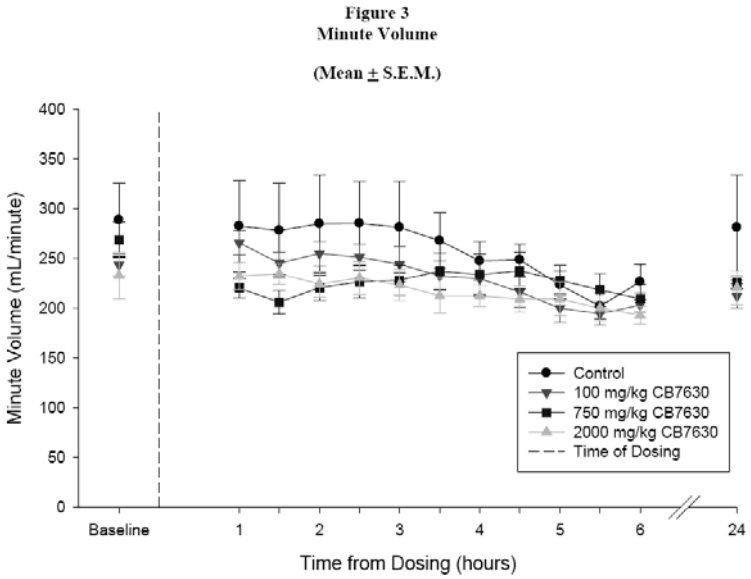


Figure 2
Respiration Rate

(Mean ± S.E.M.)





[Figures excerpted from sponsor]

Gastric irritation effects:

1632-1: An acute gastric irritation study in the male mouse, a single dose toxicity study in the male rat, a multiple dose toxicity study in the male rat and toxicokinetic study in the male rat.

Key Study Findings:
- No remarkable findings.

Report #:	1632-1
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	March 4, 2010
GLP Compliance:	Yes
QA Report:	Yes (X), No ()
Drug, lot #, and % purity:	CB7630, P 1621 M1, 98%
Doses:	0 and 800 mg/kg
Species/strain:	mice/ MFI strain
Number/sex/group or time point:	10 males/group
Route; formulation; volume; and infusion rate:	Oral gavage single dose day 1 0.5% w/v Methocel A4M + (Methylcellulose) + + NaCl + 0.1% w/v Tween 80 10 mL/kg/day
Age:	Approximately 7-9 weeks
Weight:	No information was given
Parameters:	• Environment • Body weight

Methods	• Observations and condition Necropsy 14 days after dose. Animals checked for gastrointestinal irritancy.

Results and Conclusion:

- Two groups of 10 male mice were administered a single oral dose at 0 and 800 mg/kg/bw.
- After 14 days of treatment, all animals were scheduled for necropsy and examined for gastrointestinal irritancy.
- No remarkable changes in terms of gastrointestinal irritancy were observed.

Cardiovascular effects:

692409: A Pharmacological Assessment of the Effect of CB7630 on the Cardiovascular System of the Cynomolgus Monkey Using Telemetry

Report #:	692409
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation	April 12, 2002
GLP Compliance	Yes
QA Report	Yes (X), No ()
Drug, lot #, and % purity	CB7630, 827-28-1, 95.9%
Doses:	0, 250, 750, and 2000 mg/kg
Species/strain:	Cynomolgus monkeys/ *Macaca fascicularis*
Number/sex/group or time point:	1 male/group/ time-point (4 males/dose)
Route; formulation; volume; and infusion rate:	Oral gavage once daily x 3 days; Solution consisting of Methocel A4M (0.5% w/v), Tween 80 (0.1% w/v) and NaCl (0.9% w/v) in deionized water 10 mL/kg
Age:	3-4 years
Weight:	2.5-3.0 kg
Parameters:	Clinical signs Systemic arterial blood pressures (systolic, diastolic, mean, and pulse pressure) Heart rate ECG intervals (PR, RR, QRS, and QT) ECG waveforms Body temperature

Table excerpted from sponsor

Dosing Schedule (250, 750, and 2000 mg/kg or Vehicle)				
Animal				
Number	**Dose 1**	**Dose 2**	**Dose 3**	**Dose 4**
111	vehicle	250	2420	2420
112	250	750	vehicle	750
113	750	2000	250	vehicle
114	2000	vehicle	750	250

Note: The target dose was 2000 mg/kg. Following dose formulation analysis, Animals No. 113 and 114 received 2000 mg/kg of test article, whereas Animals No. 111 and 112 received 2420 mg/kg. For the purpose of the report, the high dose will be expressed as 2000 mg/kg.

Results and Conclusion:

- Measurements were taken in conscious unrestrained animals and no deaths occurred during the study.
- Clinical signs were noted in 1 monkey at 2000 mg/kg and consisted of soft, pale, white feces the morning following the treatment and liquid, white, feces with mucoid material two days following the dose administration.
- At 250 and 750 mg/kg, pale feces was noted in 4/4 animals the day following the dose administration. The pale feces was due to the presence of test article.
- No other remarkable changes in terms of hemodynamic and electrocardiographic parameters were observed.

071018: Effects of Abiraterone and Abiraterone Acetate on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells

Report #:	071018
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation	April 11, 2008
GLP Compliance	Yes
QA Report	Yes (X), No ()
Drug, lot #, and % purity	Abiraterone acetate (CB 7630) and Abiraterone (CB 7598); 562-97-1, 99.7%
Methods	Abiraterone: 0, 10, and 27μM
Doses:	Abiraterone acetate: 0, 1.3, 3, 10, and 27μM
Study Design:	Standard hERG assay

Results and Conclusion:

- Abiraterone (CB 7598) and abiraterone acetate (CB 7630) was tested up to the maximum soluble concentration of 27μM.

- For abiraterone, the inhibition of human ether-à-go-go-related gene (hERG) tail current are listed in the following sponsor Table 2 below:
- Abiraterone inhibited the hERG K+ current at 10 and 27 µM by 2% and 6%, respectively. The inhibitory effect of abiraterone on hERG potassium current was not determined since inhibition was < 50%. The inhibition noted in this study does not appear to have physiological relevance.

Table 2: Mean Percent Inhibition of hERG current by Abiraterone
Mean percent inhibition at each abiraterone concentration (Mean), standard deviation (SD), standard error of the mean (SEM) and number of cells (n).

Concentration (µM)	Mean	SD	SEM	n
0	0.3%	0.2%	0.1%	3
10	1.6%*	0.3%	0.2%	3
27	5.7%*	0.3%	0.2%	3

* Value is statistically different than vehicle alone.

[Table excerpted from sponsor]

- For abiraterone acetate, the inhibition of human ether-à-go-go-related gene are listed in the following sponsor Table 4 below:
- The inhibition at 3, 10 and 27 µM was statistically significant (P<0.05) when compared to vehicle control values (0.3 ± 0.1% (n = 3) in control). The IC_{50} for the inhibitory effect of abiraterone acetate on hERG potassium current was 12.2 µM.

Table 4: Mean Percent Inhibition of hERG current by Abiraterone Acetate
Mean percent inhibition at each abiraterone acetate concentration (Mean), standard deviation (SD), standard error of the mean (SEM) and number of cells (n).

Concentration (µM)	Mean	SD	SEM	n
0	0.3%	0.2%	0.1%	3
1.3	1.9%	0.2%	0.1%	3
3	10.4%*	0.2%	0.1%	3
10	38.1%*	0.5%	0.3%	3
27	84.4%*	4.2%	2.1%	4

* Value is statistically different than vehicle alone.

[Table excerpted from sponsor]

- This study does not predict a high risk of QT prolongation.

2.6.2.5 Pharmacodynamic drug interactions

400378: *In vitro* evaluation of CB7630 as an inducer/suppressor of CYP1A2, 2C9 and 3A4 expression in primary cultured of human hepatocytes

Report #: 400378
Conducting Laboratory and Location: (b) (4)

(b) (4)

Date of Study Initiation	March 16, 2007
GLP Compliance	No
QA Report	Yes (), No (X)
Drug, lot #, and % purity	Abiraterone acetate (CB 7630), 562-88-1, 98.3 %
Methods:	To determine if abiraterone acetate at 0.1, 1, and 10 uM induces or suppresses CYP1A2, CYP2C9, and CYP3A4 in cultured human hepatocytes after 48 hours of treatment
Study Design:	HPLC using media from cell culture of human hepatocytes containing both phase 1 and phase 2 metabolizing enzymes from a single donor Resorufin, 4-hydroxydiclofenac and 6β-hydroxytestosterone were used as markers of CYP1A2, CYP2C9 and CYP3A4, respectively. Omeprazole was the control

Results and Conclusion:

- Abiraterone acetate (CB7630) was a weak inhibitor for suppressing CYP3A4 at all concentrations tested. The extent of inhibition was small and not concentration-dependent (See sponsor's Figure 3).
- Abiraterone acetate was not an inhibitor for CYP2C9 (See sponsor's Figure 2).
- Abiraterone acetate was a slight inhibitor for CYP1A2, however, this induction was not concentration dependent (See sponsor's Figure 1).

Figure 1 **7-Ethoxyresorufin *O*-Deethylation Activity in Hepatocytes (Donor Hu0615) Determined Following Two Days of Omeprazole or CB7630 Treatment**

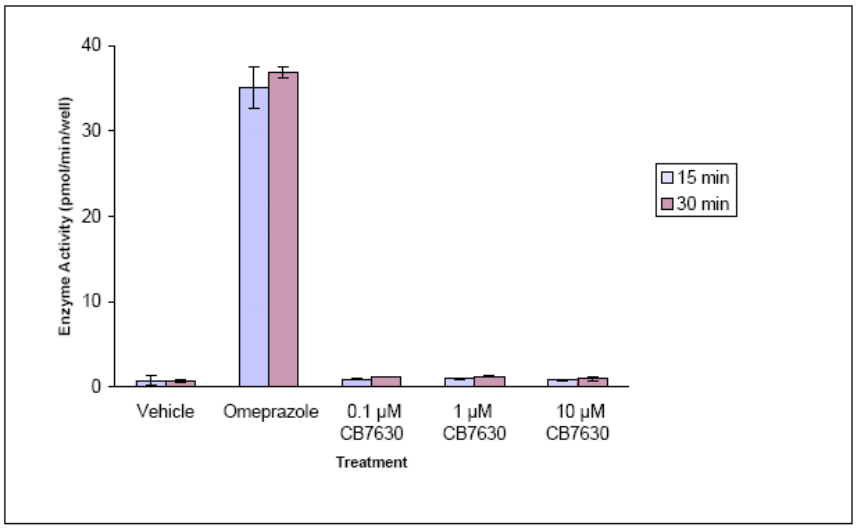


Figure 2 **Diclofenac 4-Hydroxylation Activity in Hepatocytes (Donor Hu0615) Determined Following Two Days of CB7630 Treatment**

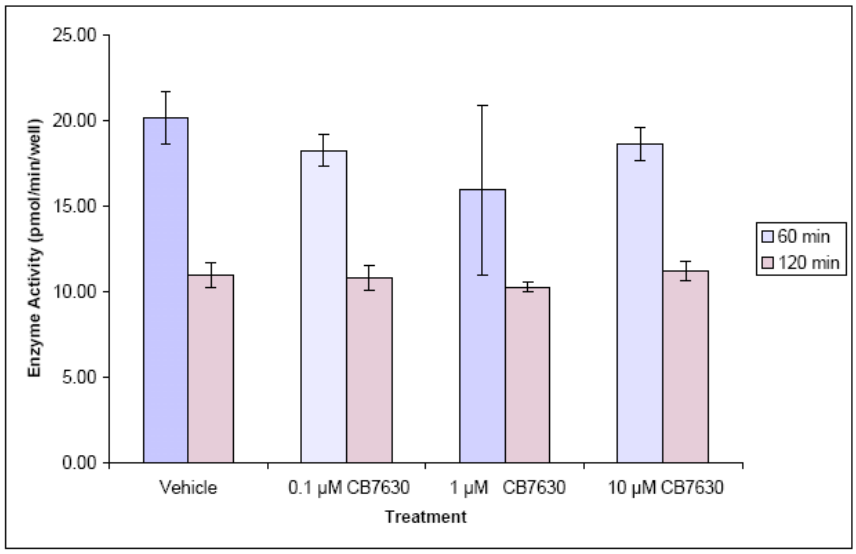
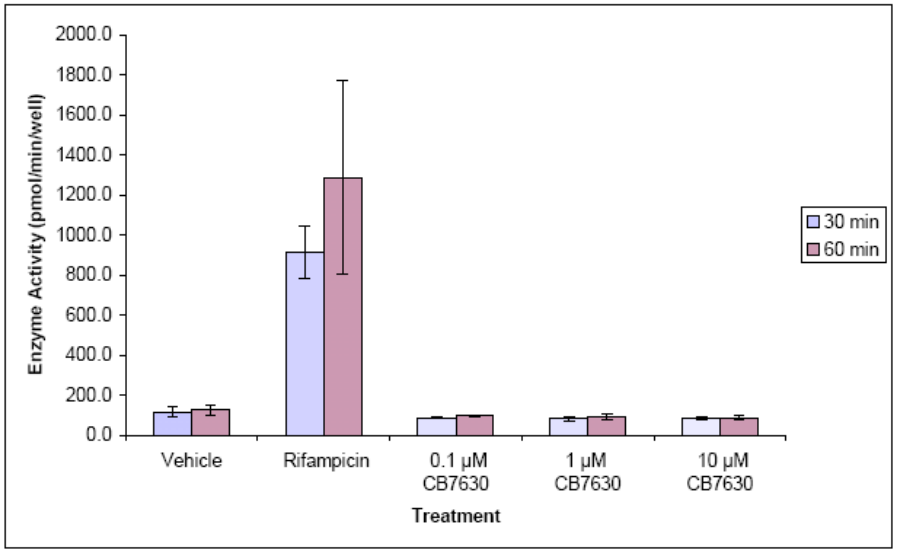


Figure 3 **Testosterone 6β-Hydroxylation Activity in Hepatocytes (Donor Hu0615) Determined Following Two Days of Rifampicin or CB7630 Treatment**



[Tables excerpted from sponsor]

400379: *In vitro* evaluation of CB7598 and CB7630 as potential inhibitors of human cytochrome P450 enzymes

Report #:	400379
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation	September 6, 2007
GLP Compliance	No
QA Report	Yes (), No (X)
Drug, lot #, and % purity	Abiraterone acetate (CB 7630), 562-88-1, 98.3 % Abiraterone (CB 7598), 562-97-1, 98.3 %
Methods:	To determine if abiraterone acetate (CB7630) and abiraterone (CB7598) at 0, 0.1, and 10 uM induces or suppresses CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in cultured human hepatocytes after 48 hours of treatment
Study Design:	HPLC using media from cell culture of human hepatocytes containing both phase 1 and phase 2 metabolizing enzymes from 50 donors. Table 1 shows overview of enzymatic

reactions.

Text Table 1 Overview of Analyzed Enzymatic Reactions for Summary

Enzyme	Enzyme Reaction	Probe Substrate Concentration (μM)	CB7598 and CB7630 Concentration (μM)	Selective Inhibitor	Inhibitor Concentration (μM)
CYP1A2	7-Phenacetin *O*-deethylase	4, 10, 20, 40, 80, 200, 400	0, 0.1, 1 and 10	α-Naphthoflavone	0.2
CYP2A6	Coumarin 7-Hydroxylase	1, 2, 5, 10, 20		Tranylcypromine	3.0
CYP2C9	Tolbutamide Methyl-Hydroxylase	125, 250, 500, 750, 1500		Sulfaphenazole	20.0
CYP2C19	*S*-Mephenytoin 4'-Hydroxylase	25, 50, 100, 200, 300		Ticlopidine	1.0
CYP2D6	Dextromethorphan *O*-Demethylase	0.25, 0.5, 2, 4, 10		Quinidine	0.25
CYP2E1	Chlorzoxazone 6-Hydroxylase	30, 60, 120, 200, 300		4-Methylpyrazole	40.0
CYP3A4/5	Testosterone 6β-Hydroxylase	25, 50, 100, 150, 200		Ketoconazole	0.1
CYP3A4/5	Midazolam 1-hydroxylase	0.5, 2, 4,10, 20		Ketoconazole	0.1

[Table excerpted from sponsor]

Results and Conclusion:

Abiraterone (CB7598):

- Abiraterone (CB7598) was not an inhibitor for CYP2A6 and CYP2E1.
- Abiraterone (CB7598) was a moderate inhibitor of CYP2C9, CYP2C19 and CYP3A4/5.
- Abiraterone (CB7598) was a strong inhibitor towards CYP1A2 (*Ki*: 0.44 μM) and CYP2D6 (*Ki*: 0.39).

Abiraterone acetate (CB7630):

- Abiraterone acetate (CB7630) was not an inhibitor for CYP2A6
- Abiraterone acetate (CB7630) was a moderate inhibitor for CYP2E1, CYP2C9 and CYP3A4/5
- Abiraterone acetate (CB7630) was a strong inhibitor for CYP1A2 (0.32 μM) and CYP2C19 (0.12 μM).

FK7476: Study on the possible induction and/or inhibition of hepatic drug metabolizing enzymes by JNJ-212082-AAA in male and female Sprague Dawley rats, after oral administration by gavage for 1 month at doses of 0, 40 and 400 mg/kg body weight/day (TOX9587)

Report #:	FK7476
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation	October 29, 2010
GLP Compliance	No
QA Report	Yes (), No (X)
Drug, lot #, and % purity	JNJ-212082-AAA, abiraterone acetate (CB 7630), CMLG-045/10-CR4, 98.3%

Methods:	To determine if JNJ-212082-AAA, abiraterone acetate (CB 7630) had any effect at inducing and/or inhibiting hepatic drug metabolizing enzymes obtained from liver microsomes in rats
Study Design:	Protein, cytochrome P-450 content, mono-oxygenase enzyme, and phase 2 enzymes were determined from liver microsomes and cytosols obtained from male and female rats dosed with JNJ-212082-AAA daily for 28 days at 0, 40, and 400 mg/kg/day.

Results:

Male rats (See Sponsor's Figure 7.1 and 7.3):

- Abiraterone acetate (JNJ-212082-AAA) had no effect on microsomal protein content.
- Abiraterone acetate (JNJ-212082-AAA) increased relative liver weight, cytochrome P-450 content, CYP1A1/2, CYP4A1, and thyroxine glucuronyltransferase (UGT) activity at the 400 mg/kg/day dose.
- Abiraterone acetate (JNJ-212082-AAA) increased DHEA sulfotransferase activity (SULT2A1) at 40 and 400 mg/kg/day.
- Abiraterone acetate (JNJ-212082-AAA) increased in sulfotransferase activity towards oxidized abiraterone (resulting in M9) at 40 and 400 mg/kg/day.
- Abiraterone acetate (JNJ-212082-AAA) decreased cytosolic protein content, aniline hydroxylase activity (CYP2E1), *N*ethylmorphine *N*-demethylase activity (CYP3A1/2), and 7-pentoxyresorufin *O*-dealkylase activity (CYP2B) the latter not dose-dependent.

Figure 7-1: Relative liver weight (RLW), microsomal protein (Prot) and cytochrome P-450 content (P450) and enzyme activities (percentages relative to the vehicle group) in liver microsomes from male rats after administration of JNJ-212082-AAA for 1 month (RLW in g liver/100 g body weight; prot in mg/g liver; P450 in nmol/mg protein; AH = aniline hydroxylase, EM = *N*-ethylmorphine *N*-demethylase, EROD = 7-ethoxyresorufin *O*-deethylase, PROD = 7-pentoxyresorufin *O*-dealkylase, LAH = lauric acid hydroxylase, T4-UGT = T4 glucuronosyltransferase, expressed as nmol or pmol of product formed per mg of protein and per min). For details on statistics, see Table 7-3 [* : P ≤ 0.05; ** : P ≤ 0.01; *** : P ≤ 0.001 and a red * indicates reduction relative to vehicle].

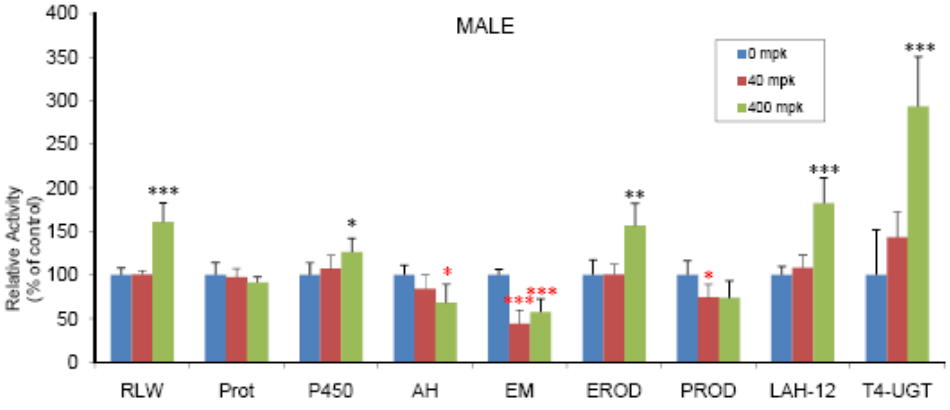
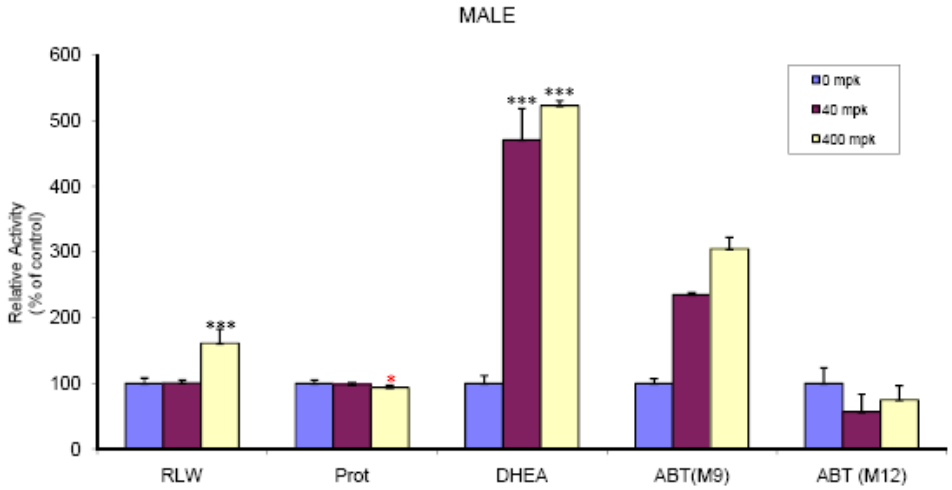


Figure 7-3: Relative liver weight (RLW), cytosolic protein (Prot) and enzyme activities (percentages relative to the vehicle group) in liver cytosolic fractions from male rats after administration of JNJ-212082-AAA for 1 month (RLW in g liver/100 g body weight; Prot in mg/g liver; DHEA = dehydroepiandrosterone sulfotransferase, ABT(M9) = sulfatase of oxidized abiraterone, ABT(M12) = sulfatase of abiraterone, expressed as nmol of product formed per mg of protein and per min). For details on statistics, see Table 7-5 [* : P ≤ 0.05; *** : P ≤ 0.001; and a red * indicates reduction relative to vehicle].



[Figures excerpted from sponsor]

Figure 7-2: Relative liver weight (RLW), microsomal protein (Prot) and cytochrome P-450 content (P450) and enzyme activities (percentages relative to the vehicle group) in liver microsomes from female rats after administration of JNJ-212082-AAA for 1 month (RLW in g liver/100 g body weight; Prot in mg/g liver; P450 in nmol/mg protein; AH = aniline hydroxylase, EM = *N*-ethylmorphine *N*-demethylase, EROD = 7-ethoxyresorufin *O*-deethylase, PROD = 7-pentoxyresorufin *O*-dealkylase, LAH = lauric acid hydroxylase, T4-UGT = T4 glucuronosyltransferase, expressed as nmol or pmol of product formed per mg of protein and per min). For details on statistics, see Table 7-4. [* : P ≤ 0.05; ** : P ≤ 0.01; *** : P ≤ 0.001 and a red * indicates reduction relative to vehicle].

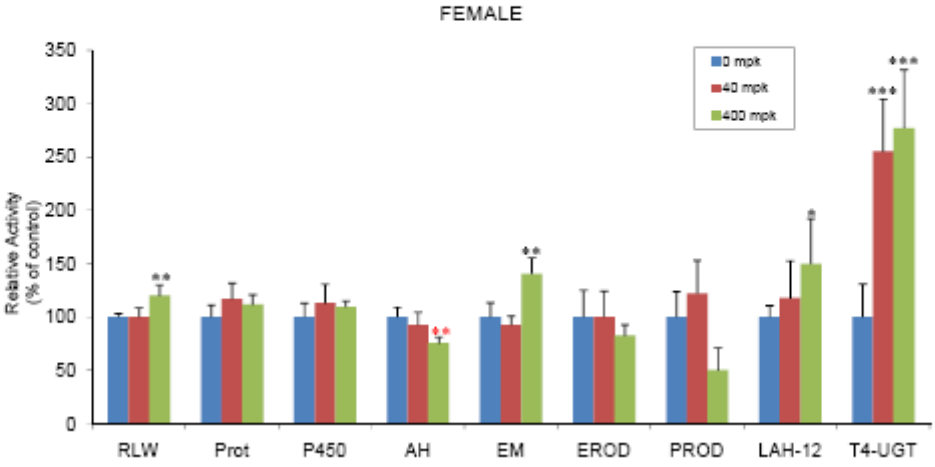
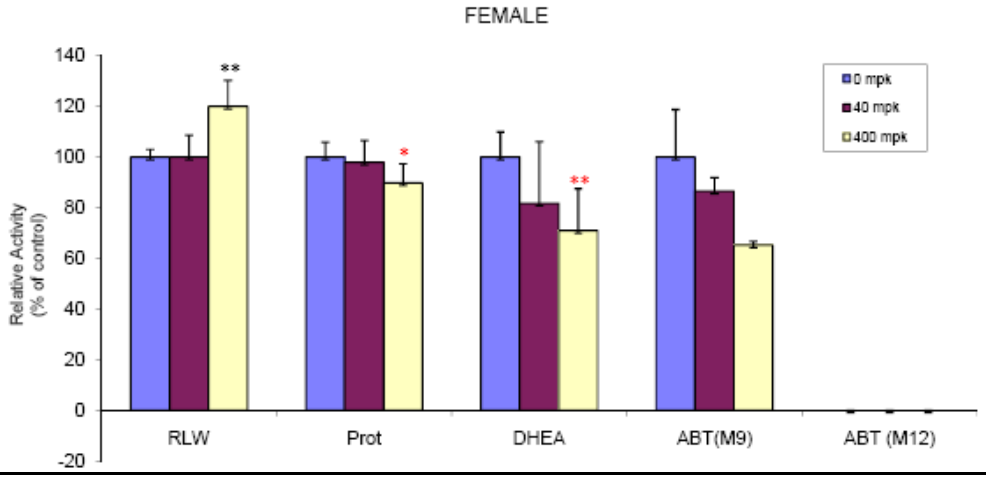


Figure 7-4: Relative liver weight (RLW), cytosolic protein (Prot) and enzyme activities (percentages relative to the vehicle group) in liver cytosolic fractions from female rats after administration of JNJ-212082-AAA for 1 month (RLW in g liver/100 g body weight; Prot in mg/g liver; DHEA = dehydroepiandrosterone sulfotransferase, ABT(M9) = sulfatase of oxidized abiraterone, ABT(M12) = sulfatase of abiraterone, expressed as nmol of product formed per mg of protein and per min). For details on statistics, see Table 7-5 [* : P ≤ 0.05; ** : P ≤ 0.01; and a red * indicates reduction relative to vehicle].



[Figures excerpted from sponsor]

Female rats (See Sponsor's Figure 7.2 and 7.4):

- Abiraterone acetate (JNJ-212082-AAA) had no effect microsomal protein content, cytochrome P-450 content, and activity of CYP1A1/2 and CYP2B.
- Abiraterone acetate (JNJ-212082-AAA) increased relative liver weight and activity of CYP3A1/2 and CYP4A1 at 400 mg/kg/day.
- Abiraterone acetate (JNJ-212082-AAA) increased UGT activity at 40 and 400 mg/kg/day.
- Abiraterone acetate (JNJ-212082-AAA) decreased cytosolic protein content, CYP2E1 activity and SULT2A1 activity towards DHEA at the highest dose of 400 mg/kg/day.
- Abiraterone acetate (JNJ-212082-AAA) decreased SULT2A1 activity towards oxidized abiraterone (resulting in M9) but not towards abiraterone, resulting in M12.

2.6.3 PHARMACOLOGY AND SAFETY PHARMACOLOGY TABULATED SUMMARIES

Study # (or Author) /Type of study	Method of Administration/ GLP?	Route	Dose	Test system	Findings
Published literature studies					
Potter et al., J. Med. Chem., 1998 / steroidal inhibition of human CYP17	*In vitro*/ No	NA	NA	Human testicular microsome	Abiraterone inhibited C17, 20-lyase and 17α-hydroxylase with IC_{50} of 2.9 and 4 nM, respectively. Abiraterone acetate was less active compared to abiraterone at inhibiting C17/20-lyase and 17α-hydroxylase with IC_{50} of 17

Study # (or Author) /Type of study	Method of Administration/ GLP?	Route	Dose	Test system	Findings
					and 18 nM, respectively. Abiraterone and abiraterone acetate did not show any activity at inhibiting aromatase and 5α-reductase.
Jarman et al., J. Med. Chem. 1998 / inhibition of human CYP17	*In vitro*/ No	NA	NA	Human testicular microsome	Abiraterone inhibited P450 17α with an IC_{50} of 4 nM No recovery of the enzyme activity was observed indicating irreversible inhibition of the enzyme
Haider et al. J. of Steroid Biochem. and Molecular Biology, 2003 / Inhibition of human and rat CYP17	*In vitro and in vivo*/ No	***In vitro:*** NA ***In vivo:*** i.p.	***In vitro:*** NA ***In vivo:*** 39.2 mg/kg/ day daily x 14 days	***In vitro:*** Rat and human testicular microsome and E. coli expressing human CYP17 and NADPH-P450 reductase ***In vivo:*** male rats	***In vitro:*** abiraterone is more active than abiraterone acetate at inhibiting P450 17α in human, rat and E. coli with IC_{50}'s of 54, 220, and 73 nM, respectively. No recovery of the enzyme activity indicating irreversible inhibition of the enzyme. ***In vivo:*** abiraterone acetate showed a ↓ ventral prostate (54-84%), complete prostate (54-84%), seminal vesicles (37-81%), and testes weights (17-36%) Abiraterone acetate decreased testosterone levels to ↓95% compared to control when given at 39.2 mg/kg/day
Haider et al. Arch. Pharm. Pharm. Med. Chem, 2001 / Inhibition of human CYP17	*In vitro*/ No	NA	NA	Human testicular microsome	abiraterone inhibited P450 17α with an IC_{50} = 73 nM
Barrie et al., J. Steroid Molec. Biol., 1994/ Effect on organ weight and hormones levels	*In vivo*/ No	i.p	0, 7.8, 39.2, and 196 mg/kg/ day daily x 14 days	Male mice	Abiraterone acetate caused a reduction of several androgen-sensitive organs (prostate, seminal vesicles, adrenals, and testes) in a dose-dependent manner Abiraterone acetate significantly decreased testosterone levels (↓92 and ↓96%) at the MD and HD

Study # (or Author) /Type of study	Method of Administration/ GLP?	Route	Dose	Test system	Findings
					when compared to control values. LH increased 212 and 325% at 39.2 and 196 mg/kg/day, respectively.
Duc et al, *J. Steroid Biochem and Mol. Bio*., 2003/Effect on organ weight and testosterone levels	*In vitro and in vivo*/ No	***In vitro:*** NA ***In vivo:*** oral gavage	***In vitro:*** NA ***In vivo:*** 50 mg/kg daily x 3 days	***In vitro:*** Rat testes microsomes ***In vivo:*** male rats	***In vitro:*** Abiraterone was more active compared to other steroidal and non-steroidal compounds at inhibiting P450 17α activity with an IC_{50} of 5.8 nM. ***In vivo:*** abiraterone acetate inhibited prostate (VP) and seminal vesicle (SV) weights with no effect on adrenal weights. Abiraterone acetate significantly increased lutenizing hormone (LH) levels (+378%). It had a modest effect at reducing testosterone (T) levels (40%).
Studies with Abiraterone and its metabolites					
8655156/ Steroid nuclear receptor binding	*In vitro*/ No	NA	NA	Cell cultures	Abiraterone (JNJ589485), ketone analog of abiraterone (JNJ46252908), abiraterone sulphate (JNJ48171188), and N-oxide abiraterone sulphate (JNJ49029578) were inactive when tested for glucocorticoid receptor binding, estrogen receptor-α binding, estrogen receptor-β binding and androgen receptor binding. Abiraterone and abiraterone sulphate produced a 52% and 69% inhibition, respectively, at binding to the human progesterone receptor. N-oxide abiraterone sulphate (JNJ49029578) was inactive at binding to the human progesterone receptor with 8% inhibition. Ketone analog of abiraterone (JNJ-46252908) inhibited progesterone and androgen

Study # (or Author) /Type of study	Method of Administration/ GLP?	Route	Dose	Test system	Findings
					receptors at 85 and 83%, respectively.
8655175/ Human PR receptor binding	*In vitro*/ No	NA	NA	Cell cultures	In the binding assay, abiraterone was more active than abiraterone sulphate at binding progesterone with IC_{50} of 230 and 400 nM, respectively when tested at concentrations ranging from 0.01 to 5 µM. In the cellular functional assay, both compounds were inactive at binding progesterone when tested at concentrations ranging from 0.01 to 5.0 µM.
2010ORB001 /Inhibition of rat CYP17; Steroid formation	*In vitro*/ No	NA	Log doses ranging from 0.003 to 10 mM	Tissue from rat testes and NCI-H295R cell line	Abiraterone sulphate (JNJ-48171188-AAA) showed a greater inhibition compared to the *N*-oxide abiraterone sulphate (JNJ-49029578) metabolite at inhibiting P450 17α in rat testes with an IC_{50} =3.6nM compared to 26.2. Abiraterone sulphate (JNJ-589485) was more active than the *N*-oxide abiraterone sulphate (JNJ-49029578) metabolite at inhibiting androstenedione, testosterone, and cortisol formation in NCI-H295R cell lines. Both metabolites had no effect on aldosterone formation. Abiraterone inhibited aldosterone formation with an IC_{50} = 2.7 µM.

Note: P450 17α = 17α-hydroxylase/C17-20 lyase or CYP17

Study #/Organ System	Method of Administration /GLP?	Species	Doses	Gender /N	Findings
TOX9587 CNS-FOB	Oral/ Yes	Rat	0, 40, and 400 mg/kg	5M/group	**40 mg/kg**: slight ↓ in alertness, ↓ pinna reflex **400 mg/kg:** slight ↓ in

Study #/Organ System	Method of Administration /GLP?	Species	Doses	Gender /N	Findings
					alertness, ↓ pinna reflex, ↓ touch escape (24hours). The observed behavioral changes noticed at 40 or 400 mg/kg are considered of minor clinical relevance. Cmax at 400 mg/kg was 3,670 ng/mL
8210847 Pulmonary	Oral/ Yes	Rat	0 100, 750, 2000 mg/kg	8M/group	No mortality **750 mg/kg** - ↓ in tidal volume (16%) 750 mg/kg. Decrease was not dose-dependent and does not appear to be toxicologically significant.
071018. DPC Gastric irritation	Oral/ Yes	Mice	0 and 800 mg/kg	10M/ group	No remarkable findings.
692409 Cardiovascular	Oral / Yes	Monkey	0 250, 750, 2000 mg/kg	1M/group	No mortality **2000 mg/kg** – clinical signs of soft, pale, white feces following treatment and liquid, white, feces with mucoid material 2 days following treatment in 1 animal. **250 and 750 mg/kg** – clinical signs of pale feces in 4/4 animals following treatment. No changes in CV parameters

Study #/Organ System	Method of Administration /GLP?	Species	Doses	Gender /N	Findings
071018 hERG current	*In vitro*/ Yes	NA	0, 1.241, and 4.137 µM	NA	Abiraterone - 5.7% inhibition of hERG function at the highest concentration tested, 27 µM. IC_{50} was not determined since inhibition was <50%. Abiraterone acetate - 84.4% inhibition at the highest concentration tested, 27 µM. IC_{50} was 12.2 µM.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Pharmacokinetics studies consisted of both *in vitro* and *in vivo* studies using abiraterone acetate. Almost all *in vivo* studies were part of the nonclinical toxicology studies in Albino Swiss mice, Sprague-Dawley (SD) rats and cynomolgus monkeys in which abiraterone acetate was dosed orally (p.o). Due to the low aqueous solubility, abiraterone acetate was dosed intravenously (iv) only once in cynomolgus monkeys and in male WHT mice. No iv and p.o. dosing studies were conducted with abiraterone. *In vitro*, abiraterone and abiraterone acetate were shown to have a low apparent permeability and are not substrates of P-glycoprotein (P- gp) in Caco-2 cell monolayers. Abiraterone showed little inhibition of P-gp whereas abiraterone acetate significantly inhibited P-gp with and IC_{50} of 10.8 µM. After a single dose of abiraterone acetate was administered to mice *in vivo*, abiraterone acetate was rapidly converted to abiraterone. Also in mice, abiraterone plasma levels were markedly higher via the iv route compared to oral dosing. Exposure to abiraterone after oral dosing of abiraterone acetate represented a bioavailability of 37% in mice which was greater than that observed in monkeys (less than 2 %).

Radio-labeled abiraterone (JNJ-589485) is highly bound ($\geq$99.8%) to proteins in plasma across multiple species (mouse, rat, rabbit, monkey and man) with no relevant species differences occurring. Furthermore, in human plasma, abiraterone was primarily or exclusively bound ($\geq$89%) to both human serum albumin (HAS) and human α1-acid glycoprotein (α1-AGP) at a concentration of 1750 ng/mL. In a clinical study, liver impairment had no impact on the unbound fraction of abiraterone in the plasma when protein binding of abiraterone was measured in male subjects with mild or moderate hepatic impairment compared to matched subjects with normal hepatic function.

In monkeys *in vivo*, the pathways proposed for metabolism of abiraterone acetate (in plasma) appeared to be initiated with hydrolysis of the acetate ester. This was followed by various multiple oxidations and direct sulphation of the free hydroxyl group of abiraterone. Combinations of hydrogenations, hydroxylation, and Phase 2 conjugation (sulfation and glucuronidation) were also observed. The main metabolite also was abiraterone sulphate. Thirty-nine metabolites were characterized in monkey plasma along with abiraterone acetate and abiraterone. Of these, several were isomers of mono-oxy-abiraterone sulfate and of dihydro-mono-oxy-abiraterone. Several metabolites were also formed by hydrogenation and mono-oxidation. In the pivotal toxicity studies in the rat (26 week) and monkey (39 week), both species were exposed major metabolites which included abiraterone sulfate metabolite, JNJ-48171188 (M45, ABT sulphate) and abiraterone oxidated sulfate metabolite, JNJ-49029578 (M31, *N*-oxide ABT sulphate). At the highest administered dose of abiraterone acetate, in both rat and monkey, exposure (Cmax and AUC) to abiraterone sulphate exceeded the exposure to abiraterone sulphate in man. At the maximum dose of abiraterone acetate in monkeys (1000 mg/kg), exposure to *N*-oxide abiraterone sulphate approximated that in man. In rats, exposure to *N*-oxide abiraterone sulphate was markedly lower (20%) at the maximum dose of abiraterone acetate (400 mg/kg). These data suggest that at the highest dose of abiraterone acetate from pivotal toxicity studies, exposure to abiraterone sulphate was qualified in both rat and monkeys while exposure to *N*-oxide abiraterone sulphate was qualified in monkey, but not in rats.

In rats, the excretion of radio-labeled abiraterone in urine was limited (< 2 % of the administered dose), and almost all radio-labeled abiraterone (89.9-93.4% by 168 h post-dose) was recovered in the feces. There was no difference in routes and rates of excretion between male and female rats. In bile duct-cannulated rats, 18.7 % of the administered radio-labeled abiraterone was excreted in bile over 120 h. For all excretion routes, almost all radio-labeled abiraterone was excreted within 24 h. In man, the majority of radiolabeled material excreted occurred predominantly (87.9%) in the feces, in which the main compound was unchanged abiraterone acetate. About 5 % of administered radio-labeled abiraterone was recovered from the urine (Studies reviewed by Margaret Brower, PhD under IND 71, 023).

The ex-vivo inducing effect of abiraterone acetate on the major oxidative enzyme activities obtained in livers from male and female Sprague Dawley rats showed that abiraterone acetate did not affect microsomal protein content in males and females, while cytochrome P-450 content was increased in males only. Abiraterone acetate had no inducing effect on the enzymatic activities including 7-ethoxyresorufin *O*-deethylase (CYP1A1/2) and 7-pentoxyresorufin *O*-dealkylase (CYP2B) in females, while in males they were significantly increased (at 400 mg/kg/day only) and decreased, respectively. A significant increase in relative liver weight and induction of lauric acid hydroxylation (CYP4A1) was observed in both genders at 400 mg/kg/day only, while thyroxine glucuronyltransferase activity (UGT) was increased at the highest dose only in

males, but at both doses in females. Aniline hydroxylase (CYP2E1; both genders) and *N*-ethylmorphine *N*-demethylase (CYP3A1/2; males only) activities were decreased, while the latter was increased in females at 400 mg/kg/day. In male rats, a statistically significant increase in DHEA sulfotransferase activity (SULT2A1) and in SULT2A1 activity towards abiraterone oxide (resulting in *N*-oxide abiraterone sulphate) was observed at both 40 and 400 mg/kg/day. In female rats a statistically significant decrease in activity was observed in SULT2A1 activity towards DHEA at the highest dose, while a dose-dependent decrease in SULT2A1 activity towards oxidized abiraterone (resulting in *N*-oxide abiraterone sulphate) was also observed.

In an *in vitro* induction study with human hepatocytes, abiraterone acetate showed a slight inhibition of CYP3A4 activity (30% when compared to rifampicin) and no effect on CYP2C9. Furthermore, in human microsomes, abiraterone acetate and abiraterone strongly inhibited CYP1A2 and CYP2D6, while CYP2C9 and CYP3A4/5 were only moderately inhibited. Abiraterone acetate strongly inhibited CYP2C19 and moderately inhibited CYP2E1 while abiraterone moderately inhibited CYP2C19 but did not inhibit CYP2E1. No inhibition was seen for CYP2A6 when testing both compounds.

2.6.4.2 Methods of Analysis – No studies reviewed

2.6.4.3 Absorption

8202265: Determination of the apparent permeability of abiraterone and abiraterone acetate through Caco-2 cell monolayers and evaluation as P-glycoprotein substrates and inhibitors

Report #:	8202265
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	May 20, 2010
GLP Compliance:	No
QA Report:	Yes (), No (X)
Drug, lot #, and % purity:	Radiolabeled: ^{14}C-Abiraterone acetate, 53459-19-27, 98% Non-radiolabeled: Abiraterone, 562-97-1, 100% Abiraterone acetate, 1453-3-1, 100%

Results:

- Both abiraterone and abiraterone acetate had low permeability in Caco-2 cell monolayers and are not substrates of P-gp.
- Abiraterone showed little inhibition on P-gp.
- Abiraterone acetate significantly inhibited P-gp with an IC_{50} of 10.8 µM.
- Both abiraterone and abiraterone acetate were stable when dosed at basolateral compartments but not stable when dosed at apical compartments.

[redacted] Pharmacokinetics of abiraterone in the mouse

Report #:	[redacted]-001
Conducting Laboratory and Location:	[redacted]
Date of Study Initiation:	12/2004
GLP Compliance:	No
QA Report:	Yes (), No (X)
Drug, lot #, and % purity:	Radiolabeled: ^{14}C-Abiraterone acetate, 53459-19-27, 98% Non-radiolabeled: Abiraterone, 562-97-1, 100% Abiraterone acetate, 1453-3-1, 100%

Methods

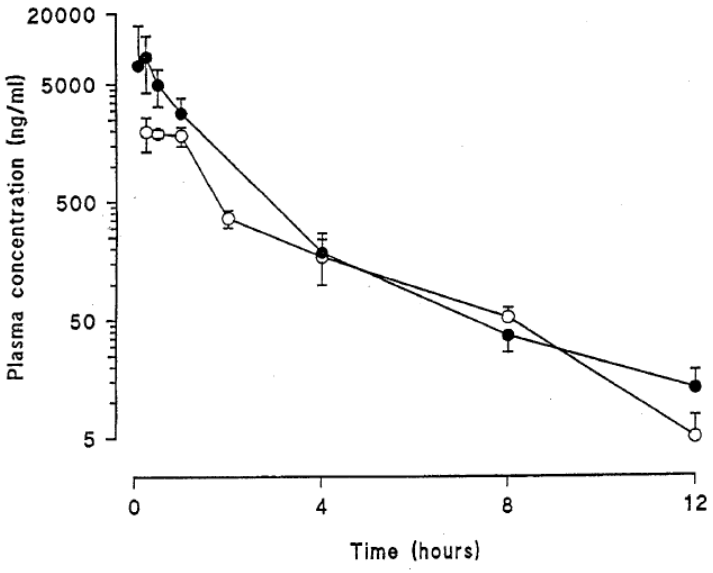
Doses:	Single Dose of 0.1 and 39.2 mg/kg (CB7630)
Species/strain:	Mice/WHT mice
Number/sex/group or time point:	4 males
Route, formulation, volume, and infusion rate:	Oral Gavage and iv infusion Saline, 15.4% ethanol and 3.8%, and Tween 80 7.84 mg/ml
Age:	9 weeks old
Weight:	~35 g
Sampling times:	5 min, 30 min, 1, 2, 4, 8, and 12 hours
Study design:	▪ To compare plasma concentrations of abiraterone or CB7598 (metabolite) after administration of oral and iv doses of abiraterone acetate (CB7630) ▪ Blood was collected by cardiac puncture at various time-points listed above.

Results:

- Abiraterone acetate (CB7630) rapidly converted to the metabolite, abiraterone (CB7598).
- Higher concentrations of abiraterone were attained in the plasma concentration when given iv compared to oral.
- AUC was higher in the iv route compared to the oral (oral route is 3807 ng/ml x h and iv route is 10251 ng/ml x h)
- Oral bioavailability was 37%.
- Summary of results are listed below in Sponsor's Figure 1.

Figure 1

Comparison of the plasma concentrations of CB7598 achieved after administration of oral (o) and intravenous (•) doses of CB7630 (0.1mmoles/kg) to male WHT mice (points represent means±s.d.; n=4)



[Figure excerpted from sponsor]

05-501648: Pharmacokinetic modeling of CB7630 and its metabolite CB7598 following oral and intravenous administration of various formulations of CB7630 to cynomolgus monkeys

Report #:	05-501648
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	11/2005
GLP Compliance:	No
QA Report:	Yes (), No (X)
Drug, lot #, and % purity:	CB7630 (drug) and CB7598 (metabolite); no other information was given

Methods

Doses:	Oral: 250 mg/kg on Days 1, 3, 5, and 7 iv: 24 mg/kg on Day 7
Species/strain:	Monkey/cynomolgus
Number/sex/group or time point:	2 males
Route, formulation, volume, and infusion rate:	Oral Gavage and iv infusion Saline, 15.4% ethanol and 3.8%, and Tween 80 7.84 mg/ml
Age:	No information given
Weight:	No information given
Sampling times:	▪ Oral: predose, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours post dose. ▪ iv: predose, 0.083, 0.25, 0.5, 1, 2, 6, 12, and 24 hours post dose.
Study design:	▪ To determine the plasma PK parameters of abiraterone acetate (CB7630), and its metabolite, abiraterone (CB7598) following oral and intravenous administration of various formulations of abiraterone acetate (CB7630) to male monkeys. ▪ For oral dosing, blood was collected on Days 1, 3, and 5 ▪ For iv dosing, blood was collected on Day 7

Results:

- There was no difference in any PK parameters for the abiraterone (CB7598) using three different oral formulations (i.e., Tablet 1, Tablet 2, and Capsule formulations) of abiraterone acetate (CB7630).
- Mean bioavailability of the oral formulations ranged from 1.5% to 1.6%.
- Mean $t_{1/2}$ estimates of abiraterone (CB7598) ranged from approximately 6.6 to 9.5 hours after oral and intravenous routes, respectively.
- Results are shown in Sponsor's Table 1 below.

- Abiraterone acetate (CB7630) plasma concentrations were measurable following intravenous and not oral administration (See Sponsor's Table 2 below).

Table 1. Group Mean (SD for N>2) Pharmacokinetic Parameters of CB7598 After Oral and Intravenous Administration of CB7630 to Male Monkeys at Dose Levels of 250 mg/animal and 24 mg/kg, Respectively

Parameter	CB7630 Formulation			
	Tablet 1	Tablet 2	Capsule	Intravenous
T_{max} (hr)	2.5 ± 1.22	2.0 ± 0.00	2.3 ± 0.82	0.22 ± 0.07
C_{max} (ng/mL)	104.6 ± 80.48	113.0 ± 52.76	77.8 ± 75.70	19783.3 ± 2291.22
AUC_{0-t} (ng·hr/mL)	570.2 ± 488.80	514.6 ± 237.97	474.0 ± 418.10	19383.9 ± 2080.82
$AUC_{0-\infty}$ (ng·hr/mL)	622.4 ± 514.18	558.0 ± 247.73	618.7 ± 407.16	19763.5 ± 2128.25
$t_{1/2}$ (hr)	7.8 ± 3.77	7.6 ± 4.25	9.5 ± 4.55	6.6 ± 1.59
F	1.6 ± 1.13	1.5 ± 0.50	1.6 ± 0.98	NA

$AUC_{0-\infty}$ = area under the plasma concentration-time curve extrapolated to infinity; AUC_{0-t} = area under the plasma concentration-time curve to the last measurable concentration during the sampling interval; C_{max} = maximum observed plasma concentration; F = % bioavailability; $t_{1/2}$ = plasma elimination half-life; T_{max} = time to reach maximum plasma concentration.

Table 2. Group Mean (SD for N>2) Pharmacokinetic Parameters of CB7630 After Oral and Intravenous Administration to Male Monkeys at Dose Levels of 250 mg/animal and 24 mg/kg, Respectively

Parameter	CB7630 Formulation			
	Tablet 1	Tablet 2	Capsule	Intravenous
T_{max} (hr)	ND	ND	ND	0.083 ± 0.00
C_{max} (ng/mL)	ND	ND	ND	10788.3 ± 3127.97
AUC_{0-t} (ng·hr/mL)	ND	ND	ND	1697.9 ± 463.18
$AUC_{0-\infty}$ (ng·hr/mL)	ND	ND	ND	1693.7 ± 459.14
$t_{1/2}$ (hr)	ND	ND	ND	1.6 ± 0.45

$AUC_{0-\infty}$ = area under the plasma concentration-time curve extrapolated to infinity; AUC_{0-t} = area under the plasma concentration-time curve to the last measurable concentration during the sampling interval; C_{max} = maximum observed plasma concentration; ND = not determined because plasma concentration values were below the LLOQ; $t_{1/2}$ = plasma elimination half-life; T_{max} = time to reach maximum plasma concentration.

[Tables excerpted from sponsor]

2.6.4.4 Distribution

8282443: Pharmacokinetics, distribution, metabolism and excretion of radioactivity following oral administration of 14C-Abiraterone Acetate to rats (Reviewed by Margaret Brower, PhD under IND 71, 023)

Key Study Findings

- Systemic exposure greater in males; half-life extended in females
- Abiraterone acetate crosses blood:brain barrier; detected in CNS for 24h postdose
- Highest drug distribution: GI contents, liver, bile, kidney, urine, melanin containing tissues (e.g. eye)
- Major route of elimination was feces; excretion within 24h post-dose
- Parent drug primarily detected in feces (33%), abiraterone and abiraterone sulfate detected at 13-25%
- Abiraterone sulfate primary metabolite in plasma

8202266: *In vitro* protein binding of 14C-Abiraterone to plasma proteins from rat, monkey, and human and to human serum albumin (HAS) and α1-acid glycoprotein (AAG) (Reviewed by Margaret Brower, PhD under IND 71, 023)

Key Study Findings

- Protein binding of abiraterone acetate *in vitro*: human (98.8-99.1%) ≥ rat (98.9-99%) ≥ monkey (97.4-98.3%).

FK7603: The binding of ^{14}C-abiraterone to proteins in plasma from mouse, rat, rabbit, monkey and man and to human serum albumin (HSA) and α1-acid glycoprotein (α1-AGP)

Report #:	FK7603
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	10/1/2010
GLP Compliance:	No
QA Report:	Yes (), No (X)
Drug, lot #, and % purity:	Radiolabeled abiraterone (^{14}C -JNJ-589485), Lot # 2560, 99.9%

Methods

Study design:

- To determine the extent of binding of radiolabeled abiraterone (JNJ-589485) on the following using equilibrium dialysis:
- 1) proteins in the plasma of mice, rats, rabbits, monkeys and humans
- 2) isolated human plasma proteins (HAS and α1-acid glycoprotein (α1-AGP)
- In mice, rats, rabbits and monkeys, free fractions were tested at 350 and 3500 ng/mL
- In humans, free fractions were tested at 175, 350 and 1750 ng/mL.

Results:

- Abiraterone (JNJ-589485) is highly bound (≥99.8%) to proteins in plasma across multiple species (mouse, rat, rabbit, monkey and man).
- No relevant species differences were observed.
- No concentration-dependent correlation between abiraterone (JNJ-589485) concentration and the free fraction was observed.
- Results are shown in sponsor's Table 6.1 below.

Table 6-1: Binding and fraction unbound (%) of different concentrations of ^{14}C-JNJ-589485 to proteins in plasma from various species after 1 h equilibrium dialysis.

Species	Final concentration ^{14}C-JNJ-589485 in plasma (ng/ml)							
	175		350		1750		3500	
	Unbound (%)	Bound (%)	Unbound (%)	Bound (%)	Unbound (%)	Bound (%)	Unbound (%)	Bound (%)
mouse (male)	-	-	0.111 ± 0.012	99.9	-	-	0.101 ± 0.003	99.9
rat (male)	-	-	0.182 ± 0.009	99.8	-	-	0.181 ± 0.003	99.8
rabbit (female)	-	-	0.147 ± 0.011	99.9	-	-	0.165 ± 0.009	99.8
monkey (male)	-	-	0.147 ± 0.003	99.9	-	-	0.128 ± 0.002	99.9
human (male)	0.086 ± 0.010	99.9	0.079 ± 0.009	99.9	0.077 ± 0.005	99.9	-	-

[Table excerpted from sponsor]

- Abiraterone (JNJ-589485) is highly bound to protein to both human plasma proteins (α1-AGP and HAS) at a concentration of 1750 ng/mL (See sponsor's Table 6.2).

Table 6-2 Binding (%) and fraction unbound (%) of ^{14}C-JNJ-589485 at a concentration of 1750 ng/mL to purified human α1-acid glycoprotein (AGP) and human serum albumin (HSA).

Conditions	unbound (%)	bound (%)
α1-AGP, 0.07% (w/v)	10.6 ± 1.075	89.4
α1-AGP, 0.10% (w/v)	7.57 ± 0.437	92.4
α1-AGP, 0.20% (w/v)	5.56 ± 0.346	94.4
HSA, 4.3 % (w/v)	0.121 ± 0.007	99.9
HSA, 4.3 % (w/v) + AGP, 0.07 % (w/v)	0.114 ± 0.009	99.9

[Table excerpted from sponsor]

FK7448: The protein binding of ^{14}C-JNJ-589485 in human pre-dose plasma of subjects of a phase 1 single dose open-label pharmacokinetic study (COU-AA-11)

Report #: FK7448

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation:	7/15/2005
GLP Compliance:	No
QA Report:	Yes (), No (X)
Drug, lot #, and % purity:	Radiolabeled abiraterone (14C -JNJ-589485), Lot # 2525, 99.7%

Methods

Study design:

- To determine the unbound fraction of radio-labeled abiraterone (^{14}C-JNJ-589485) in pre-dosed plasma samples from a phase 1 single dose open-label PK study in male subjects with mild or moderate hepatic impairment compared to subjects with normal hepatic function.
- Radio-labeled abiraterone (14C-JNJ-589485) in plasma and buffer was analyzed by LSC and fraction of unbound radiolabeled abiraterone was calculated.

Results:

- The unbound fraction of abiraterone in plasma was slightly higher in subjects with mild hepatic impairment compared to subjects with moderate hepatic impairment and normal hepatic function (See sponsor's Table 6.1 below).
- Overall, liver impairment had no impact on the unbound fraction of abiraterone in plasma.

Table 6-1 % unbound abiraterone (^{14}C-JNJ-589485) in pre-dosed plasma after a 1 h equilibrium dialysis.

Mild Hepatic Impairment Child-Pugh Class A		Moderate Hepatic Impairment Child-Pugh Class B		Normal Hepatic Function	
Subject number	% unbound fraction	Subject number	% unbound fraction	Subject number	% unbound fraction
001	0.15	009	0.27	017	0.13
002	0.50	010	0.18	018	0.31
003	0.14	011	0.12	019	0.20
004	0.12	012	0.15	020	0.14
005	0.21	013	0.16	021	0.26
006	0.27	014	0.18	022	0.15
007	0.14	015	0.19	023	0.17
008	0.22	016	0.29	024	0.12
Mean	**0.22**	**Mean**	**0.19**	**Mean**	**0.19**
Standard deviation	**0.12**	**Standard deviation**	**0.06**	**Standard deviation**	**0.07**

[Table excerpted from sponsor]

2.6.4.5 Metabolism

400380: Metabolite profiling of abiraterone acetate in cryopreserved hepatocytes from humans (Reviewed by Margaret Brower, PhD under IND 71, 023)

Key Study Findings

– Major *in vitro* biotransformation pathway: deacetylation producing CB7598 (abiraterone), followed by sulfate and glucuronic acid conjugation, alone or in combination with oxidation

8214362: Identification of metabolites of abiraterone acetate in selected human plasma samples (Reviewed by Margaret Brower, PhD under IND 71, 023)

Key Study Findings

Major metabolites: conjugate of abiraterone sulfate, isomers of mono oxyabiraterone sulfate and abiraterone, primarily identified from 2 to 96 hours postdose

FK7389: *In vitro* metabolism of ^{14}C-JNJ-212082 in liver microsomes and hepatocytes of male and female mouse, male and female rat, male dog, female rabbit, male monkey and man -Preliminary report. (Reviewed by Margaret Brower, PhD under IND 71, 023)

Key Study Findings

- The extent of metabolism in human hepatocytes *in vitro* most closely matched the male rat at 76 minutes.
- The major in vitro metabolic pathway of abiraterone acetate in human, mice, and female liver microsomes following 60min incubation was ester hydrolysis producing abiraterone, followed by mono- and di-oxidation of abiraterone.
- Hydrolysis of abiraterone acetate to abiraterone was the result of esterase activity, not CYP-mediated.

FK7532: Structural characterization of metabolites of abiraterone acetate in selected monkey plasma samples from (b) (4) Study No. 7777-103 (39-Week oral gavage toxicity study with CB7630 in cynomolgus monkeys with a 4-week recovery period)

Report #:	FK7532
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	December 12, 2006
GLP Compliance:	No
QA Report:	Yes, No (X)
Drug, lot #, and % purity:	Abiraterone (CB7598), 562-97-1, 98.2% Abiraterone acetate (CB7630), 1274-43-2, 98.2%
Doses:	500 mg/kg daily x 39-weeks (274 days)
Species/strain:	Cynomolgus monkeys /*Macaca fascicularis*
Number/sex/group or time point:	3/sex/dose
Route, formulation, volume, and infusion rate:	Oral gavage; DCDS4501A (20 mM histidine acetate, pH 5.5, 240 mM sucrose, and 0.02% polysorbate 20) 10 mL/kg on Day 1 and changing to 5 mL/kg on Day 29
Age:	~ 5-7 years
Weight:	~ 3-7 kg
Sampling times:	0.5, 1, 2, 4, and 8 hours post-dose
Study design:	Samples transferred were collected from N=4 male monkeys at 500 mg/kg/day at specified times following the first dose of abiraterone acetate.

Results:

- The pathways proposed for abiraterone acetate in monkey plasma appeared to be initiated with hydrolysis of the acetate ester. This was then followed by various multiple oxidations and direct sulfation of the free hydroxyl group of abiraterone. Combinations of hydrogenations, hydroxylation, and Phase 2 conjugation (sulfation and glucuronidation) were also observed.
- Thirty-nine metabolites were characterized/identified in plasma along with abiraterone acetate and abiraterone. Of these, several were isomers of mono-oxy-abiraterone sulfate and of dihydro-mono-oxy-abiraterone. Several metabolites were also formed by hydrogenation and mono-oxidation.
- Metabolites M70, M18, M78, M35, M83, M61, M42, M88, M87, M89, M90, M92, M93, M95 and abiraterone acetate were not detected at 0.5 hours. However, they were detectable between hours 1 and 8 of this study
- Metabolites M92 and abiraterone acetate were only detectable after 2 hours. M93 was only detected after 4 hours and M75 was not detected at 1 and 4 hour timpoints.
- Metabolite M50 was detected at all time-points.
- Summary of results are shown in sponsor table below.

The following table summarizes the metabolites identified at each time point:

LC-MS Peak Designation	Final Metabolite Designation	Proposed Identification	Plasma time points (hours) 0.5	1	2	4	8
559B	M69	Dihydro-di-oxy-Abiraterone glucuronide	X	X	X	X	X
605	M70	Sulfated-Abiraterone glucuronide	ND	X	X	X	X
463A	M18	Dihydro-di-oxy-Abiraterone sulfate	ND	X	X	X	X
461B	M23	Di-oxy-Abiraterone sulfate	X	X	X	X	X
383B	M78	Dihydro-di-oxy-Abiraterone	ND	X	X	X	X
383	M27	Dihydro-di-oxy-Abiraterone	X	X	X	X	X
461G	M72	Di-oxy-Abiraterone sulfate	X	X	X	X	X
463D	M79	Dihydro-di-oxy-Abiraterone sulfate	X	X	X	X	X
543B	M30	Dihydro-mono-oxy-Abiraterone glucuronide	X	X	X	X	X
381D	M80	Di-oxy-Abiraterone	X	X	X	X	X
445G	M81	Mono-oxy-Abiraterone sulfate	X	X	X	X	X
445A	M31	N-oxide Abiraterone sulfate	X	X	X	X	X
447A	M61	Dihydro-mono-oxy-Abiraterone Sulfate	ND	X	X	X	X
381A	M62	Di-oxy-Abiraterone	X	X	X	X	X
541	M82	Mono-oxy-Abiraterone glucuronide	X	X	X	X	X
445C	M65	Mono-oxy-Abiraterone sulfate	X	X	X	X	X
445D	M35	Mono-oxy-Abiraterone sulfate	ND	X	X	X	X
445I	M77	Mono-oxy-Abiraterone sulfate	X	X	X	X	X
541B	M83	Mono-oxy-Abiraterone glucuronide	ND	X	X	X	X
445F	M38	Mono-oxy-Abiraterone sulfate	X	X	X	X	X
525B	M84	Abiraterone glucuronide	X	X	X	X	X
527	M85	Dihydro-Abiraterone glucuronide	X	X	X	X	X
527B	M86	Dihydro-Abiraterone glucuronide	X	X	X	X	X
365A	M42	Mono-oxy-Abiraterone	ND	X	X	X	X
429	M45	Abiraterone sulfate	X	X	X	X	X
367C	M87	Dihydro-mono-oxy-Abiraterone	ND	X	X	X	X
365C	M88	Mono-oxy-Abiraterone	ND	X	X	X	X
367D	M89	Dihydro-mono-oxy-Abiraterone	ND	X	X	X	X
367A	M90	Dihydro-mono-oxy-Abiraterone	ND	X	X	X	X
365B	M91	Mono-oxy-Abiraterone	X	X	X	X	X
367B	M92	Dihydro-mono-oxy-Abiraterone	ND	ND	X	X	X
431B	M93	Dihydro-Abiraterone sulfate	ND	ND	ND	X	X
431	M46	Dihydro-Abiraterone sulfate	X	X	X	X	X
347	M94	3-keto-17-(3-pyridyl)-androsta-4,16-diene	X	X	X	X	X
Abiraterone	M50	Abiraterone	X	X	X	X	X
351A	M51	Dihydro-Abiraterone	X	X	X	X	X
351B	M52	Dihydro-Abiraterone	X	X	X	X	X
349	M95	5-alpha-17-(3-pyridyl)-16-androstene-3-one	ND	X	X	X	X
351C	M96	Dihydro-Abiraterone	X	X	X	X	X
Abiraterone Acetate	Abiraterone Acetate	Abiraterone Acetate	ND	ND	X	X	X
331	M75	Dehydrated-Abiraterone	X	ND	X	ND	X

X Metabolite detected and identified.
ND Metabolite not detected or present at a concentration too low to allow identification.

[Table excerpted from sponsor]

BA1732: Comparison of the exposure of selected metabolites of JNJ-212082 (abiraterone acetate) between human and animal species used for safety evaluation of JNJ-212082.
Report #: BA1732
Conducting Laboratory and Location: (b) (4)

(b) (4)

Date of Study Initiation: 7/6/2010
GLP Compliance: No
QA Report: Yes (), No (X)
Drug, lot #, and % purity: JNJ-212082 (abiraterone acetate), JNJ-589485 (abiraterone), JNJ-48171188 (M45, ABT sulphate), JNJ-49029578 (M31, *N*-oxide ABT sulphate)

Methods

Study design:

- Selected metabolites of abiraterone acetate (JNJ-212082) obtained from a human mass balance study, COU-AA-007, were quantified in a selection of samples from 26-week rat and 39-week monkey plasma.
- The two selected metabolites from human mass balance study (COU-AA-007) include: abiraterone sulfate metabolite, JNJ-48171188 (M45, ABT sulphate) and abiraterone oxidated sulfate metabolite, JNJ-49029578 (M31, *N*-oxide ABT sulphate)
- The exposure of the metabolites were compared between animal and human samples
- In addition, a selection of samples from clinical trial COU-AA-006 was analyzed (both after single and repeated administration).
- Animal-to-human exposure ratios were calculated using a human dose of 1000 mg once daily as reference

Results:

- At the highest administered dose in rats (400 mg/kg/day) and monkeys (1000 mg/kg/day), exposure (Cmax and AUC) to abiraterone sulfate metabolite (JNJ-48171188) exceeded human exposure at 1000 mg o.d.
- Exposure to abiraterone oxidated sulfate metabolite (JNJ-49029578) is approximately equivalent to the human exposure at 1000 mg once daily in monkeys. However, exposure to this metabolite in rats is 20% of the human exposure.
- See sponsor's tables below.

JNJ-48171188 (M45, sulphate metabolite)

Sex	Male monkey			Female monkey		
Dose (mg/kg/day)	250	500	1000	250	500	1000
Day	273			273		
C_{max} monkey / C_{max} man	1.2	2.7	7.2	1.3	3.4	5.8
$AUC_{0-24\ h}$ monkey / $AUC_{0-24\ h}$ man	0.8	2.2	13.1	0.4	4.7	5.7

JNJ-49029578 (M31, oxidated sulphate metabolite)

Sex	Male monkey			Female monkey		
Dose (mg/kg/day)	250	500	1000	250	500	1000
Day	273			273		
C_{max} monkey / C_{max} man	0.2	0.3	0.9	0.2	0.4	0.9
$AUC_{0-24\ h}$ monkey / $AUC_{0-24\ h}$ man	0.1	0.1	0.7	0.1	0.2	0.4

JNJ-48171188 (M45, sulphate metabolite)

Sex	Male rat			Female rat		
Dose (mg/kg/day)	50	150	400	50	150	400
Day	183			183		
C_{max} rat / C_{max} man	0.2	0.4	1.4	0.5	1.6	2.6
$AUC_{0-24\ h}$ rat / $AUC_{0-24\ h}$ man	0.2	0.5	2.7	NC	1.3	3.9

JNJ-49029578 (M31, oxidated sulphate metabolite)

Sex	Male rat			Female rat		
Dose (mg/kg/day)	50	150	400	50	150	400
Day	183			183		
C_{max} rat / C_{max} man	0.038	0.1	0.2	0.1	0.2	0.3
$AUC_{0-24\ h}$ rat / $AUC_{0-24\ h}$ man	NC	0.045	0.2	NC	0.1	0.2

2.6.2.7 Other Pharmacokinetic Studies – None

2.6.3 PHARMACOKINETICS TABULATED SUMMARY

Study # /Type of study	Method of Administration/GLP	Route	Dose	Test system	Findings
Absorption					
8202265/ Drug permeability and transport	*In vitro*/ No	NA	NA	Caco-2-cells	Abiraterone and abiraterone acetate were shown to have a low apparent permeability and are not substrates of P-glycoprotein (P-gp). Abiraterone did not inhibit P-gp Abiraterone acetate significantly inhibited P-gp with an IC_{50} of 10.8 µM.
(b) (4)-001/ Plasma kinetics and bioavailability	*In vivo*/ No	Oral and iv	Single dose of 0.1 and 39.2 mg/kg	Male mice	After oral dosing, peak concentrations of the metabolite (CB7598) were achieved quickly (15 minutes) and then fell rapidly thereafter. After iv dosing, a much higher concentration of the metabolite (CB7598) was attained in the first 30 minutes (4 to 5 fold greater than after oral dosing). AUC for oral and iv route for prodrug was 3807 and 10251 ng/ml x h, respectively giving an oral bioavailability of 37%
05-501648/ Plasma kinetics	*In vivo*/ No	Oral and iv	Oral: 250	Male monkey	**Abiraterone (CB7598):** ▪ No differences in any PK

Study # /Type of study	Method of Administration/GLP	Route	Dose	Test system	Findings
and bioavailability			mg/kg iv: 24 mg/kg		parameter when three different oral formulations of CB7630 (Tablet 1, Tablet 2, and Capsule formulations) were compared. ▪ The mean absolute bioavailability of the oral formulations ranged from 1.5% to 1.6%. ▪ Mean $T_{1/2}$ estimates of the metabolite (CB7598) ranged from approximately 6.6 to 9.5 hours **Abiraterone acetate (CB7630):** ▪ Plasma concentrations were only measurable following intravenous administration
Distribution					
FK7603/ Protein binding	*In vitro*/ No	NA	NA	Mice, rat, monkey, rabbit, and human subjects	Radio-labeled abiraterone is highly bound (≥99.8%) to proteins in plasma across multiple species (mouse, rat, rabbit, monkey and man). No relevant species differences were observed.
FK7448/ Protein binding	*In vitro*/ No	NA	NA	Human plasma from mild or moderate hepatic impaired/normal hepatic function	Liver impairment had no impact on the unbound fraction of abiraterone in plasma.
Metabolism					
FK7532/ Metabolism	*In vivo*/ No	Oral	500 mg/kg	Male monkey (N=4)	The pathways proposed for abiraterone acetate in monkey plasma appeared to be initiated with hydrolysis of the acetate ester. Thirty-nine metabolites were identified in monkey plasma including isomers of mono-oxy-abiraterone sulfate, dihydro-mono-oxy-abiraterone, hydrogenation and mono-oxidation.
BA1732/ Comparison	*In vivo*/ No	Oral	Rat: 400	Rat, monkey, and human	At the highest administered dose of abiraterone acetate,

Study # /Type of study	Method of Administration/GLP	Route	Dose	Test system	Findings
exposure human and animal species			mg/kg, monkey: 1000 mg/kg	subjects	in both rat and monkey, exposure to abiraterone sulphate exceeded the exposure to abiraterone sulphate in man. At the maximum dose of abiraterone acetate in monkeys,exposure to *N*-oxide abiraterone sulphate approximated that in man. In rats, exposure to *N*-oxide abiraterone sulphate was markedly lower (20%).
Pharmacodynamic drug interactions					
400378/ Enzyme induction/ suppression	*In vitro*/ No	NA	0, 0.1, and 10 uM	Cultured human hepatocytes from single donor	**Abiraterone acetate (CB7630)** Weak inhibitor for suppressing CYP3A4 at all concentrations tested. Not an inhibitor for CYP2C9 Slight inhibitor for CYP1A2
400379/ Enzyme inhibition	*In vitro*/ No	NA	0, 0.1, and 10 uM	Cultured human hepatocytes from 50 donors	**Abiraterone (CB7598)** Not an inhibitor for CYP2A6 and CYP2E1. Moderate inhibitor of CYP2C9, CYP2C19 and CYP3A4/5. Strong inhibitor for CYP1A2 (0.44 µM) and CYP2D6 (0.39). **Abiraterone acetate (CB7630)** Not an inhibitor for CYP2A6 Moderate inhibitor for CYP2E1, CYP2C9 and CYP3A4/5 Strong inhibitor for CYP1A2 (0.32 µM) and CYP2C19 (0.12 µM).
FK7476/ Induction/ Inhibition	*In vitro*/ No	NA	0, 40, and 400 mg/kg/ day	Liver microsomes from male and female rats from 1-month study (TOX9587)	**Male rats** Significant increase in relative liver weight and CYP450 content Significant increase in activity of CYP1A1/2, CYP4A1 and

Study # /Type of study	Method of Administration/GLP	Route	Dose	Test system	Findings
					UGT at the highest dose of 400 mg/kg/day. Statistically significant increase in SULT2A1 activity towards DHEA was at 40 and 400 mg/kg/day. Dose-dependent increase in SULT2A1 activity towards abiraterone oxide (resulting in M9) (but not towards abiraterone, resulting in M12) at 40 and 400 mg/kg/day. Statistically significant decrease in cytosolic protein content and activity of CYP2E1, CYP3A1/2 and CYP2B (latter not dose-dependent). **Female rats** No effect on microsomal protein content, cytochrome P-450 content, and activity of CYP1A1/2 and CYP2B. Significant increase in relative liver weight and activity of CYP3A1/2 and CYP4A1 at 400 mg/kg/day. Significant increase in UGT activity at 40 and 400 mg/kg/day. A decrease in cytosolic protein content, CYP2E1 activity and SULT2A1 activity towards DHEA at the highest dose of 400 mg/kg/day. A decrease in SULT2A1 activity towards oxidized abiraterone (resulting in M9) (but not towards abiraterone, resulting in M12).

2.6.4.9 Tables and figures to include comparative TK summary

The following studies are reviewed in the Toxicology section (Section 2.6.6) including repeat-dose toxicity (2.6.6.3). These studies include the following:

13and 26-week studies in the rat (Study Nos. 7777-100 and 7777-105) and 13- and 39-week studies in the monkey (Study Nos. 7777-101 and 7777-103).

Dose (mg/kg/day)	Sex	Cmax (μg/mL) range		AUC_{0-t} (ng h/mL)		Ratio of animal to human exposure
		Day 1	End of study	Day 1	End of study	AUC
Rat 13-week						
250/50	M F	2377 159	142 113	18482 629	664 274	0.68 0.29
750/250	M F	3713 312	291 278	42759 1777	1770 1155	1.81 1.18
2000/750	M F	3617 532	NC NC	46334 3404	NC NC	NC NC
Rat 26-week						
50	M F	729 77	138 132	3006 240	1132 710	1.15 0.72
150	M F	3243 189	251 276	16397 1063	2220 1734	2.27 1.78
400	M F	3537 500	494 291	49610 1889	5586 3106	5.72 3.18
Monkey 13-week						
250	M F	519 261	146 103	2004 1326	1252 947	1.28 0.97
750	M F	526 360	62 100	3148 1765	774 999	0.80 1.02
2000	M F	478 597	115 243	2737 4100	1604 2961	1.64 3.03
Monkey 39-week						
250	M F	114 151	139 87	594 628	610 489	0.625 0.500
500	M F	226 482	183 466	1577 3408	1139 2945	1.17 3.02
1000	M F	238 471	270 185	1637 4154	2095 1223	2.14 1.25

Note:

- Human exposure ratio was calculated based on total drug AUC values and a human AUC of 976 hr*ng/mL (Day 1; Cycle 2) Information based on Study No. COU-AA-006 where abiraterone acetate was given daily at 1000 mg in patients with mCRPC.
- For all studies, ratio of animal to human exposure was calculated from end of study AUC values
- For 13-week rat study, Dosing suspended in HD males administered 2000mg/kg on D9. Dosing resumed on D12 at lower dose of 750mg/kg, followed by reducing low-dose and mid-dose to 50 and 250mg/kg, respectively, on D10.
- For 13-week rat and monkey study, end of study Cmax and AUC values were at taken at end of Week 13.
- For 26 and 39-week rat and monkey studies, end of study Cmax and AUC values were at taken at end of Week 26 and 39.
- NC = not calculated due to mortality

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

The general toxicology of abiraterone acetate (AA or CB7630) was examined in rats and monkeys. Single dose studies were conducted in the rat while repeat dose studies were conducted in both rats and monkeys with abiraterone acetate administered through the oral route (via oral gavage). The dose levels for all toxicology studies are expressed in mg abiraterone acetate per kg body weight (mg/kg). The rat was considered an appropriate species to study the *in vitro* and *in vivo* metabolic profiling of abiraterone acetate while the monkey was an appropriate species to show similarities between the human reproductive endocrine system (since abiraterone acetate main target is on androgen-sensitive organs). As discussed in the pharmacology portion of this review, abiraterone acetate is converted to abiraterone *in vivo*. With the exception of some genotoxicity studies, all toxicology studies in rats and monkeys were conducted with abiraterone acetate. Single dose, 13-week and 26-week studies in rats were reviewed under IND 71,023 by Margaret Brower, Ph.D (which is attached at the end of this review). In the current submission, 13-week and 39-week studies in monkeys have been submitted and reviewed.

In the 13 and 39-week monkey studies, abiraterone acetate was administered once per day at 0, 250, 750, and 2000 mg/kg/day (13-week study) and 0, 250, 500, and 1000 mg/kg/day (39-week study). At the end of dosing and/or drug treatment, animals at the control and high dose were assessed a 4-week recovery period to examine the reversibility, persistence or delayed occurrence of any effects of abiraterone acetate.

Target organs of toxicity were somewhat similar in both studies. At 13-weeks, the main target organs of toxicity were seen in the liver, adrenals glands and male mammary glands. At 39-weeks, the main target organs of toxicity were seen male and female reproductive organs (epididymis, seminal vesicle, prostate, testes, uterus, and ovaries), liver, and adrenal glands. All toxicities were consistent with the pharmacological activity of the abiraterone acetate and most but not all toxicities were reversed or partially reversed after a 4-week recovery period.

In monkeys, repeated oral dosing did not lead to mortality when dosed up to 2000 mg/kg for 13-weeks or up to 1000 mg/kg when dosed for 39-weeks. There were, however, non-drug-related deaths in the 39-week study in four animals (1 M at the low dose of 250 mg/kg and 3 M at the high dose of 1000 mg/kg). These deaths were related to accidental dose aspiration and/or gavage accident. After reducing the daily administered volume from 10 to 5 mL/kg (from Day 29 of the dosing phase onwards), all animals survived until the end of the study.

In both studies, common clinical signs related to abiraterone acetate administration were white color discharge across all dose groups. Non-formed

feces were also seen at both 13 (MD and HD) and 39 (all dose groups) weeks, however, the incidence was greater in males at 39-weeks compared to females. 13-week studies also showed white color feces at ≥750 mg/kg. These signs appear to reflect the presence of test article in the vomit and feces. Alopecia in the arms was a common sign in both males and females at the high dose (1000 mg/kg) when abiraterone acetate was given for a longer duration (39 weeks). Females of all dose groups including controls showed the presence of menses cycle at both 13 and 39-weeks. In both studies, all clinical signs were not present at the end of the recovery period.

No significant changes on body weight or body weight gain were observed when abiraterone acetaete was given for 13-weeks. At 39-weeks, however, slight but significant body weight loss (≤22%) was noted in males across at all dose levels. During the first 2 week of recovery, body weight loss was still significantly lower (26.6%) for high dose males (1000 mg/kg) only. However by the end of recovery, body weight was comparable to controls. The pharmacological action of decreasing testosterone concentrations occurring in males may have contributed to changes in body weight. Unlike body weight changes, lower food consumption (>20%) was seen across all dose groups in both males and females in the 39-week study only. During the beginning of the recovery period, food consumption changes was still decreased (↓≥20%) but tended to be at control levels by the end of 4-weeks.

In the 39-week study, ECG assessments showed infrequent ventricular premature complexes in three male monkeys at the high dose of 1000 mg/kg. The toxicological relevance of these arrhythmias was considered limited and were not drug related based on the following. First, there were no abnormalities at the ECG recording at the end of the treatment period. Secondly, no ECG abnormalities were seen in the 13-week monkey study at comparable abiraterone plasma exposure. Moreover, no effects were noted in a cardiovascular safety study in the male telemetered monkey after a single oral dose up to 2000 mg/kg (Safety pharmacology Study No. 692409). Finally, there was no histological evidence of cardiomyopathy in both 13 and 39-week studies.

At both 13 and 39-weeks, hormone analysis showed increasing levels of adrenocorticosterone (ACTH), aldosterone (ALDS) and progesterone (PROG) levels and lower cortisol (CORT) and dehydroepiandrosterone (DHEA) levels across all dose groups. Luteinizing hormone (LH) increased in males only at 13-weeks (LD-HD: ↑146, ↑489, and ↑152%). However, a clear effect was not observed since this increase was not dose-dependent and was not present in the 39-week study. Estrogen and testosterone levels also decreased in both studies, however, to a greater extent at 39-weeks (list levels). Estrogen levels were not fully reversed by the end of the 4-week recovery period (list how much). In both studies, increased ACTH hormone concentrations corresponded with brown discolorations of the adrenal cortex, increased adrenal weights, and microscopic evidence of adrenocortical hypertrophy in both males and females at all dose levels. In the 39-week study, increased progesterone concentrations corresponded with uterine findings of pseudodecidual endometrium (moderate to

marked in severity) while decreased DHEA hormone concentrations corresponded to vaginal epithelium mucosal atrophy (slight in severity). Histopathology findings were not present at 13-weeks despite increases in these both hormones possibly indicating a delayed toxicity effect. At 13-weeks, most hormone levels exhibited reversibility except for ACTH (males: ↑186%; females: ↑169%) and ALDS levels (males: ↑207%) that remained slightly higher for animals dosed the high dose of 2000 mg/kg.

The liver was one of the major organs affected in both studies but to a greater extent when abiraterone acetate was given for a longer duration (39 weeks). At both 13 and 39-weeks, doses of ≥250 mg/kg led to increased triglycerides and total bilirubin. Total bilirubin increases were around 2-fold in the 39-week study at 1000 mg/kg and 6-fold in the 13-week study at 2000 mg/kg. Increases in relative liver weights (including both relative to body and brain weight) were also seen in both studies. At 13-weeks, liver weights elevated in a dose dependent manner with slightly greater increases seen in males compared to females (males: ↑49-112%; females: ↑24-72%; LD-HD, respectively). At 39-weeks, increases were similar with the exception of females at the high dose, 1000 mg/kg, had greater increases compared to males. At 13-weeks, histopathological findings included bile duct hyperplasia was evident at all dose levels in both males and females ranging from minimal changes at 250 mg/kg to slight to moderate changes at 750 and 2000 mg/kg. At 39-weeks, bile duct/oval cell hyperplasia was seen at all dose levels without a clear dose-related effect and males were affected to a greater extent compared to females. The bile duct hyperplasia was slightly less in severity compared to the 13-week study. The increase in liver weights from both studies were directly associated with histological correlation of bile duct/oval cell hyperplasia. 39-week studies also showed a significant but reversible increase in ALP in both males and females at 500 and 1000 mg/kg. This increase reached above 400% in females at 500 mg/kg (↑421%) on Week 26 and above 200% in both sexes (males: ↑243%; females: ↑245%) by Week 39. ALP levels were also elevated at 13-weeks in females at 750 mg/kg but were not biologically significant (increases were not above 200%) despite histological, gross pathology and organ weight changes. Liver findings at both 13 and 39 weeks tended to improve after a 4-week recovery period but were not completely reversed. At 13-weeks, all males and most females still had minimal to slight bile duct hyperplasia and liver weights were still elevated but to a lesser extent (males: ↑32%; females: ↑34%). At 39-weeks, males still had a 93% increase in ALP levels and both males and females had increases in relative liver weights (males: ↑30%; females: ↑37%).

Shorter term treatment of abiraterone acetate did not have a major effect on reproductive organs with the exception of the ovaries. In the 13-week study, increases in relative ovary weights (relative to both body and brain) were observed. Increases reached up to ↑140 and 154% (relative to body weight and relative to brain weight, respectively). Relative ovary weights (relative to body: ↑264% and relative to brain: ↑333%) and gross pathology lesions of ovarian cysts were also present at the end of recovery. However, no histopathology findings were observed in the ovaries during drug treatment and recovery. Unlike 13-

week studies, 39-week administration of abiraterone acetate led to a number of male and female reproductive organs being affected. This was the result of the anti-androgen pharmacology of abiraterone acetate and the longer duration of treatment. At 39-weeks, target organs of toxicity in males included the testes (atrophy of the spermatogenic epithelial lining of testicular tubules and interstitial cell hyperplasia), epididymis, (aspermia or hypospermia; increased quantity of tubular debris), seminal vesicles (increased mineralization), prostate (epithelial atrophy). In females, target organs included the vagina (epithelial atrophy), uterus (endometrial pseudodecidual change associated with thickening seen at necropsy with hypertrophy and hyperplasia of stromal cells, endometrial gland atrophy, and increased number of endometrial granular lymphocytes) and ovaries (follicular cysts). Most if not all findings were across all dose groups and many were correlated to organ weight changes.

The male mammary gland was an additional organ affected the 13-week study only. Histological changes included minimal to slight hyperplasia in all males at the low dose, 200 mg/kg, and fewer numbers being affected at higher doses (750 and 2000 mg/kg). This hyperplasia was often accompanied by minimal to moderate periductal edema with or without fibrosis. Moderate to marked atrophy in male mammary glands was also observed in 13-week rat studies. Since changes only occurred in one species, the exact cause of these findings could not be determined. At 39-weeks, the thymus was affected in males. This thymic involution exhibited in control males is a natural age-related process that commences at puberty and androgen ablation has been reported to activate thymic regeneration. Although biological variation cannot be ruled out, the anti-androgen activity of abiraterone acetate may have protected or enabled regeneration of the thymus.

Toxicokinetic (TK) data from both 13 and 39-week monkey studies show that the metabolite, abiraterone (CB7598), was the only species detected. Abiraterone acetate concentrations were generally below the lower limit of quantitation and TK analysis could not be conducted. Abiraterone exposure did not increase with increasing dose. After reaching Cmax, abiraterone concentrations declined from Day 1 to the end of the study. Monkeys did not display overt gender-related differences in Cmax and AUC of abiraterone. Values for mean Cmax and AUC_{0-24} were generally similar from Day 1 to the end of the study indicating no accumulation of abiraterone occurring after multiple dosing. Male rats had higher plasma exposure than female rats, especially after single dosing. At the rat MTD and at the highest dose tested in long-term repeated monkey studies (2,000 mg/kg/day), plasma exposure (as measured by AUC) to abiraterone was comparable between both species and the calculated animal to human abiraterone exposure ratios were slightly higher than 1.

Genetic toxicology:
In vitro and *in vivo* genetic toxicology studies were conducted; the Ames assay, the *in vitro* chromosomal aberration assay in mammalian cells, and the *in vivo* mouse micronucleus assay. Two different formulations of abiraterone acetate were tested, as well as abiraterone (CB-7598), the active metabolite. At the

doses tested in these studies, with and without metabolic activation, abiraterone acetate and abiraterone were not mutagenic or clastogenic. In addition, QSAR analysis for genetic toxicity was conducted for the (b) (4) impurity of abiraterone acetate. Predicted genetic toxicity for the impurity was found to be negative.

Carcinogenicity:
Carcinogenicity studies were not conducted and are not necessary to support the safety of abiraterone acetate for the proposed metastatic cancer indication.

Reproductive toxicology:
Reproductive toxicology studies were not conducted and are not necessary to support the safety of abiraterone acetate for the proposed metastatic cancer indication in males.

Special toxicology studies – Studies on impurities:
Impurities of abiraterone acetate which include a (b) (4) were present at low concentrations in one or more of the drug substance batches tested in toxicology studies. Both *in vitro* assays (Ames and the chromosomal aberration assay in mammalian cells) showed no evidence of mutagenic activity when abiraterone acetate was spiked with the 5 impurities at nominal levels (to give a total of (b) (4) impurities – See Table below). When abiraterone acetate spiked with 5 impurities was administered to rats at 40 and 400 mg/kg/day daily for 1 month (oral gavage), no toxicologically relevant differences between groups dosed with five impurities and the groups without impurities for all examined parameters.

Toxicological Qualification Limits f Specified Impurities of Abiraterone acetate Drug Substance

Impurity	Nominal impurity level (% w/w of abiraterone acetate dose)
(b) (4)	

In addition, Ames assays were conducted with pure impurities including a (b) (4) 2 additional synthesis by products impurities of abiraterone acetate (b) (4) and an (b) (4) impurity to evaluate its potential toxicity and/or to determine if these (pure) impurities present a structural alert. The (b) (4) (b) (4) impurity ((b) (4) was present at (b) (4) in one of the drug substance batches used in the 13-week rat and 13-week monkey study and at (b) (4) in one of the batches used in the 26-week rat and 39-week monkey study. Both (b) (4) (b) (4) (b) (4) (b) (4) (b) (4) impurities (b) (4) were not mutagenic *in vitro* (Ames) while the (b) (4)

(b) (4) did not produce any mutagenicity alerts in DEREK. The (b) (4) impurity ((b) (4) was also not clastogenic in an *in vitro* chromosome aberration assay. In a 28-day repeat dose toxicity study in rats, the toxicity profile of abiraterone acetate was similar in absence and presence of 5 spiked impurities which included the (b) (4) impurity. All but the (b) (4) impurity ((b) (4) were shown not to be muatagenic in the presence and absence of S9 activation.

2.6.6.2 Single-dose toxicity: No studies reviewed

2.6.6.3 Repeat-dose toxicity

7777-100: 13-week oral gavage toxicity and toxicokinetic study with CB7630 in rats with a 4-week recovery period (Reviewed by Margaret Brower, PhD under IND 71, 023)

Key Study Findings:

- CB7630 was lethal at doses ≥750mg/kg
- Bile duct hyperplasia in males and females following dosing and recovery
- Adrenal hypertrophy in males and females at all doses
- Pituitary hyperplasia in males following dosing and recovery
- Atrophy of testes, seminal vesicles, epididymes, prostate, and male mammary gland at all doses; incidence and severity decreased following recovery
- Hypospermia at all doses; incidence and severity decreased following recovery
- Atrophy of uterus and cervix at all doses
- Lung infiltrate deposition (test material) in males and females at all doses
- Expected pharmacological effect: increase in LH, decrease in testosterone, and atrophy of male reproductive organs

7777-105: 26-week oral gavage toxicity and toxicokinetic study with CB7630 in rats with a 4-week recovery period (Reviewed by Margaret Brower, PhD under IND 71, 023)

Key Study Findings:

- CB7630 was lethal at doses ≥400mg/kg, the highest dose tested.
- Dose related incidence of cataracts in males and females following dosing and recovery
- Adrenal atrophy/hypertrophy dosed males following dosing and recovery; incidence less in dosed females
- Bile duct hyperplasia at HD
- Dose related incidence of liver hypertrophy in males and females
- Dose related incidence of lung inflammation in males and females following dosing, and lung infiltrate deposition following recovery
- Atrophy of testes, prostate, seminal vesicles, and male mammary gland, and ovarian hyperplasia following dosing; incidence decreased following recovery
- Aspermia at all doses following dosing; aspermia and hypospermia following recovery

– Expected pharmacological effect: increase in LH, decrease in testosterone, and atrophy of male reproductive organs

7777-101: 13-Week Oral Gavage Toxicity Study with CB7630 in Cynomolgus Monkeys with a 4-Week Recovery Period

Study no.:	7777-101
Study report location:	Cougar Biotechnology, Inc. 10990 Wilshire Boulevard, Suite 1200 Los Angeles, California 90024 United States of America
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8/16/2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CB7630,1274-43-2 and 1274-61-2, 97.9 and 98.2%

- No drug-related mortality occurred throughout the study.
- Females were more affected than males in terms of WBC parameters (neutrophils, lymphocytes, and eosinophil) at ≥750 mg/kg.
- Significant increases in total bilirubin, triglycerides, cortisone, ACTH, ALDS, LH, estrogen, progestin, DHEA and testosterone levels were observed across all doses levels at Weeks 13 and 26. All were recoverable with the exception of ALDS in males and estrogen levels in females.
- Target organs of toxicity: adrenal glands, liver, ovary, seminal vesicle and male mammary gland.
- With the exception of ovary weights, all changes/findings were reversible following 4-week recovery period.
- TK data showed no drug accumulation of the metabolite abiraterone which was detected with values of Cmax and AUC that decreased after multiple doses.
- Study was designed to select doses for future 39-week study.

Methods

Doses:	250, 750, and 2000 mg/kg/day (3000, 9000, and 24000 mg/m^2/day)
Frequency of dosing:	Daily for 13 weeks (or daily for 91 days)
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	DCDS4501A (20 mM histidine acetate, pH 5.5, 240 mM sucrose, and 0.02% polysorbate 20)
Species/Strain:	Cynomolgus monkeys /*Macaca fascicularis*
Number/Sex/Group:	4/sex/dose; 3 sex/dose (recovery control and HD only)
Age:	5-6 years
Weight:	M: 3-4 kg/F: 2-3 kg
Satellite groups:	4-7/sex/group (TK)
Unique study design:	None
Deviation from study protocol:	– Terminal sacrifice on Week 13 (Study Day 91) – Recovery sacrifice on Week 17 on Control and HD animals

Experimental design:

Group	Dose (mg/kg/day)/ mg/m^2/day)	# animals		Sacrifice			
				Main study		Recovery	
		M	F	M	F		
1	0/0	7	7	4	4	3	3
2	250/3000	4	4	4	4	0	0
3	750/9000	4	4	4	4	0	0
4	2000/24000	7	7	4	4	3	3
5	0/0	7	7	4	4	3	3
6	250/3000	4	4	4	4	0	0
7	750/9000	4	4	4	4	0	0
8	2000/24000	7	7	4	4	3	3

Observations:

Mortality:	Twice daily
Clinical signs:	Twice daily
Menses cycle:	2 weeks predose and daily during dosing and recovery
Body weights:	Predose, prior to dosing, and weekly during dosing and recovery
Food consumption:	Predose, prior to dosing, and weekly during dosing and recovery
FOB	Predose, Weeks 6 and 17 (recovery)
Ophthalmoscopy:	Predose, Weeks 6 and 17 (recovery)
Hematology:	Weeks 6, 13, and 17 (recovery)
Clinical chemistry:	Weeks 6, 13, and 17 (recovery)
Coagulation	Weeks 6, 13, and 17 (recovery)
Urinalysis:	Weeks 6, 13, and 17 (recovery)

Gross pathology:	Conducted on all animals including those found dead, euthanized in extremis, and those euthanized at study termination and end of recovery.
Organ weights:	Week 13 and 17 (recovery)
Histopathology:	Adequate Battery: yes (X), no () Peer review: yes (X), no ( )
Toxicokinetics:	N=4-7 sex/dose at 0.25, 0.5, 1, 2, 4, 8, 12,and 24 hours post-dose on Days 1 and 91

Results:
Mortality: None

Clinical signs:

Index	**No. of animals affected**			
Dose (mg/kg/day)	**0**	**250**	**750**	**2000**
Dose (mg/m^2/day)	**0**	**3000**	**9000**	**24000**
No. of animals	**7**	**4**	**4**	**7**
Discharge				
– Vomit, white color		2M/2F	2M/4F	5M/6F
– Vomit, yellow			2F	3F
– Vomit, with food				4F
Feces				
– White in color			3M/4F	7M/7F
Excretion				
– Non-formed feces			3F	2F
Menses				
– Normal, YES	2F	2F		2F

Note:
- Recovery animals were included in the Control and 2000 mg/kg groups.

Body weight: unremarkable.

Food consumption: unremarkable.

FOB: unremarkable.

Ophthalmoscopy: unremarkable.

Hematology: **(Recovery in bold and in parenthesis)**

Index	**% change from control**							
Dose (mg/kg)	**0**		**250**		**750**		**2000**	
Dose (mg/m^2)	**0**		**3000**		**9000**		**24000**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
CLOTTING PARAMETERS								
PLTS								

Index	% change from control							
Dose (mg/kg)	0		250		750		2000	
Dose (mg/m^2)	0		3000		9000		24000	
Gender	M	F	M	F	M	F	M	F
Week 6			↑20	↑18		↑50	↑33	↑22
Week 13			↑21		↑21	↑70	↑50	↑30
WHITE BLOOD CELL PARAMETERS								
NEUT								
Week 6						↓46		↓36
LYMPH								
Week 6						↑38		↑47
Week 13				↑25		↑28		↑44
Recovery								**(↑49)**
ESO								
Week 6						↑107		↑122
Week 13				↑43	↑54		↑61	↑46
Recovery							**(↑52)**	

Note:

- All values were statistically significant from controls $p \leq 0.05$
- Recovery consisted of control and HD (2000 mg/kg) animals only.

Clinical Chemistry: **(Recovery in bold and in parenthesis)**

Index	% change from control							
Dose (mg/kg)	0		250		750		2000	
Dose (mg/m^2)	0		3000		9000		24000	
Gender	M	F	M	F	M	F	M	F
INDICATOR METABOLITES								
TBILI								
Week 6			↑500	↑500	↑500	↑700	↑600	↑700
Week 13			↑250	↑250	↑350	↑350	↑500	↑350
TRIGLY								
Week 6			↑35	↑27	↑85	↑95	↑93	↑95
Week 13			↑85	↑62	↑169	↑138	↑256	↑184
ALP								
Week 6						↑70		
Week 13						↑86		
HORMONES								
CORT								
Week 6			↓80	↓85	↓85	↓88	↓84	↓86
Week 13			↓72	↓69	↓79	↓63	↓76	↓61
ACTH								
Week 6			↑245	↑560	↑331	↑734	↑308	↑691
Week 13			↑384	↑605	↑761	↑417	↑583	↑475
							↑186	↑169
ALDS								
Week 6			↑120	↑193	↑189	↑207	↑142	↑314

Index	**% change from control**							
Dose (mg/kg)	**0**		**250**		**750**		**2000**	
Dose (mg/m^2)	**0**		**3000**		**9000**		**24000**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Recovery							**(↑207)**	
LH								
Week 6			↑146		↑489		↑152	
ESTR								
Week 13				↓74		↓75		↓72
PROG								
Week 6			↑2267	↑12900	↑1433	↑11400	↑2166	↑20900
Week 13				↑195		↑135		↑475
DHEA								
Week 6			↓95	↓94	↓96	↓94	↓96	↓94
Week 13			↓95	↓92	↓95	↓87	↓96	↓31
TEST								
Week 6						↓63		↓56
Week 13							↓75	↑218

Note:

- All values were statistically significant from controls $p<0.05$
- Recovery consisted of control and HD animals.

Gross Pathology: **(Recovery in bold and in parenthesis)**

Index	**Macroscopic observations**							
Dose (mg/kg/day)	**0**		**250**		**750**		**2000**	
Dose (mg/m^2/day)	**0**		**3000**		**9000**		**24000**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Adrenal glands								
– Brown discoloration			1/4				1/4	
– Large							1/4	
Liver								
– Discolored	1/4			1/4				
– Mass						1/4		
Seminal vesicle								
– Discolored	1/4	**--**		**--**	1/4	**--**		**--**
– Mass		**--**		**--**		**--**		**--**
Ovary								
– Cysts	**--**	**(2/3)**	**--**		**--**	1/4	**--**	1/4 **(1/3)**

Note:

- Recovery consisted of control and HD (2000 mg/kg) animals only.

Organ weights: **(Recovery in bold and in parenthesis)**

Index	**% change from control**			
Dose (mg/kg/day)	**0**	**250**	**750**	**2000**
Dose (mg/m^2/day)	**0**	**3000**	**9000**	**24000**

Gender	M	F	M	F	M	F	M	F
Adrenal glands								
– Relative to body wt.			↑37		↑25		↑42	
– Relative to brain wt.			↑42		↑22		↑60	
Liver								
– Relative to body wt.			↑50	↑36	↑77	↑68	↑95	↑72
– Relative to brain wt.			↑49	↑24	↑71	↑66	↑112 **(↑32)**	↑67 **(↑34)**
Ovary								
– Relative to body wt.	**--**		**--**	↑82	**--**	↓45	**--**	↑154 **(↑264)**
– Relative to brain wt.	**--**		**--**	↑70	**--**	↓44	**--**	↑140 **(↑333)**

Note:
- All values were the ratio (%) of organ weights.
- Recovery consisted of control and HD (2000 mg/kg) animals only.
- All values were statistically significant from controls p<0.05

Histopathology:

Organ	Observation	Microscopic Observations							
		0 mg/kg		250 mg/kg		750 mg/kg		2000 mg/kg	
		M	F	M	F	M	F	M	F
	No. of animals	**4(3)**	**4(3)**	**4**	**4**	**4**	**4**	**4(3)**	**4(3)**
Adrenal, cortex	Hypertrophy, diffuse, zona fasciculate/reticularis								
	– *minimal*			2/4	4/4	2/4	3/4	3/4	1/4
	– *slight*				1/4		1/4		1/4
Liver	Hyperplasia, bile duct								
	– *minimal*			2/4	3/4	1/4		**(3/3)**	1/4
	– *slight*					3/4	1/4		3/4
								1/4	**(2/3)**
	– *moderate*						1/4	1/4	
Mammary gland (male)	Hyperplasia								
	– *minimal*		--	4/4	--	1/4	--	2/4	--
	– *slight*		--		--	1/4	--	1/4	--
	Edema/fibrosis, periductal stroma								
	– *minimal*		--	1/4	--		--		--
	– *slight*		--	3/4	--	1/4	--	1/4	--
	– *moderate*		--		--	1/4	--		--

Note:
- Recovery consisted of control and HD (2000 mg/kg) animals only.

Toxicokinetics:

Toxicokinetic Parameters in Monkeys Following 13 Weeks of CB7630 Administration		
	Males	**Females**

Toxicokinetic Parameters in Monkeys Following 13 Weeks of CB7630 Administration						
	Males			Females		
Dose (mg/kg/day)	**250**	**750**	**2000**	**250**	**750**	**2000**
Dose (mg/m^2/day)	**3000**	**9000**	**24000**	**3000**	**9000**	**24000**
C_{max} (ng/mL)						
Day 1	519	526	478	261	360	597
Day 275	146	62	115	103	100	243
AUC_{0-24} (ng*hr/mL)						
Day 1	2004	3148	2737	1326	1765	4100
Day 275	1252	774	1604	947	999	2961
Tmax (hr)						
Day 1	1.25	2.5	2.4	1.25	1.25	1.3
Day 275	8	2	5.4	2.5	3.7	8.6
T1/2 (hr)						
Day 1	8	7	8	11	11	7
Day 275	NA	NA	9	4	8	NA

Note:
NA – Sample was below the lower limit of quantitation.

- The metabolite CB7598 (abiraterone) was the only species detected. CB7630 (abiraterone acetate) concentrations were generally below the lower limit of quantitation and TK analysis could not be performed.
- The metabolite exposure did not increase with increasing dose.
- Mean Tmax values ranged from 1.25 to 2.5 hours on Day 1 and 1.9 to 8.6 hours on Day 91.
- After reaching Cmax, the metabolite concentrations declined from Day 1 to Day 91.
- There were no apparent gender-related differences. However, on Day 91 females had higher Cmax values (>2 fold) compared to males at the 2000 mg/kg dose.
- Values for Cmax and AUC decreased after multiple dosing.
- Values for mean Cmax and AUC_{0-24} were generally similar on Days 1 and 273 indicating no accumulation of abiraterone occurred after multiple dosing.

7777-103: 39-Week Oral Gavage Toxicity Study with CB7630 in Cynomolgus Monkeys with a 4-Week Recovery Period

Study no.:	7777-103
Study report location:	Cougar Biotechnology, Inc. 10990 Wilshire Boulevard, Suite 1200 Los Angeles, California 90024 United States of America
Conducting laboratory and location:	(b) (4)
Date of study initiation:	7/15/200
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CB7630,1274-43-2 and 1274-61-2, 97.9 and 98.2%

Key study findings:

- No drug-related mortality occurred throughout the study.
- Males exhibited a dose-dependent decrease in body weight (≥20%) due to CB7630 pharmacological action of decreasing testosterone. Not recoverable during the first 2 weeks of recovery.
- EKG showed the presence of infrequent ventricular premature complexes in 3 animals at the high dose of 1000 mg/kg dose. There was no effect on PR and QRS intervals at any dose levels.
- Females were more affected than males in terms of WBC parameters (monocytes, leukocytes, and eosinophil) at ≥250 mg/kg.
- Significant increases in total bilirubin, triglycerides, ACTH, ALDS, DHEA, cortisone, and progesterone, was observed across all doses levels. Many parameters were higher at the end of dosing (Week 39) compared to the start of dosing. All were recoverable with the exception of total bilirubin and estrogen levels.
- Liver toxicity was present as evidenced by increases in ALP activity in females at ≥500 mg/kg and males at 1000 mg/kg. These changes were recoverable.
- Target organs of toxicity: adrenal glands, liver, thymus, male (epididymus, prostate, testes, and seminal vesicle) and female (ovary, uterus, and vagina) reproductive organs.
- With the exception of epididymus and testes weights being elevated during recovery, all changes/findings were reversible following 4-weeks.
- TK data showed no drug accumulation with the metabolite was detected with values of Cmax and AUC being similar after multiple dosing.

Methods

Doses:	250, 500, and 1000 mg/kg/day (3000, 6000, and 12000 mg/m^2/day)
Frequency of dosing:	Daily for 39 weeks (or daily for 274 days)
Route of administration:	Oral gavage
Dose volume:	10 mL/kg on Day 1 and changing to 5 mL/kg on Day 29
Formulation/Vehicle:	DCDS4501A (20 mM histidine acetate, pH 5.5, 240 mM sucrose, and 0.02% polysorbate 20)
Species/Strain:	Cynomolgus monkeys /*Macaca fascicularis*
Number/Sex/Group:	4/sex/dose; 3 sex/dose (recovery control and HD only)
Age:	5-7 years
Weight:	M: 3-7 kg/F: 2-4 kg
Satellite groups:	4-7/sex/group (TK)
Unique study design:	None
Deviation from study protocol:	– Terminal sacrifice on Week 39 (Study Day 275) – Recovery sacrifice on Week 45 on Control and HD animals

Experimental design:

Group	Dose (mg/kg/day)/ mg/m^2/day)	# animals		Sacrifice			
				Main study		Recovery	
		M	F	M	F		
1	0/0	7	7	4	4	3	3
2	250/3000	4	4	4	4	0	0
3	500/6000	4	4	4	4	0	0
4	1000/12000	7	7	4	4	3	3
5	0/0	7	7	4	4	3	3
6	250/3000	4	4	4	4	0	0
7	500/6000	4	4	4	4	0	0
8	1000/12000	7	7	4	4	3	3

Animals replaced:

Dose (mg/kg/day/ mg/m^2/day)	# animals replaced	Gender	Day of replacement	Reason for replacement
250/3000	1	M	15	Dose aspiration
1000/12000	1	M	12	Dose aspiration
1000/12000	1	M	12	Dose aspiration
1000/12000	1	M	18	no replacement cause of death was gavage related trauma

Note: Death was related to accidental dose aspiration and/or gavage accident and not drug related. After reducing the daily dosing volume from 10 to 5 mL/kg on Day 29 and onwards, all animals survived until scheduled necropsy.

Observations:

Mortality:	Twice daily
Clinical signs:	Twice daily
Menses cycle:	2 weeks predose and daily during dosing and recovery
Body weights:	Predose, prior to dosing, and weekly during dosing and recovery
Food consumption:	Predose, prior to dosing, and weekly during dosing and recovery
FOB	Predose, Weeks 1, 13, 26, 39, and 43 (recovery)
Ophthalmoscopy:	Predose, Weeks 27, 39, and 43 (recovery)
Hematology:	Weeks 3, 13, 26, 39, and 43 (recovery)
Clinical chemistry:	Weeks 3, 13, 26, 39, and 43 (recovery)
Coagulation	Weeks 3, 13, 26, 39, and 43 (recovery)
Urinalysis:	Weeks 3, 13, 26, 39, and 43 (recovery)
Gross pathology:	Conducted on all animals including those found dead, euthanized in extremis, and those euthanized at study termination and end of recovery.
Organ weights:	Study Days 26 and 64 (Recovery Week 39)
Histopathology:	Adequate Battery: yes (X), no () Peer review: yes (X), no ( )
Toxicokinetics:	N=4-7 sex/dose at 0.25, 0.5, 1, 2, 4, 8, 12,and 24 hours post-dose on Days 1 and 273

Results:

Mortality: none

Clinical signs:

Index	**No. of animals affected**			
Dose (mg/kg/day)	**0**	**250**	**500**	**1000**
Dose (mg/m^2/day)	**0**	**3000**	**6000**	**12000**
No. of animals	**7**	**4**	**4**	**7**
Discharge				
– Vomit, white color	2M	4M/1F	3M/2F	6M/5F
Excretion				
– Non-formed feces	4M/4F	3M/1F	2M/1F	3M/5F
Menses				
– Normal, YES	7F	4F	4F	6F
Skin and pelage				
– Alopecia arms	3F	1M	3M	3F

Note:

- Recovery animals were included in the Control and 1000 mg/kg/day (12000 mg/m^2/day) groups.

Body weight (See graphs excerpted from the sponsor's submission):

Males at 250, 500 and 1000 mg/kg/day (3000, 6000, and 12000 mg/m^2/day) lost 0.3, 0.6 and 0.3 kg of body weight, respectively. The mean body weight differences were 20.9, 22.4, and 16.4% at the end of the dosing phase when compared to control with corresponding significantly lower mean body weight change. During the first 2 weeks of recovery, the mean body weight was significantly lower at 1000 mg/kg/day (12000 mg/m^2/day) males when compared to control. The mean body weight increased during the last 2 weeks of recovery although the differences were still 26.6% when compared to control. The CB7630 pharmacological action of decreasing testosterone concentrations may have had an effect on body weight of males. There was no effect in body weight for females at any dose levels.

Gender	Dose (mg/kg/day)	Observations
Treatment		
Males	250	Statistically significant ↓ (20.9%) compared to control from Week 1 through Week 40.
	500	Statistically significant ↓ (22.4%) compared to control from Week 1 through Week 40.
	1000	Statistically significant ↓ (16.4%) compared to control from Week 1 through Week 40.
Females	250	Unremarkable
	500	Unremarkable
	1000	Unremarkable
Recovery		
Males	250	N/A
	500	N/A
	1000	Statistically significant ↓ (26.6%) during the first 2 week of recovery but increased to control values during the last 2 weeks.
Females	250	N/A
	500	N/A
	1000	Unremarkable

Note:

- Recovery animals were included in the Control and 1000 mg/kg/day (12000 mg/m^2/day) groups.

Figure 1
Mean Body Weights - Males

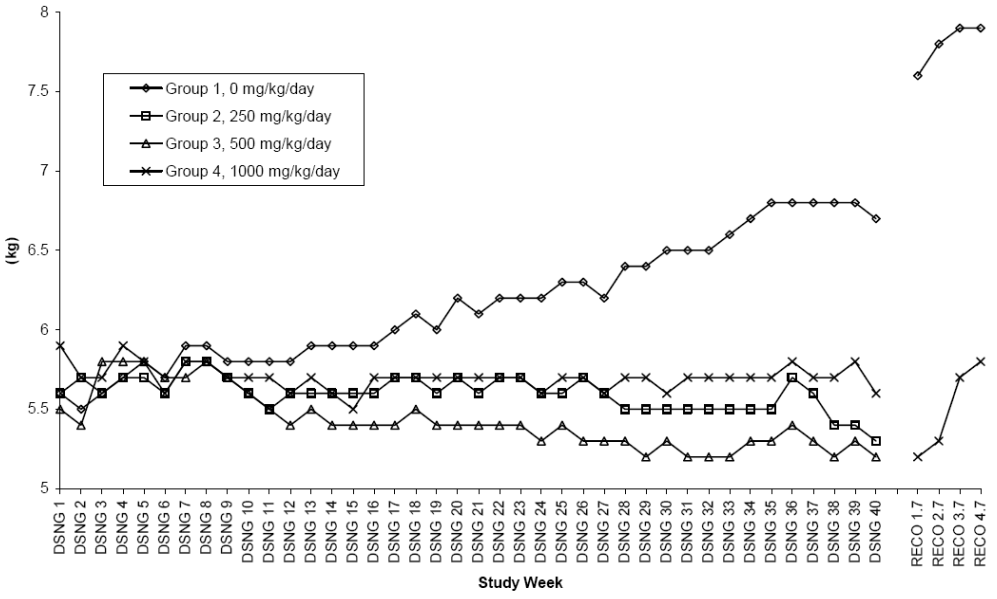
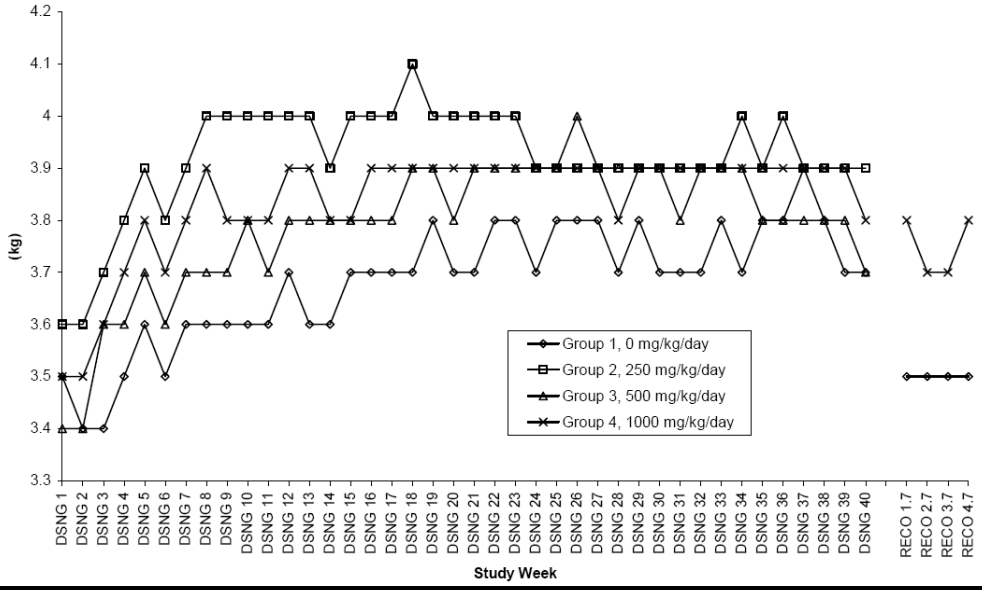


Figure 2
Mean Body Weights - Females



Food consumption:

Low food consumption occurred across all groups including control during the dosing and recovery phases. No other information was available in the sponsor report.

Gender	Dose (mg/kg/day)	Observations
Treatment		

Males	250	Statistically significant ↓ (≥20%) compared to control from Week 1 through Week 40.
	500	Statistically significant ↓ (≥20%) compared to control from Week 1 through Week 40.
	1000	Statistically significant ↓ (≥20%) compared to control from Week 1 through Week 40.
Females	250	Unremarkable
	500	Unremarkable
	1000	Unremarkable
Recovery		
Males	250	N/A
	500	N/A
	1000	Statistically significant ↓ (≥20%) compared to control during recovery.
Females	250	N/A
	500	N/A
	1000	Unremarkable

Note:
Recovery animals were included in the Control and 1000 mg/kg/day (12000 mg/m^2/day) groups.

Ophthalmoscopy: Unremarkable.

EKG:
- There was no effect on PR or QRS intervals at any dose levels.
- The mean QTc intervals were significantly higher for males at 500 mg/kg in Week 39 predose; for males at 1000 mg/kg in Week 39 predose and postdose; for females at 500 mg/kg in Week 13 predose; and for females at 1000 mg/kg in Week 13 predose. No other significances were noted for electrocardiographic data.
- Infrequent ventricular premature complexes were found in animals at the 1000 mg/kg dose and included animal #: 64490 (Week 1 predose), 64494 (Recovery), and 64495 (Week 1 predose, Week 13 predose, Week 13 postdose, Week 26 postdose and recovery).
- Ventricular premature complexes can result from a primary myocardial effect or from the indirect effects related to systemic metabolic changes.
- No other remarkable changes were observed.

Hematology: **(Recovery in bold and in parenthesis)**

Index	**% change from control**							
Dose (mg/kg)	**0**		**250**		**500**		**1000**	
Dose (mg/m^2)	**0**		**3000**		**6000**		**12000**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
RED CELL PARAMETERS								
RETIC								
Week 3							↑22	
Week 13				↑45		↑52	↑117	↑39

Index	% change from control							
Dose (mg/kg)	0		250		500		1000	
Dose (mg/m^2)	0		3000		6000		12000	
Gender	M	F	M	F	M	F	M	F
Week 26				↑130			↑27	↑19
Week 39					↑28		↑23	↑30
WHITE BLOOD CELL PARAMETERS								
WBC								
Week 3					↓23			
Week 13					↓25			
Week 39					↓26		↓20	
NEUT								
Week 3			↓43		↓51		↓31	
Week 13			↓33		↓49	↓32	↓20	
Week 26			↓39	↓26	↓30	↓22	↓26	↓23
Week 39			↓46		↓58	↓19	↓50	↓26
LYMPH								
Week 3			↑23	↑36			↑31	↑25
Week 13			↑27	↑32		↑27	↑26	
Week 26						↑19		↑27
MONO								
Week 3			↑47	↑30		↑37	↑42	↑47
Week 13			↑29	↑26		↑31	↑35	
Week 26				↑46		↑57		↑39
Week 39				↑60		↑84		
LUC								
Week 3				↑75		↑50		↑125
Week 13				↑100		↑40		↑80
Week 26				↑62		↑25		↑62
Week 39				↑62		↑37		↑25 **(↑120)**
ESO								
Week 3			↑78	↑169	↓58			↑175
Week 13				↑87	↓21		↑50	↑148
Week 26				↑23	↓35		↑41	↑176
Week 39				↑41	↑38		↑69	↑123

Note:

- All values were statistically significant from controls $p<0.05$
- Recovery consisted of control and HD (1000 mg/kg/day) animals only.

Clinical Chemistry: **(Recovery in bold and in parenthesis)**

Index	% change from control							
Dose (mg/kg)	0		250		500		1000	
Dose (mg/m^2)	0		3000		6000		12000	
Gender	M	F	M	F	M	F	M	F
PROTEINS								

Index	% change from control							
Dose (mg/kg)	0		250		500		1000	
Dose (mg/m^2)	0		3000		6000		12000	
Gender	M	F	M	F	M	F	M	F
CREA								
Week 13			↓20		↓20		↓27	
Week 26			↓20		↓30		↓27	
Week 39			↓27		↓20		↓27	
INDICATOR METABOLITES								
TBILI								
Week 3				↑200		↑100		↑100
Week 13			↑100	↑50	↑50	↑50	↑200	↑50
Week 26			↑200	↑100	↑50	↑50	↑50	↑100
Week 39			↑200	↑67	↑50	↑100	↑100 **(↑200)**	↑67 **(↑100)**
TRIGLY								
Week 3								↑53
Week 13							↑34	↑50
Week 26							↑41	↑65
Week 39					↑42	↑64	↑82	↑148
ALP								
Week 26						↑100	↑92	↑144
Week 39						↑421	↑243 **(↑93)**	↑245
HORMONES								
ACTH								
Week 3			↑300	↑180	↑500	↑335	↑624	↑708
Week 39			↑1000	↑800	↑337	↑801	↑343	↑461
ALDS								
Week 3			↑129	↑211	↑186	↑135	↑226	↑294
Week 39			↑80	↑230	↑60	↑132	↑100	↑307
DHEA								
Week 3			↓85	↓82	↓94	↓89	↓95	↓93
Week 39			↓91	↓87	↓96	↓85	↓96	↓91
CORT								
Week 3			↓76	↓70	↓66	↓76	↓79	↓76
Week 39			↓77	↓71	↓77	↓73	↓80	↓78
ESTR								
Week 3				↓85		↓80		
Week 39			↓55	↓67	↓33	↓69	↓33 **(↓50)**	↓64 **(↓71)**
PROG								
Week 3			↑1487	↑400	↑3475	↑1700	↑1400	↑1950
Week 39			↑1390		↑1800		↑1460	↑85
TEST								
Week 3			↓93		↓94		↓96	

Index	% change from control							
Dose (mg/kg)	0		250		500		1000	
Dose (mg/m^2)	0		3000		6000		12000	
Gender	M	F	M	F	M	F	M	F
Week 39			↓89		↓91		↓97	↓48

Note:
- All values were statistically significant from controls p<0.05
- Recovery consisted of control and HD (1000 mg/kg/day) animals only.

Coagulation: Unremarkable

Urinalysis: Unremarkable

Gross Pathology: **Unscheduled deaths**

Index	Macroscopic observations							
Dose (mg/kg)	0		250		500		1000	
Dose (mg/m^2)	0		3000		6000		12000	
Gender	M	F	M	F	M	F	M	F
Lung								
– Adhesion	--	--	--	--	--	--	1	--
– Discolored	--	--	--	--	--	--	1	--
Jejunum								
– Distended	--	--	--	--	--	--	1	--
LN, mandibular								
– Thickening	--	--	--	--	--	--	1	--
Cavity, thoracic								
– Containing fluid	--	--	--	--	--	--	1	--

Note:
- These deaths (in males) at 1000 mg/kg (12000 mg/m^2) were not considered drug related.

Gross Pathology:

Index	Macroscopic observations							
Dose (mg/kg)	0		250		500		1000	
Dose (mg/m^2)	0		3000		6000		12000	
Gender	M	F	M	F	M	F	M	F
Adrenal cortex								
– Brown discoloration			2/4	1/4		2/4	1/4	1/4
Ovary								
– Small, cysts	--		--		--		--	1/4
Uterus								
– Thickening	--		--	1/4	--		--	2/4

Note:
- Recovery – unremarkable.

Organ weights: **(Recovery in bold and in parenthesis)**

Index	% change from control							
Dose (mg/kg)	0		250		500		1000	
Dose (mg/m^2)	0		3000		6000		12000	
Gender	M	F	M	F	M	F	M	F
Adrenal glands								
– Relative to body wt.			↑50	↑137	↑71	↑106	↑28 **(↑54)**	↑75 **(↑17)**
– Relative to brain wt.			↑33	↑110	↑42	↑70	↑25	↑60
Liver								
– Relative to body wt.			↑41	↑47	↑64	↑53	↑44 **(↑30)**	↑70 **(↑37)**
– Relative to brain wt.			↑16	↑24	↑31	↑30	↑31	↑48
Epididymus								
– Relative to body wt.		--	↓40	--	↓41	--	↓40 **(↓42)**	--
– Relative to brain wt.		--	↓52	--	↓56	--	↓50 **(↓56)**	--
Seminal vesicles								
– Relative to body wt.		--	↓61	--	↓60	--	↓44 **(↓43)**	--
– Relative to brain wt.		--	↓69	--	↓70	--	↓53	--
Prostate								
– Relative to body wt.		--	↓34	--	↓40	--	↓38	--
– Relative to brain wt.		--	↓47	--	↓53	--	↓47	--
Testis								
– Relative to body wt.		--	↓30	--	↓32	--	↓21 **(↓47)**	--
– Relative to brain wt.		--	↓34	--	↓37	--	↓20 **(↓60)**	--
Uterus								
– Relative to body wt.	--		--		--	↓32	--	↑42
– Relative to brain wt.	--		--		--	↓40	--	↑20
Ovary								
– Relative to body wt.	--		--	↑92	--	↓31	--	↑107
– Relative to brain wt.	--		--	↑60	--	↓21	--	↑85

Note:
- All values were the ratio (%) of organ weights.
- Recovery consisted of control and HD (1000 mg/kg) animals only.
- All values were statistically significant from controls p<0.05

Histopathology: **(Recovery in bold and in parenthesis)**

Organ	Observation	Microscopic Observations							
		0 mg/kg		250 mg/kg		500 mg/kg		1000 mg/kg	
		M	F	M	F	M	F	M	F
	No. of animals	**4(3)**	**4(3)**	**4**	**4**	**4**	**4**	**4(3)**	**4(3)**
Adrenal,	Hypertrophy, diffuse								

	Observation	Microscopic Observations							
		0 mg/kg		250 mg/kg		500 mg/kg		1000 mg/kg	
		M	F	M	F	M	F	M	F
glands	– *minimal*			1	1	1	1	1	1
	– *slight*			3	3	3	3	2	3
Epididymus	Aspermia								
	– *present*		--	1	--		--	**(1)**	--
	Hypospermia								
	– *slight*		--		--		--	**(1)**	--
	– *marked*		--	1	--	2	--	1	--
Liver	Hyperplasia, bile duct/oval cell								
	– *minimal*			1		3	1	2	1 **(1)**
	– *slight*								1
Thymus	Involution								
	– *slight*	1							
	– *moderate*	2							
Prostate	Atrophy								
	– *slight*		--	3	--	2	--	3	--
Seminal Vesicle	Increased mineralization								
	– *minimal*		--		--	1	--	1	--
	– *slight*		--	1	--		--	1	--
	– *moderate*		--		--	1	--		--
Testes	Atrophy, spermatogenic epithelium								
	– *present*		--	2	--	2	--	3	--
	Hyperplasia, interstitial cells								
	– *slight*		--	4	--	4	--	3	--
Ovary	Cyst, follicle								
	– *slight*	--		--	3	--	4	--	4
	– *moderate*	--		--	1	--		--	
Uterus	Endometrium, pseudodecidual								
	– *moderate*	--		--	2	--	1	--	
	– *marked*	--		--	2	--	3	--	4
Vagina	Atrophy, epithelium								
	– *slight*	--		--	3	--	2	--	3

Note:

– Recovery consisted of control and HD (1000 mg/kg) animals only.

Toxicokinetics:

Toxicokinetic Parameters in Monkeys Following 39 Weeks of CB7630 Administration						
	Males			**Females**		
Dose (mg/kg/day)	**250**	**500**	**1000**	**250**	**500**	**1000**
Dose (mg/m^2/day)	**3000**	**6000**	**12000**	**3000**	**6000**	**12000**

C_{max} (ng/mL)						
Day 1	114	226	238	151	482	471
Day 273	139	183	270	87	466	185
AUC_{0-24} (ng*hr/mL)						
Day 1	594	1577	1637	628	3408	2945
Day 273	610	1139	2095	489	4154	1223
Tmax (hr)						
Day 1	0.87	2.5	3	1.7	1.2	3
Day 273	1.5	3.7	4	4.7	3.7	3
T1/2 (hr)						
Day 1	5	5	5.5	7	6	5
Day 273	8	NA	6	7	10	9

Note:
No NA – Sample was below the lower limit of quantitation.

- The metabolite CB7598 was only detected. CB7630 concentrations were generally below the lower limit of quantitation and TK analysis could not be performed.
- Exposure increased with increasing dose from the LD to the MD (250 to 500 mg/kg/day). However, exposure did not increase with increasing dose at the HD (1000 mg/kg/day).
- Mean concentrations were generally similar after multiple dosing.
- Abiraterone readily appeared in plasma, with mean Tmax values ranging from 0.875 to 3.14 hours on Day 1 and from 1.50 to 4.75 hours on Day 273. After reaching Cmax, abiraterone concentrations readily declined, with mean t1/2 values ranging from 5.03 to 6.87 hours on Day 1, and from 5.82 to 9.95 hours on Day 273.
- Females generally had higher mean Cmax and AUC0-24 values than males with greater than 2-fold differences observed at the 500 mg/kg/day dose level.
- Values for mean Cmax and AUC0-24 were generally similar on Days 1 and 273 indicating no accumulation of abiraterone occurred after multiple dosing.

Other: none

Histopathology inventory

Study	**7777-101**	**7777-103**
Species	Monkey	Monkey
Adrenals	X	X*
Aorta	X	X
Bone Marrow femur	X	X
Bone (femur)	X	X
Brain	X*	X*
Cecum	X	X
Cervix	X	X*
Colon	X	X

Duodenum	X	X
Epididymis	X	X*
Esophagus	X	X
Eye	X	X
Fallopian tube		
Feet		
Gall bladder	X*	
Gross (abnormal) lesions	X	X
Harderian gland		X
Heart	X*	X*
Ileum	X	X
Injection site		
Jejunum	X	X
Kidneys	X*	X*
Lachrymal gland		X
Larynx		
Liver	X*	X*
Lungs	X	X
Lymph nodes, inguinal		X
Lymph nodes mandibular		X
Lymph nodes, mesenteric		X
Mammary Gland	X	X
Nasal cavity	X	
Optic nerves	X	X
Ovaries	X*	X*
Pancreas	X	X
Parathyroid	X	X
Paws/hands	X	X
Peripheral nerve		
Pharynx		
Pituitary	X	X
Prostate	X	X*
Rectum		X
Salivary gland	X	X
Sciatic nerve	X	X
Seminal vesicles	X	X*
Skeletal muscle	X	X
Skin	X	X
Spinal cord		X
Spleen	X*	X*
Sternum	X	X

Stomach	X	X
Teeth		X
Testes	X*	X*
Thymus	X*	X*
Thyroid	X	X
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X	X
Vagina	X	X
Zymbal gland		

X, histopathology performed, *organ weight obtained

2.6.6.4 Genetic toxicology

Summary of Results are shown in Section 2.6.6.10

CTBR 960666: CB7630 (Lot No.562-51-3): Bacterial Mutation Test (Reviewed by Margaret Brower, PhD under IND 71, 023)

CTBR 960736: CB7630 (Lot No.CML-121/05-CR1): Bacterial Mutation Test. (Reviewed by Margaret Brower, PhD under IND 71, 023)

CTBR 960744: CB-7598: Bacterial Mutation Test. (Reviewed by Margaret Brower, PhD under IND 71, 023)

CTBR 960667: CB7630 (Lot No.562-51-3): Chromosome Aberration Test. (Reviewed by Margaret Brower, PhD under IND 71, 023)

CTBR 960737: CB7630 (Lot No.CML-121/05-CR1): Chromosome Aberration Test. (Reviewed by Margaret Brower, PhD under IND 71, 023)

CTBR 960745: CB-7598: Chromosome Aberration Test. (Reviewed by Margaret Brower, PhD under IND 71, 023)

CTBR 960747: CB7630: Rat Micronucleus Test- initial CML process (Reviewed by Margaret Brower, PhD under IND 71, 023)

CTBR 960747: CB7630: Rat Micronucleus Test-current CML process. (Reviewed by Margaret Brower, PhD under IND 71, 023)

CTBR 960747: CB-7598: Rat Micronucleus Test. Reviewed by Margaret Brower, PhD under IND 71, 023)

2.6.6.5 Reproductive toxicology – No studies submitted

2.6.6.6 Carcinogenicity – No studies submitted

2.6.6.7 Local tolerance – No studies submitted

2.6.6.8 Special toxicology studies

Studies on Impurities

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

TOX9589: JNJ-212082-AAA spiked with (b) (4) impurities: Bacterial Reverse Mutation Test

Report #: TOX9589
Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: March 2010
GLP Compliance: Yes
QA Report: Yes (X), No ()
Drug, lot #, and % purity: JNJ-212082-AAA (95%); CMLG-045/10-CR4; 100%

Impurity	Nominal impurity level (%w/w of abiraterone acetate dose)	Lot No.	Purity ;
(b) (4)		1676-48-1	98.6%
		JVDU1-01-010-1	96.4%
		KPAESHUY-01-020-2	95.3%
		JVDU1-01-014-6	99.7%
		KPAESHUY-01-024-2	99.4%
Total impurities:	(b) (4)		

Key Study Findings:
JNJ-212082-AAA spiked with (b) (4) impurities was not mutagenic in both initial and confirmatory assays up to the maximum concentration tested (5000 µg/plate).

Methods:
Strains: *S. typhimurium* TA1535; TA1537, TA98, TA100, WP2 *uvr*A

Concentrations in definitive study:	Initial assay: 50, 150, 500, 1000, 2000, 3000, 4000 and 5000 µg/plate Confirmatory study: 80, 160, 320, 640, 1250, 2500 and 5000 µg/plate.
Basis of concentration selection:	5 highest concentration below toxic level, or if nontoxic, at 5 levels up to standard limit of 5000 µg/plate
Negative control:	100% ethanol
Positive control:	Sodium azide; 9-Aminoacridine; 2-Nitrofluorene; 4-Nitroquinoline-1-oxide; 2-Aminoanthracene; Benzo[a]pyrene
Formulation/Vehicle:	100% ethanol
Incubation and sampling time:	Incubated at 37°C for approximately 72 hours

Study Validity:
For an assay to be considered valid, the mean revertant colony counts of the vehicle/negative controls for each strain should lie close to or within the current historical control range of the laboratory. All positive control articles (with S9 where required) should produce increases in revertant colony numbers to at least twice the concurrent vehicle control levels with the appropriate bacterial strain (1.5× for strain TA100).

8200783: Bacterial Reverse Mutation Assay with a Confirmatory Assay

Report #:	8200783
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation	Dec. 10, 2008
GLP Compliance	Yes
QA Report	Yes (X), No ()
Drug, lot #, and % purity	Abiraterone acetate mesylate impurity; 1318-37-1; 99.14%

Key Study Findings:
- Positive increases in the mean number of revertants per plate were observed with tester strains TA98 (3.7-fold) and TA100 (6.0-fold) in the absence of S9, and with tester strain TA100 (3.7-fold) in the presence of S9. No positive increases were observed with any of the other tester strains in the presence or absence of S9 mix.
- In conclusion, abiraterone acetate mesylate impurity was positive in the bacterial reverse mutation assay with a confirmatory assay in the presence and absence of S9.

Methods:

Strains:	*S. typhimurium* TA98, TA100, TA1535; TA1537, WP2 *uvr*A
Concentrations in definitive study:	Initial assay: 10, 33.3, 100, 333, 1000, and 3330 µg/plate

	Confirmatory study: 10, 25, 50, 75, 100, 150, 200, 333, and 1000 µg/plate
Basis of concentration selection:	Based on dose range-finding assay using tester strains TA100 and WP2*uvr*A. Test article was evaluated at doses of 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, and 5000 µg/plate with and without S9 (one plate per dose).
Negative control:	100% ethanol
Positive control:	2-Nitrofluorene, Sodium azide, ICR-191, 4-Nitroquinoline-N-oxide, Benzo[a]pyrene, 2-Aminoanthracene
Formulation/Vehicle:	Ethanol
Incubation and sampling time:	Incubated at 37°C for approximately 52 hours

Study Validity:
For an assay to be considered valid, the mean revertant colony counts of the vehicle/negative controls for each strain should lie close to or within the current historical control range of the laboratory. All positive control articles (with S9 where required) should produce increases in revertant colony numbers to at least twice the concurrent vehicle control levels with the appropriate bacterial strain (1.5× for strain TA100).

TOX9597: *In Vitro* Bacterial Reverse Mutation Test with (b) (4) in *Salmonella typhimurium*

Report #:	TOX9597
Conducting Laboratory and Location:	Johnson & Johnson Pharmaceutical Research & Development B-2340 Beerse, Belgium
Date of Study Initiation	Feb. 26, 2010
GLP Compliance	Yes
QA Report	Yes (X), No ()
Drug, lot #, and % purity	(b) (4) (b) (4); KPAESHUY-01-024-2; 99.4%

Key Study Findings:
(b) (4) ((b) (4) a synthesis impurity of abiraterone acetate, is not mutagenic in the presence and absence of S9 up to the maximum concentration tested (1000 µg/plate).

Methods:

Strains:	*S. typhimurium* TA1535; TA1537, TA98, TA100, TA102
Concentrations in definitive study:	Initial assay: 15.6, 31.2, 62.5, 125, 250, 500 and 1000 µg/plate

	Confirmatory study: 4.1, 10.2, 25.6, 64, 160, 400 and 1000 µg/plate.
Basis of concentration selection:	5 highest concentration below toxic level, or if nontoxic, at 5 levels up to standard limit of 1000 µg/plate
Negative control:	DMSO
Positive control:	Sodium azide; 9-Aminoacridine; 2-Nitrofluorene; 4-Nitroquinoline-N-oxide; 2-Aminoanthracene
Formulation/Vehicle:	Ethanol
Incubation and sampling time:	Incubated at 37°C for approximately 48-72 hours

Study Validity:
For an assay to be considered valid, the mean revertant colony counts of the vehicle/negative controls for each strain should lie close to or within the current historical control range of the laboratory. All positive control articles (with S9 where required) should produce increases in revertant colony numbers to at least twice the concurrent vehicle control levels with the appropriate bacterial strain (1.5× for strain TA100).

TOX9598: *In Vitro* Bacterial Reverse Mutation Test with (b) (4) in *Salmonella typhimurium*

Report #:	TOX9598
Conducting Laboratory and Location:	Johnson & Johnson Pharmaceutical Research & Development B-2340 Beerse, Belgium
Date of Study Initiation	Feb. 26, 2010
GLP Compliance	Yes
QA Report	Yes (X), No ()
Drug, lot #, and % purity	Synthesis impurity of abiraterone acetate ((b) (4) JVDU1-01-026-3; 99.4%

Key Study Findings:
Synthesis impurity of abiraterone acetate ((b) (4) a synthesis impurity of abiraterone acetate, is not mutagenic in the presence and absence of S9 up to the maximum concentration tested (3000 µg/plate).

Methods:

Strains:	*S. typhimurium* TA1535; TA1537, TA98, TA100, TA102
Concentrations in definitive study:	Initial assay: 23.4, 46.88, 93.75, 187.5, 375, 750 and 1500 µg/plate Confirmatory study: 62.5, 125, 250, 500, 1000, 2000 and 3000 µg/plate
Basis of concentration selection:	5 highest concentration below toxic level, or if nontoxic, at 5 levels up to

	standard limit of 3000 µg/plate
Negative control:	DMSO
Positive control:	Sodium azide; 9-Aminoacridine; 2-Nitrofluorene; 4-Nitroquinoline-N-oxide; 2-Aminoanthracene
Formulation/Vehicle:	Ethanol
Incubation and sampling time:	Incubated at 37°C for approximately 48-72 hours

Study Validity:
For an assay to be considered valid, the mean revertant colony counts of the vehicle/negative controls for each strain should lie close to or within the current historical control range of the laboratory. All positive control articles (with S9 where required) should produce increases in revertant colony numbers to at least twice the concurrent vehicle control levels with the appropriate bacterial strain (1.5× for strain TA100).

TOX9599: *In Vitro* Bacterial Reverse Mutation Test with [redacted] in *Salmonella typhimurium*

Report #:	TOX9599
Conducting Laboratory and Location:	Johnson & Johnson Pharmaceutical Research & Development B-2340 Beerse, Belgium
Date of Study Initiation	Feb. 26, 2010
GLP Compliance	Yes
QA Report	Yes (X), No ()
Drug, lot #, and % purity	[redacted] TRAMMELO-01-043-1; 100%

Key Study Findings:
- [redacted] a synthesis impurity of abiraterone acetate, is not mutagenic in the presence and absence of S9 up to the maximum concentration tested (2000 µg/plate).
- [redacted], a synthesis impurity of abiraterone acetate, is mutagenic properties towards strain TA1537 (> 3-fold) in the presence of rat liver S9 but is not mutagenic towards stains TA98, TA100, TA1535 and TA102 in the presence of S9.

Methods:

Strains:	*S. typhimurium* TA1535; TA1537, TA98, TA100, TA102
Concentrations in definitive study:	Initial assay: 31.25, 62.5, 125, 250, 500, 1000 and 2000 µg/plate Confirmatory study: 31.25, 62.5, 125, 250, 500 and 1000 µg/plate
Basis of concentration selection:	5 highest concentration below toxic level, or if nontoxic, at 5 levels up to

	standard limit of 2000 µg/plate
Negative control:	DMSO
Positive control:	Sodium azide; 9-Aminoacridine; 2-Nitrofluorene; 4-Nitroquinoline-N-oxide; 2-Aminoanthracene
Formulation/Vehicle:	Ethanol
Incubation and sampling time:	Incubated at 37°C for approximately 48-72 hours

Study Validity:
For an assay to be considered valid, the mean revertant colony counts of the vehicle/negative controls for each strain should lie close to or within the current historical control range of the laboratory. All positive control articles (with S9 where required) should produce increases in revertant colony numbers to at least twice the concurrent vehicle control levels with the appropriate bacterial strain (1.5× for strain TA100).

TOX9725: *In Vitro* Bacterial Reverse Mutation Test with (b) (4) in *Salmonella typhimurium*

Report #:	TOX9725
Conducting Laboratory and Location:	Johnson & Johnson Pharmaceutical Research & Development B-2340 Beerse, Belgium
Date of Study Initiation	Aug. 16, 2010
GLP Compliance	Yes
QA Report	Yes (X), No ()
Drug, lot #, and % purity	(b) (4) (b) (4) tvanhoeg-07-019-1; 99.75%

Key Study Findings:
(b) (4) (b) (4) a synthesis impurity of abiraterone acetate, is not mutagenic in the presence and absence of S9 up to the maximum concentration tested (2500 µg/plate).

Methods:

Strains:	*S. typhimurium* TA1535; TA1537, TA98, TA100, TA102
Concentrations in definitive study:	Initial assay: 6.17, 18.52, 55.5, 166.6, 500, 1500 and 2500 µg/plate Confirmatory study: 3.43, 10.29, 30.86, 92.59, 277.7, 833.3 and 2500 µg/plate
Basis of concentration selection:	5 highest concentration below toxic level, or if nontoxic, at 5 levels up to standard limit of 2000 µg/plate
Negative control:	DMSO
Positive control:	Sodium azide; 9-Aminoacridine; 2-Nitrofluorene; 4-Nitroquinoline-N-oxide; 2-Aminoanthracene

Formulation/Vehicle:	Ethanol
Incubation and sampling time:	Incubated at 37°C for approximately 48-72 hours

Study Validity:
For an assay to be considered valid, the mean revertant colony counts of the vehicle/negative controls for each strain should lie close to or within the current historical control range of the laboratory. All positive control articles (with S9 where required) should produce increases in revertant colony numbers to at least twice the concurrent vehicle control levels with the appropriate bacterial strain (1.5× for strain TA100).

***In Vitro* Chromosome Aberration Test in Human Lymphocytes:**

TOX9602: *In Vitro* Mammalian Chromosome Aberration Test with JNJ-212082-AAA spiked with (b) (4) impurities in Human Lymphocytes

Report #:	TOX9602
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation	March 29, 2010
GLP Compliance	Yes
QA Report	Yes (X), No ()
Drug, lot #, and % purity	JNJ-212082-AAA (AD04FY); CMLG-045-CR4; 100%

Impurity	Nominal impurity level (%w/w of abiraterone acetate dose)	Lot No.	Purity ;
(b) (4)		1676-48-1	98.6%
		JVDU1-01-010-1	96.4%
		KPAESHUY-01-020-2	95.3%
		JVDU1-01-014-6	99.7%
		KPAESHUY-01-024-2	99.4%
Total impurities:	(b) (4)		

Key Study Findings:
JNJ-212082-AAA (AD04FY) spiked with (b) (4) impurities was negative for the induction of structural and numerical chromosome aberrations in both the non-activated and the S9-activated test systems in the *in vitro* mammalian chromosome aberration test using human peripheral blood lymphocytes.

Methods:	
Strains:	Human peripheral blood lymphocytes
Concentrations in definitive study:	With and without S9: 5, 10, 25, 35, 50, 75, 100, 150, 200 and 300 µg/mL
Basis of concentration selection:	The highest dose level selected for evaluation was the dose which induced at least 50% toxicity, as measured by mitotic inhibition, relative to the solvent control, with a sufficient number of scorable metaphase cells. Two additional lower dose levels were included in the evaluation.
Negative control:	Ethanol
Positive control:	Mitomycin C (without activation) and Cyclophosphamide (with activation)
Formulation/Vehicle:	Ethanol
Incubation and sampling time:	20 hr without S9; 4 hr with 16 hr recovery period with and without S9
Number of cells analyzed	500 cells for mitotic index and 200 metaphase spreads for chromatid-type and chromosome-type aberrations.

Study Validity:
For an assay to be considered valid, the vehicle/negative control results should lie within or close to the historical control range, while the positive control should produce a significant increase in the incidence of aberrant cells compared to concurrent controls.

Results:
JNJ-212082-AAA (AD04FY) spiked with (b) (4) impurities did not significantly increase the percentage of cells with structural or numerical aberrations in the test article-treated group relative to the solvent control at any dose levels (using Fisher's Exact test). The proportion of cells with structural or numerical aberrations for all groups was within the laboratory historical control range. No increase in the incidence of chromatid or chromosome gaps or polyploidy were observed.

***In Vivo* Toxicity Assay in Rodents:**

TOX9587: 1-month Repeated Dose Oral Toxicity Study of JNJ-212082-AAA in the Rat with Integrated Irwin Observations (impurity qualification).

Study no.:	TOX9587
Study report location:	Janssen Research & Development, A division of Janssen Pharmaceutica NV B-2340 Beerse Belgium
Conducting laboratory and location:	Global Preclinical Development Beerse site, Turnhoutseweg 30 2340 Beerse, Belgium
Date of study initiation:	March 4, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Abiraterone acetate (CB7630) or JNJ-212082-AAA, CMLG-045/10-CR4, 98.2%

Impurity	Nominal impurity level (%w/w of abiraterone acetate dose)	Lot No.	Purity ;
(b) (4)		1676-48-1	98.6%
		JVDU1-01-010-1	96.4%
		KPAESHUY-01-020-2	95.3%
		JVDU1-01-014-6	99.7%
		KPAESHUY-01-024-2	99.4%
Total impurities:	(b) (4)		

Key study findings:

- No significant differences were seen between the high dose groups with and without impurities for any of the examined parameters, except for the low incidence of swollen spleens and an increased spleen weight (≤20%) in the high dose female group with impurities.
- Overall, no differences were observed at 40 and 400 mg/kg/day when abiraterone acetate was spiked with (b) (4) impurities (b) (4) in its formulation.

Methods

Doses:	40 and 400 mg/kg/day (240 and 2400 mg/m^2/day)
Frequency of dosing:	Daily for 1 month (28 days)
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% w/v Methocel A4M + 0.1% w/v Tween 80 in demineralised water
Species/Strain:	Rats/ SPF, Sprague Dawley
Number/Sex/Group:	10 sex/dose
Age:	8 weeks
Weight:	183 to 331 grams
Satellite groups:	3/sex/dose
Basis of dose selection:	Based on 26-week oral gavage toxicity study with CB7630 (JNJ-212082-AAA) in rats (Study No. 7777-105). Doses administered were 50, 150, and 400 mg/kg/day. 15/17 deaths were drug related at the 400 mg/kg/day group. Mortality was seen from Week 8 and onwards in males and Week 15 and onwards in females. Target organs of toxicity included adrenals, liver, lung, male and female reproductive organs and male mammary glands. There was also a dose related incidence of cataracts in males and females following dosing and recovery. Most changes and findings exhibited partial recovery with few exceptions which included severe seminiferous tubule atrophy at 150 and 400 mg/kg, cataracts in males at 150 and 400 mg/kg and females at 400 mg/kg and minimal to moderate biliary hyperplasia in both males and females at 400 mg/kg. Based on these results, a high dose of 400 mg/kg was selected and a low dose of 40 mg/kg.
Unique study design:	To assess the potential toxicity of 5 drug substance impurities of JNJ-212082-AAA (abiraterone acetate) when administered once daily by the oral route (via gavage) to rats, for 1 month. These impurities were given at (b) (4) [redacted] in total of the JNJ-212082-AAA formulation, namely (b) (4) [redacted], (b) (4) [redacted] J [redacted] and (b) (4) [redacted]
Deviation from study protocol:	– None

Observations:

Mortality:	Twice daily
Clinical signs:	Twice daily
Body weights:	Predose and Day 0 and at weekly intervals during the study.

Food consumption:	Weekly intervals during the dosing period.
Ophthalmoscopy:	Days 28 and 29, prior to necropsy
Hematology:	Days 28 and 29, prior to necropsy
Clinical chemistry:	Days 28 and 29, prior to necropsy
Coagulation	Days 28 and 29, prior to necropsy
Urinalysis:	Days 28 and 29, prior to necropsy
Gross pathology:	Conducted on all animals including those found dead, euthanized in extremis, and those euthanized at study termination and end of recovery.
Organ weights:	Days 28 and 29, prior to necropsy
Histopathology:	Adequate Battery: yes (X), no () Peer review: yes (X), no ( )
Toxicokinetics:	Days 28 and 29, prior to necropsy

Results:
Mortality: None

Clinical signs: Unremarkable.

Body weight:
No relevant differences were seen between the group dosed with and without impurities.

Food consumption**:** Unremarkable.

Ophthalmoscopy: Unremarkable.

EKG: not done

Hematology:
No significant differences were noted between the groups dosed with or without impurities.

Clinical Chemistry:
No significant differences were seen between the groups with impurities and without impurities.

Coagulation: Unremarkable

Urinalysis: Unremarkable

Gross Pathology**:** Unremarkable.

Organ weights: Unremarkable.

Histopathology**:** Unremarkable.

Toxicokinetics:

- Toxicokinetics showed that the absorption was fast to moderate, with Tmax mostly observed between 0.5 and 2 h after dosing.
- In general, exposure increased less than dose-proportional and was lower in male after repeated dosing than after single dosing, but similar in females.
- The exposure in males was much higher than in females.
- Results are shown in Sponsor's Table below.

	Male		Female	
Dose (mg/kg/day)	40	400	40	400
	Day 0			
C_{max} (ng/ml)	703	3670	52.2	127
T_{max} (h)	1-2	4-8	1-2	2
$AUC_{0\text{-}Inf}$ (ng.h/ml)	2834	32906	174	795[1]
	Day 27			
C_{max} (ng/ml)	237	520	44.8	152
T_{max} (h)	0.5-2	2-4	0.5-2	1-2
$AUC_{0\text{-}24\ h}$ (ng.h/ml)	996	4398	151	798[2]

[1] n=2; [2] $AUC_{0\text{-}last}$ (t_{last} was 8 or 24 h)

Results:

- No significant differences were seen between the high groups with and without impurities for any of the examined parameters, except for the low incidence of swollen spleens and an increased spleen weight (≤20%) in the high dose female group with impurities.

2.6.6.9 Discussion and Conclusion

Androgens stimulate the development and progression of prostate cancer. Currently available androgen deprivation therapies block the testicular source of testosterone, but not the adrenal or peripheral sources, which continue to supply androgens to prostate tumor cells and promote disease progression. There is an unmet medical need for improved therapy for patients with metastatic advanced prostate cancer (also referred to as castration resistant prostate cancer [CRPC]) that can lower androgen levels more effectively than available androgen deprivation therapies without the adverse events associated with cytotoxic chemotherapy. Abiraterone acetate is a prodrug of abiraterone, which blocks androgen production by the irreversible inhibition of cytochrome P450 17α-hydroxylase/C17,20-lyase (CYP17). This enzyme is a key enzyme in the production of androgens and is found in the testes, adrenals, and in prostate tumors.

CYP 17 catalyzes two reactions that are rate limiting to the biosynthesis of androgens: 17α hydroxylation of C21 steroids and cleavage of the C17,20 bond of C21 steroids. The 17α hydroxylation activity is also a required step in cortisol biosynthesis, whereas the C17,20 bond side chain cleavage is specific to the biosynthesis of androgens. This enzyme catalyzes the conversion of pregnenolone or progesterone into dehydroepiandrosterone (DHEA) or

androstenedione, respectively, two precursors of testosterone. Abiraterone results in reductions of androgen levels by specifically inhibiting 17α-hydroxylase/C17,20-lyase.

The majority of the toxicities observed with abiraterone acetate in rat and monkey are likely related to the pharmacodynamic properties of the compound, i.e. related to interference of abiraterone acetate with the steroid metabolism due to selective CYP17 inhibition. In male animals these effects were primarily noted in male reproductive organs (testes, prostate, seminal vesicles, and epididymides) and mammary gland. In females, most notable changes were seen in the ovaries, but also in the uterus and vagina. Associated with the effect on steroidogenesis is the ACTH-related adrenocortical hypertrophy. These pharmacological effects were partially to fully reversible.

Another target organ of toxicity after prolonged treatment with abiraterone acetate in monkeys is the liver as evidenced by bile duct/oval cell hyperplasia, in serum associated with increases in ALP, total bilirubin.

In rats, cataract was seen ophthalmologically in a dose-dependent manner at the end of the 26-week treatment period, without evidence of reversibility. The mechanism is unclear although a species-specific effect cannot be excluded since abiraterone acetate-related cataract was neither observed in monkeys when dosed for 39 weeks (at abiraterone plasma exposure comparable to clinical exposure). The mechanism of cataract formation is unknown.

In the battery of genotoxicity studies, abiraterone acetate was not mutagenic or clastogenic *in vitro*. Abiraterone acetate was not clastogenic (induction of micronuclei) in the *in vivo* rat micronucleus study.

There was no reproductive toxicology studies conducted to investigate the effect of abiraterone acetate on reproductive function since the general toxicity studies with abiraterone acetate provide sufficient information to assess the effect on reproductive organs.

2.6.6.10 Tables and Figures

See text of review for pertinent tables and figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

Repeat Dose Toxicity Studies						
Study #	**Species**	**Route/ duration**	**N/sex/ dose**	**mg/kg/ day**	**mg/m^2**	**Significant findings**
7777-100	Rat	Oral gavage Daily x 13 Weeks 4-week recovery	15 (main)/ 5 (recovery)	250/50 750/250 2000/750	1500/3000 4500/1500 12000/4500	**1500/3000 mg/m^2/day**: ↓ TRIGLY, testosterone and ↑ LH M only and ↑TBILI F only. ↓ Male reproductive organ weights (epididymis, prostate, testes, and seminal vesicles), uterus, and thymus. Hyperplasia in pituitary M only, atrophies in seminal vesicles, prostate, testes, and epididymis. **4500/1500 mg/m^2/day:** ↑ ALB, A/B ratio, ↓ TRIGLY and ↑ LH M only and TBILI F only. ↓ Male reproductive organ weights (epididymis, prostate, testes, and seminal vesicles), uterus, and thymus. ↑ liver weights. Hyperplasia in pituitary M only, atrophy in male mammary glands, prostate, testes, epididymis, hypertrophy in adrenals, **12000/4500 mg/m^2/day:** Mortality in 3 M and 10 F. Dose was reduced on Day 9. Hunched posture, swollen ventral abdomen, alopecia, pale body, rough hair-coat, reduced feces. ↓ BW in males (25-32%). ↓ FC in males (16%). Males: ↑ WBC (13%), PLTS (32%), RETIC (1.4 fold). Females: ↑ WBC (46%), PLTS (35%), RETIC (94%). Bile duct hyperplasia in liver, infiltrate in lungs M only, hyperplasia in pituitary M only, atrophy in prostate, testes, epididymis, cervix, and uterus, and hypertrophy in adrenals **Recovery:** Swollen ventral abdomen and rough hair-coat. ↑ LH M only. Hyperplasia in liver and pituitary. Atrophy in uterus and cervix.
7777-101	Monkey	Oral gavage Daily x 13 Weeks	4 main/ 3 recovery	250 750 2000	3000 9000 24000	**3000 mg/m^2/day**: White color vomit. ↑ PLTS M only and ↑ LYMPH and ESO F only. ↑ TBILI, TRIGLY, CORT, ACTH, ALDS, LH (M only), PROG, and DHEA. ↓ ESTR (F only). Brown discoloration in adrenal cortex (M only) and discolored liver (F only). Increase in adrenal (M only), liver, and ovary organ weights. Minimal to slight hypertrophy in

Repeat Dose Toxicity Studies						
Study #	**Species**	**Route/ duration**	**N/sex/ dose**	**mg/kg/ day**	**mg/m^2**	**Significant findings**
		4-week recovery				adrenals; minimal to slight bile duct hyperplasia in liver; minimal to slight hyperplasia and edema/fibrosis in male mammary gland. **9000 mg/m^2/day**: White and yellow color vomit; white and non-formed feces. ↑ PLTS M only and ↑ LYMPH and ESO and ↓ NEUT F only. ↑ TBILI, TRIGLY, CORT, ACTH, ALDS, LH (M only), ALP (F only) PROG, and DHEA. ↓ ESTR and TEST (F only). Mass in liver (F only). Discoloration in seminal vesicle and cysts in ovaries. Increase in adrenal (M only), liver, and ovary organ weights. Minimal to slight hypertrophy in adrenals; minimal to moderate bile duct hyperplasia in liver; minimal to slight hyperplasia and minimal to moderate edema/fibrosis in male mammary gland. **24000 mg/m^2/day:** White, yellow and food in vomit; white and non-formed feces. ↑ PLTS M only and ↑ LYMPH and ESO (M and F) and ↓ NEUT F only. ↑ TBILI, TRIGLY, CORT, ACTH, ALDS, LH (M only), PROG, and DHEA. ↓ ESTR (F only) and TEST. Discolored and large adrenal cortex and cysts in ovaries. Increase in adrenal (M only), liver, and ovary organ weights. Minimal to slight hypertrophy in adrenals; minimal to moderate bile duct hyperplasia in liver; minimal to slight hyperplasia and minimal to moderate edema/fibrosis in male mammary gland. **Recovery:** ↑ LYMPH (F) and ESO (M). ↑ ALDS (M only). Cysts in ovaries. Minimal to slight bile duct hyperplasia. Increase in liver organ and ovary organ weights. Minimal to slight bile duct hyperplasia in liver.
7777-105	Rat	Oral gavage Daily x 26-weeks	20 main/10 recovery	50 150 400	300 900 2400	**300 mg/m^2/day**: ↓ WBC (M only), TRIGLY and testosterone and ↑ LH. ↓ Male reproductive organ weights (epididymis, prostate, testes, and seminal vesicles), ovary and uterus and ↑ in pituitary. Atrophy in adrenals, male mammary glands, seminal vesicles, prostate, and testes; cellular debris in epididymis; hypertrophy in pituitary (M only) and ovaries; swelling of lens fiber in eye (M only).

Repeat Dose Toxicity Studies						
Study #	**Species**	**Route/ duration**	**N/sex/ dose**	**mg/kg/ day**	**mg/m^2**	**Significant findings**
		4-week recovery				**900 mg/m^2/day**: ↓ BW change (50%) (M only). ↓ WBC (M only), TRIGLY and testosterone. ↑ ALP and LH. ↓ Male reproductive organ weights (epididymis, prostate, testes, and seminal vesicles), ovary and uterus and ↑ in pituitary. Cataracts (M>F). Atrophy in adrenals, male mammary glands, seminal vesicles, prostate, and testes; hypertrophy in pituitary (M only), liver and ovaries; edema in testes, cellular debris in epididymis; swelling of lens fiber in eye (M only). **2400 mg/m^2/day**: Mortality in 10 M and 11 F. Hunched posture, emaciation, swollen ventral abdomen, hypoactivity, clear oral discharge, few feces, opacity and squinted eyes, irregular respiration, rough hair-coat, scabs perioral area/nose/paws, and ataxia (F only). ↓ BW change (50%) (M only). Cataracts (diffuse, multifocal, and/or posterior cortical) and corneal dystrophy. ↓ TRIGLY, PLTS, RETIC (F>M) and testosterone. ↑ GGT M only, TBILI, ALP, LH and urinary volume. ↓ Male reproductive organ weights (epididymis, prostate, testes, and seminal vesicles), uterus, ovary and ↑ liver weight and pituitary. Atrophy in adrenals, male mammary glands, seminal vesicles, prostate, and testes; hypertrophy in pituitary (M only), liver and ovaries; inflammation in lung; edema in testes, cellular debris in epididymis; swelling of lens fiber in eye (M only). **Recovery:** Cataracts at HD. ↓ WBC, TRIGLY and testosterone M only. ↓ RBC at HD. ↑ LH at MD and HD males only. ↓ male reproductive organ weights (epididymis, prostate, testes, and seminal vesicles). Atrophy/hyperplasia/edema of testes; aspermia/hypospermia/cellular debris of epididymis; hypertrophy/hyperplasia of ovaries; cataract and swelling of eyes.
7777-105	Monkey	Oral gavage Daily x 39-week recovery	4 main/3 recovery	250 500 1000	3000 6000 12000	**3000 mg/m^2/day**: White color vomit; non-formed feces, presence of menses, and alopecia in skin. ↓ BW loss of 21% and ↓ FC (≥20%) from Week 1 through Week 40 (M only). ↑ RETIC (F only), LYMPH, MONO, LUC (F only), ESO (F only) and ↓ NEUT. ↓ CREA (M only),

Repeat Dose Toxicity Studies						
Study #	**Species**	**Route/ duration**	**N/sex/ dose**	**mg/kg/ day**	**mg/m^2**	**Significant findings**
		weeks 4-week recovery				DHEA, CORT, ESTR, TEST and ↑ TBILI, ACTH, ALDS, and PROG. Brown discoloration in adrenals and thickening of uterus. ↑ Adrenals, liver, and ovary weights and ↓ in male reproductive organs weights. Minimal to slight hypertrophy in adrenals, minimal to moderate hypospermia/hyperplasia/atrophy in male reproductive organs, minimal to slight hyperplasia in liver, slight to moderate cyst in ovaries, moderate to marked pseudodecidual in uterus, and slight atrophy in vagina. **6000 mg/m^2/day**: White color vomit; non-formed feces, presence of menses, and alopecia in skin. ↓ BW loss of 22% and ↓ FC (≥20%) from Week 1 through Week 40 (M only). ↑ RETIC (F only), LYMPH, MOMO, LUC (F only), ESO (M only) and ↓ WBC (M only) and NEUT. ↓ CREA (M only), DHEA, CORT, ESTR, TEST and ↑ TRIGLY, TBILI, ACTH, ALDS, and PROG. Brown discoloration in adrenals. ↑ Adrenals, liver, uterus and ovary weights and ↓ in male reproductive organs weights. Minimal to slight hypertrophy in adrenals, minimal to moderate hypospermia/hyperplasia/atrophy in male reproductive organs, minimal to slight hyperplasia in liver, slight to moderate cyst in ovaries, moderate to marked pseudodecidual in uterus, and slight atrophy in vagina. **12000 mg/m^2/day**: White color vomit; non-formed feces presence of menses, and alopecia in skin. ↓ BW loss of 16% and ↓ FC (≥20%) from Week 1 through Week 40 (M only). ↑ RETIC, LYMPH, MOMO, LUC (F only), ESO and ↓ WBC (M only) and NEUT. ↓ CREA (M only), DHEA, CORT, ESTR, TEST and ↑ ALP, TRIGLY, TBILI, ACTH, ALDS, and PROG. Brown discoloration in adrenals, thickening of uterus, and small cysts in ovaries. ↑ Adrenals, liver, uterus and ovary weights and ↓ in male reproductive organs weights. Minimal to slight hypertrophy in adrenals, minimal to moderate hypospermia/hyperplasia/atrophy in male reproductive organs, minimal to slight hyperplasia in liver, slight to moderate cyst in ovaries,

Repeat Dose Toxicity Studies						
Study #	**Species**	**Route/ duration**	**N/sex/ dose**	**mg/kg/ day**	**mg/m^2**	**Significant findings**
						moderate to marked pseudodecidual in uterus, and slight atrophy in vagina. **Recovery:** ↓ BW loss of 27% and ↓ FC (≥20%) during first 2 weeks of recovery (M only). ↓ LUC (F only). ↑ TBILI, ALP (M only) and ↓ ESTR. Presence aspermia and slight hypospermia in epididymis.

Note:

- In toxicology studies, findings occurred in both males and females, unless otherwise stated.
- In rat studies, recovery consisted of all dose groups.
- In monkey studies, recovery consisted of control and HD animals only.
- In the 13-week rat study, dosing was suspended in HD males administered 2000 mg/kg on D9. Dosing resumed on D12 a lower dose of 750 mg/kg followed by reducing LD and MD to 50 and 250 mg/kg, respectively, on D10.

Genetic Toxicology Studies		
Study/Study No.	**Concentration/ Doses**	**Results**
***In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)**		
CB7630(Lot No.562-51-3): Bacterial Mutation Test (Ames)/ CTBR 960666	Without S9: 320,640, 1250, 2500,5000 µg/plate With S9 (30% activation):160, 320, 640,1250,2500,5000 µg/plate	CB7630 (initial CML process of abiraterone acetate) is not mutagenic in the presence and absence of S9.
CB7630(Lot No.CML-121/05-CR1): Bacterial Mutation Test/ CTBR 960736	Without S9: 320, 640, 1250, 2500,5000 µg/plate With S9 (30% activation):320, 640, 1250, 2500,5000	CB7630 (current CML process of abiraterone acetate) is not mutagenic in the presence and absence of S9.

Genetic Toxicology Studies		
Study/Study No.	**Concentration/ Doses**	**Results**
	µg/plate	
CB-7598: Bacterial Mutation Test/ CTBR 960744	Without S9: 50,158, 500, 1581,5000 µg/plate With S9: 50,158, 500, 1581, 5000 µg/plate	CB7598 (abiraterone metabolite) is not mutagenic in the presence and absence of S9.
***In Vitro* Chromosomal Aberration Assays in Mammalian Cells**		
CB7630(Lot No.562-51-3): Chromosome Aberration Test/ CTBR 960667	Without S9: 12.8, 25.6, 50, 100 µg /mL; With S9: 12.8, 25.6, 50, 100, 200 µg /mL	CB7630 (initial CML process of abiraterone acetate) was not clastogenic in the presence and absence of S9.
CB7630(Lot No.CML-121/05-CR1): Chromosome Aberration Test/ CTBR 960737	Without S9: 12.8, 25.6, 50, 100ug/mL at 4h; 6.4, 12.8, 25.6 µg /mL at 21h. With S9: 50, 100, 200 µg /mL	CB7630 (current CML process of abiraterone acetate) was not clastogenic in the presence and absence of S9.
CB-7598: Chromosome Aberration Test / CTBR 960745	With and without S9: 800, 1600, 3495 µg /mL	CB-7598 (abiraterone metabolite) was not clastogenic in the presence and absence of S9.
***In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)**		
CB7630: Rat Micronucleus Test-initial CML process / CTBR 960747	Single dose by oral gavage at 24h sampling time: 500, 1000, 2000mg/kg 48h sampling time: 2000mg/kg	CB7630 (initial and current CML process of abiraterone acetate) and CB-7598(abiraterone metabolite) were not clastogenic.

Special Toxicology Studies – Studies with Impurities of Abiraterone Acetate Toxicology Studies		
Study/Study No.	**Concentration/ Doses**	**Results**

Special Toxicology Studies – Studies with Impurities of Abiraterone Acetate Toxicology Studies		
Study/Study No.	**Concentration/ Doses**	**Results**
***In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)**		
Abiraterone acetate spiked with (b) (4) impurities /TOX9589	**Initial:** 50, 150, 500, 1000, 2000, 3000, 4000 and 5000 µg/plate with and without S9 **Confirmatory:** 80, 160, 320, 640, 1250, 2500 and 5000 µg/plate with and without S9	Abiraterone acetate spiked with (b) (4) impurities is not mutagenic in the presence and absence of S9.
Confirmatory assay with (b) (4) impurity ((b) (4) /8200783	**Initial assay:** 10, 33.3, 100, 333, 1000, and 3330 µg/plate with and without S9 **Confirmatory assay:** 10, 25, 50, 75, 100, 150, 200, 333, and 1000 µg/plate with and without S9	The (b) (4) impurity ((b) (4) was positive in the bacterial reverse mutation assay with a confirmatory assay in the presence and absence of S9
(b) (4) of abiraterone acetate /TOX9597	**Initial assay:** 15.6, 31.2, 62.5, 125, 250, 500 and 1000 µg/plate with and without S9 **Confirmatory assay:** 4.1, 10.2, 25.6, 64, 160, 400 and 1000 µg/plate with and without S9	The (b) (4) (b) (4) ((b) (4) is not mutagenic in the presence and absence of S9.
Synthesis impurity of abiraterone acetate ((b) (4) / TOX9598	**Initial assay:** 23.4, 46.88, 93.75, 187.5, 375, 750 and 1500 µg/plate with and without S9 **Confirmatory assay:** 62.5, 125, 250, 500, 1000, 2000 and 3000 µg/plate with and without S9	The synthesis impurity of abiraterone acetate ((b) (4) is not mutagenic in the presence and absence of S9.

Special Toxicology Studies – Studies with Impurities of Abiraterone Acetate Toxicology Studies		
Study/Study No.	**Concentration/ Doses**	**Results**
Synthesis impurity of abiraterone acetate ([redacted] /TOX9599	**Initial assay:** 31.25, 62.5, 125, 250, 500, 1000 and 2000 µg/plate **Confirmatory assay:** 31.25, 62.5, 125, 250, 500 and 1000 µg/plate	The synthesis impurity of abiraterone acetate is not mutagenic in the absence of S9. However, it was mutagenic towards the strain TA1537 but was not mutagenic in other strains tested in the presence of S9
[redacted] ([redacted])of abiraterone acetate /TOX9725	**Initial assay:** 6.17, 18.52, 55.5, 166.6, 500, 1500 and 2500 µg/plate **Confirmatory assay:** 3.43, 10.29, 30.86, 92.59, 277.7, 833.3 and 2500 µg/plate	The [redacted] ([redacted] is not mutagenic in the presence and absence of S9.
***In Vitro* Chromosome Aberration Assay in Human Lymphocytes**		
Abiraterone acetate spiked with [redacted] impurities /TOX9602	With and without S9: 5, 10, 25, 35, 50, 75, 100, 150, 200 and 300 µg/mL	Abiraterone acetate spiked with [redacted] impurities was not clastogenic in the presence and absence of S9
***In Vivo* Assay in Rodents**		
Repeated dose oral toxicity study of abiraterone acetate spiked with [redacted] impurities /TOX9587960747	Oral gavage at 40 and 400 mg/kg/day for 1 month	No significant differences between abiraterone acetate spiked with [redacted] impurities and abiraterone acetate groups without impurities at both 40 and 400 mg/kg/day

Note:
1. The [redacted] impurities consists of the following:
- The [redacted] impurity = [redacted] at [redacted]
- The [redacted] impurity = [redacted] at [redacted]
- The [redacted] impurity = [redacted] at [redacted]
- The [redacted] impurity = [redacted] at [redacted]
- The [redacted] = [redacted] at [redacted]

2. The [redacted] impurity ([redacted] ([redacted] impurity [redacted] impurity [redacted] and [redacted] impurity ([redacted] are all synthesis and were isolated in pure form.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The non-clinical program of abiraterone acetate identified the target areas of toxicity to be the liver, male and females reproductive system, adrenals, male mammary glands (26-and 39-week rat and monkey studies), pituitary (rats only), and eye (26-week rat study only). Abiraterone acetate was not genotoxic in both *in vitro* and *in vivo* assays. However, abiraterone mesylate, an impurity product of abiraterone was found to be genotoxic in the Ames assay. There was a significant amount of toxicities in the male and female reproductive organs at all doses in rats and monkeys. Administration of the drug, therefore, may affect fertility in humans. However, the non-clinical studies submitted in this application are sufficient to recommend approval from a pharmacology/toxicology perspective.

Unresolved toxicology issues (if any): None

Recommendations: None

Suggested labeling: Presented in a separate labeling review

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/s/

ROBEENA M AZIZ
04/19/2011

ROBERT T DORSAM
04/20/2011

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 202379 **Applicant: Centocor Ortho Biotech, Inc** **Stamp Date: December 18, 2010**

Drug Name: abiraterone acetate **NDA/BLA Type: NDA**

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		No reproductive toxicology studies were submitted in this application. Based on the sponsor meeting on 5/26/10, reproductive toxicology studies will not be required for this indication (metastatic hormone refractory prostate cancer) since the target population would be surgically or chemically castrated. Reproductive toxicology studies will be required if the patient population changes. This would a post-marketing requirement.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		The proposed labeling sections appear to be generally appropriate. At this time there are no human dose multiples expressed
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		The impurity profile is being discussed with the chemist. At present, no issues have been identified.
11	Has the applicant addressed any abuse potential issues in the submission?		X	n/a
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?		X	n/a

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

This NDA is fileable from a pharmacology/toxicology perspective.

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

At the present time, there are no potential review issues to be forwarded to the Applicant.

Robeena M. Aziz, MPH, PhD	1/12/11
Reviewing Pharmacologist	Date
Whitney S. Helms, PhD (Acting for S. Leigh Verbois, PhD)	1/12/11
Team Leader/Supervisor	Date

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

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/s/

ROBEENA M AZIZ
01/14/2011

WHITNEY S HELMS
01/14/2011