

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

202971Orig1s000

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number: **202971**
Supporting document/s: Original-1
Applicant's letter date: 9/24/2011
CDER stamp date: 9/26/2011
Product: Aripiprazole extended release suspension for injection
Indication: Maintenance treatment of schizophrenia
Applicant: Otsuka Pharmaceutical Company, Ltd
2440 Research Boulevard,
Rockville, Maryland 20850

Review Division: Psychiatric Drug Products
Reviewer: Sonia Tabacova, Ph.D.
Supervisor/Team Leader: Aisar Atrakchi, Ph.D.
Division Director: Thomas Laughren, MD
Project Manager: Sandeep Saini, Pharm.D.

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of **202971** are owned by Otsuka Pharmaceutical Company, Ltd or are data for which Otsuka Pharmaceutical Company has obtained a written right of reference.

Any information or data necessary for approval of NDA 202971 that Otsuka does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 202971.

TABLE OF CONTENTS

| | | |
|-----------|--|-----------|
| 1 | EXECUTIVE SUMMARY | 3 |
| 1.1 | INTRODUCTION | 3 |
| 1.2 | BRIEF DISCUSSION OF NONCLINICAL FINDINGS | 3 |
| 1.3 | RECOMMENDATIONS..... | 6 |
| 2 | DRUG INFORMATION | 6 |
| 2.1 | DRUG..... | 7 |
| 2.2 | RELEVANT INDS, NDAS, BLAS AND DMFs..... | 7 |
| 2.3 | DRUG FORMULATION..... | 7 |
| 2.4 | COMMENTS ON NOVEL EXCIPIENTS | 7 |
| 2.5 | COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN | 8 |
| 2.6 | PROPOSED CLINICAL POPULATION AND DOSING REGIMEN | 8 |
| 2.7 | REGULATORY BACKGROUND..... | 8 |
| 3 | STUDIES SUBMITTED..... | 8 |
| 3.1 | STUDIES REVIEWED | 13 |
| 3.2 | STUDIES NOT REVIEWED | 13 |
| 3.3 | PREVIOUS REVIEWS REFERENCED | 13 |
| 4 | PHARMACOLOGY | 14 |
| 4.1 | PRIMARY PHARMACOLOGY | 14 |
| 4.2 | SECONDARY PHARMACOLOGY | 14 |
| 4.3 | SAFETY PHARMACOLOGY | 14 |
| 5 | PHARMACOKINETICS/ADME/TOXICOKINETICS | 15 |
| 5.1 | PK/ADME..... | 15 |
| 5.2 | TOXICOKINETICS..... | 26 |
| 6 | GENERAL TOXICOLOGY..... | 27 |
| 6.1 | SINGLE-DOSE TOXICITY | 27 |
| 6.2 | REPEAT-DOSE TOXICITY..... | 30 |
| 7 | GENETIC TOXICOLOGY | 57 |
| 8 | CARCINOGENICITY | 58 |
| 9 | REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY | 58 |
| 10 | SPECIAL TOXICOLOGY STUDIES..... | 59 |
| 11 | INTEGRATED SUMMARY AND SAFETY EVALUATION..... | 84 |
| 12 | APPENDIX/ATTACHMENTS..... | 89 |

1 Executive Summary

1.1 Introduction

Aripiprazole (co-developed by Otsuka Pharmaceutical Company and Bristol Myers Squibb) has been approved and marketed in tablet, oral solution, and orally disintegrating tablet formulations for the treatment of schizophrenia, and in an injectable IM formulation for the treatment of agitation associated with schizophrenia or bipolar disorder. The primary NDA for aripiprazole, NDA 21-436 (schizophrenia, oral tablet) was approved on 15 November, 2002. Supplemental NDA's included an oral solution formulation for schizophrenia (NDA 21-713, approved 10 Dec, 2004), an oral disintegrating tablet formulation for schizophrenia (NDA 21-729, approved 7 June, 2006), and an injectable formulation for schizophrenia and bipolar disorder (NDA 21-866, approved 20 Sept, 2006).

The present NDA, submitted under section 505(b), seeks approval for Aripiprazole monohydrate extended-release suspension for injection [Intramuscular (IM) Depot] for the maintenance treatment of schizophrenia. This IM Depot product is an extended-release injectable suspension designed to deliver 300 mg or 400 mg of aripiprazole over a period of 4 weeks. The sponsor's clinical rationale for the development of this IM Depot formulation is the efficacy and safety/tolerability of once-a-month treatment. It was developed under IND 67380 (13 May 2003).

1.2 Brief Discussion of Nonclinical Findings

Note: The clinical formulation was used in all of the nonclinical studies.

Studies conducted with aripiprazole IM depot formulation

Pharmacokinetics: After injection of aripiprazole IM depot formulation in rats, the C_{max} and AUC of aripiprazole increased with the dose increment, and there was no gender difference in plasma concentrations. Aripiprazole injected as a depot formulation was stable at the injection site without being metabolized or decomposed. Based on the residual amount of aripiprazole in the injection site, the absorption of aripiprazole increased approximately from 39% up to 84% from 168 hours to 1008 hrs post injection, indicating a controlled release of the drug into systemic circulation. The absolute bioavailability (assessed in mini pigs) indicated that aripiprazole was completely bioavailable from IM and SC routes (111 % and 102%, respectively) and incompletely bioavailable by oral route (22.3%), which is suggestive of the extensive first pass metabolism and/or incomplete absorption of aripiprazole following PO administration.

Assessment of aripiprazole metabolites OPC-14857, DM-1451, DM-1452, OPC-3373 and 1-(2, 3 dichlorophenyl)piperazine (DCPP) following single IM injections of aripiprazole IM depot formulation to rats, showed that the plasma concentrations of DM-1451 increased nonlinearly with the dose increment while the rest of the assessed metabolites (OPC-14857, DM-1452, OPC-3373 and DCPP) were below the lower limit of quantification (LLQ). The rank order of the C_{max} and AUC_{0-t} for aripiprazole and its metabolites is aripiprazole > DM-1451 > OPC-3373 > OPC-14857.

Toxicology:

The nonclinical testing strategy for intramuscular aripiprazole was abbreviated since it was supported, in part, by results from previous in vitro and in vivo nonclinical studies conducted to support other formulations and indications for aripiprazole.

Studies conducted specifically for the IM depot formulation include general toxicity and local irritation profile, characterized in single- and repeat-dose regimens in rats, dogs, monkeys and rabbits. The carcinogenicity as well as reproductive and developmental toxicity and juvenile toxicity of aripiprazole were previously evaluated following oral and/or intravenous administration described in the primary and supplemental NDAs. Since the systemic human plasma exposure to aripiprazole at MRHD for the intramuscular depot formulation (400 mg; 200 mg BID) did not exceed the systemic exposure at the oral MRHD (30 mg/day), carcinogenicity and reproductive and developmental toxicity studies using the intramuscular depot formulation of aripiprazole would not have provided any further information to assess the potential hazard in humans. Therefore, a carcinogenicity study of the intramuscular depot formulation was not conducted in accordance with the Executive CAC recommendation (Executive CAC Minutes, September 26, 2008) and a waiver for undertaking developmental and reproductive toxicity studies was granted by the FDA to Otsuka, June 23, 2010.

Studies conducted specifically for the IM depot formulation

The following toxicology studies were conducted specifically for the IM depot formulation of aripiprazole and submitted with the present NDA:

General toxicity

Single-Dose: Dogs (2 studies)

Repeat-Dose:

- Preliminary 4 wk and 26-wk toxicity studies in rats and dogs
- 52-wk study in dogs
- 2-wk and 4-wk studies in monkeys

The pivotal repeat dose general toxicity studies supporting the clinical development of intramuscular aripiprazole IM depot formulation were performed in rats (26-weeks), dogs (52 weeks) and monkeys (4 weeks). Aripiprazole was administered weekly in the 26-week study in rats and the 52-week study in dogs, whereas daily administrations were used for the 4-week monkey study. All pivotal toxicity studies were conducted in compliance with GLP regulations. Dose selection for pivotal studies was based on results from preceding intramuscular exploratory or range-finding studies.

Rats: Weekly intramuscular injections of aripiprazole (OPC-14597) depot formulation to rats at maximal dose of 100 mg/kg for a period of 26-weeks resulted in granulomatous inflammation to the deposited drug at the injection site. There was no morphologic evidence of drug-related skeletal muscle necrosis in any of the dosed animals. Morphological changes in female reproductive and mammary tissues and atrophy of pituitary pars intermedia in both genders were present at all dose levels, and were likely pharmacologically mediated as a consequence of D2 partial agonistic activity of aripiprazole. The NOAEL was 50 mg/kg/week in the males and 100 mg/kg/week in the females since low body weight and decreased food consumption were observed in the males given 100 mg/kg, but not in the treated females under the conditions of the present study. At the NOAEL, the C_{max} and AUC_{7d} of aripiprazole at week 26 were 98.1ng/mL and 598.6 ng.d/mL, respectively for males and 1135.3 ng/mL and 4336.2 ng.d/mL, respectively for females.

Dogs: Administration of aripiprazole depot formulation to beagle dogs by weekly IM injections at doses of 10, 20 and 40 mg/kg of aripiprazole for 52 weeks resulted in localized granulomatous inflammation at the injection site in the males at all dosages and in HD females, and in necrosis of muscle fibers involved in the granulomatous inflammation in 1 HD female at the end of the 52-week dosing period. The inflammation consisted of accumulation of macrophages, eosinophilic deposits, foreign body giant cells, lymphocytes and polymorphonuclear leukocytes, in association with deposits of birefringent crystal-like material (interpreted as drug). The gross- and histopathology changes at the injection site were reversible by the end of the 26-week recovery period. There were no clinical signs, and no drug-related changes in body weight, food consumption, hematology, blood chemistry, urinalysis, ophthalmology, audiology, electrocardiography, body temperature, no drug-related changes in organ weights or in gross- and histopathology of the systemic organs. The NOAEL was 40 mg/kg/week with mean C_{max} and AUC_{7d} of aripiprazole at Week 52 of 438ng/mL and 2460 ng.d/mL, respectively for males, and 306 ng/mL and 1820 ng.d/mL, respectively for females.

Monkeys: Intramuscular daily administration of aripiprazole depot formulation at doses of 2, 4, or 7.5 mg/kg to Cynomolgus monkeys once daily for 29 days, resulted, at all doses, in CNS-related clinical signs (reduced activity, most likely pharmacologically mediated) and reversible skeletal muscle injury at the injection site. Small increases in serum aspartate aminotransferase at mid- and high dose were likely a consequence of injection site injury. Microscopically, increased incidence and/or severity of skeletal muscle necrosis (minimal to mild), degeneration (mild to moderate), and regeneration (mild to moderate) were observed at injection sites at all doses, while injection site changes associated with the control article and/or intramuscular injection procedure were generally minimal in severity. All changes at the injection site showed evidence of reversibility. Following a 1-month post-dose recovery period, there was no residual fibrosis at the injection sites of monkeys given either the control article or aripiprazole formulations. Exposures to aripiprazole and its pharmacologically active dehydro-metabolite (BMS-337044) were dose-proportional with no apparent sex differences. Small accumulation in systemic exposure occurred upon repeated dosing for 29 days. A NOAEL was not reached in this study (lowest tested dose of 2 mg/kg/day) since injection site skeletal muscle injury was present at all doses.

Local Tolerance: Local tolerance studies were conducted in rats, rabbits, dogs and monkeys with different IM depot formulations to determine a suitable formulation for use in toxicity studies. A formulation based on carboxymethyl cellulose (CMC) was found to be well tolerated in the rat, rabbit and dog, but not in the monkey. Another formulation of aripiprazole (in 15% Captisol) was examined in the rat and rabbit and was also found to be well tolerated. Microscopically, the primary finding at the injection site from these studies was a localized, granulomatous inflammatory response to deposited drug consistent with a foreign-body reaction in response to deposited drug (polymorphic, birefringent crystalline material). This inflammation was not completely resolved by termination of the studies.

Studies cross-referenced from previous NDAs

Pharmacology (including safety pharmacology), genotoxicity, carcinogenicity, oral reproductive and developmental toxicity, and juvenile toxicity of aripiprazole are cross-referenced from previously approved NDAs, primarily NDA 21-436. The primary NDA for aripiprazole, NDA 21-436 (schizophrenia, oral tablet; submitted 31 October 2001), contained the majority of the

nonclinical studies; some of these studies, as described below, are also supporting the present application and are cross-referenced from the original NDA.

- The genotoxic and carcinogenic potentials of aripiprazole were assessed in support of aripiprazole oral tablet formulation. A waiver for conducting nonclinical carcinogenicity studies was granted to OTSUKA by the Division based on Executive CAC recommendation that the proposed by the sponsor 2-year carcinogenicity study was not necessary (Executive CAC Minutes dated 26 September 2008).
- The reproductive and developmental toxicity of aripiprazole was previously evaluated in rats and rabbits following oral or IV administration. Additional developmental and reproductive toxicity studies were not conducted with the IM depot formulation. A waiver for undertaking these studies was granted by the Division (email by Keith Kiedrow, 23 June 2010) because the systemic exposure after IM depot dosing was much lower than that after oral or intravenous dosing (as shown by repeated dose TK studies of the IM depot formulation).
- Other studies: Dermal sensitization, phototoxicity, antigenicity, immunotoxicity, and physical dependence and abuse potential of aripiprazole were previously assessed (NDA 21 436).

1.3 Recommendations

1.3.1 Approvability

Approvable

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Section 13.2., under "Intramuscular Aripiprazole"

Reviewer's note: The safety margin between the NOAEL in rats (50 and 100 mg/kg for males and females, respectively) and the maximum recommended human dose (400 mg), (1 and 2 times for male and female rats, respectively, based on mg/m² body surface) was calculated by the sponsor without taking into account the difference in administration periods between humans and animals (weekly in rats vs. once in every 4 weeks in humans). If the human dose is recalculated from once in 4 weeks to weekly administration (in order to match the dosing in rats), then the MRHD should be divided by 4, and the corresponding safety margins would be 4 times higher than those stated in the label. However, we accept the more conservative safety margin as stated by the sponsor since it better corresponds to the difference in systemic exposure (AUC) between rats (at NOAEL) and humans (at MRHD), adjusted for administration period. Thus, no change in this part of labeling is recommended.

2 Drug Information

2.1 Drug

Generic Name: **Aripiprazole**

Code Name: OPC-14597, OPC-31, BMS-337039

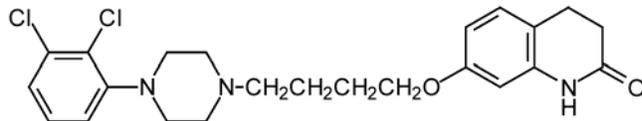
Synonyms: Throughout this NDA, the subject drug has been referred to as:

- aripiprazole extended release suspension for injection
- aripiprazole intramuscular (IM) depot
- Intramuscular (IM) Depot Formulation
- IM depot formulation of aripiprazole (Abilify[®], OPC-14597, BMS-337039)
- Intramuscular (IM) injection
- Depot

Chemical Name: 7-{4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy}-3,4-dihydro-2(1H)-quinolinone

Molecular Formula/Molecular Weight: 448.39

Structure:



Pharmacologic Class: 2nd generation antipsychotic (partial D₂ and 5HT_{1A} agonist, 5HT₂ antagonist)

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 67380; NDA 21-436

2.3 Drug Formulation

Extended release aqueous suspension for gluteal injection to deliver 300- or 400 mg of aripiprazole in 300- or 400 mg/vial strength.

2.4 Comments on Novel Excipients

Aripiprazole drug substance used for Aripiprazole IM Depot is sterile aripiprazole monohydrate manufactured by (b) (4) aripiprazole (b) (4) drug substance used for the approved drug products that are ABILIFY[®] tablets, Oral Solution, Orally Disintegrating Tablets. (b) (4) is the only solvent that is used in the commercial process of sterile aripiprazole monohydrate.

Aripiprazole IM Depot product is a sterile, single-dose, lyophilized cake for reconstitution in (b) Sterile Water for Injection. No novel excipients are used in the formulation. The commercial formulation is comprised of Sterile Aripiprazole Monohydrate, Carboxymethylcellulose Sodium USP, Mannitol USP, Sodium Phosphate Monobasic Monohydrate USP, Sodium Hydroxide NF, (b) (4) and Water for Injection, USP.

2.5 Comments on Impurities/Degradants of Concern: none

2.6 Proposed Clinical Population and Dosing Regimen

Adult patients with schizophrenia to be injected into the gluteal muscle with 300 or 400 mg aripiprazole in an aqueous suspension.

2.7 Regulatory Background

Aripiprazole (discovered by Otsuka Pharmaceutical Company and co-developed with Bristol Myers Squibb) has been approved in the US and is currently marketed in tablet, oral solution, and orally disintegrating tablet formulations for the treatment of schizophrenia, and in an injectable IM formulation for the treatment of agitation associated with schizophrenia or bipolar disorder, as listed below.

| NDA # | Formulation | Indication | Submission Date | Approval Date |
|--------------------------------|---|------------------------------------|-----------------|---------------|
| <i>Original:</i> NDA 21-436 | ABILIFY® (aripiprazole) Tablets | Schizophrenia | Oct 31, 2001 | Nov 15, 2002 |
| <i>Supplemental:</i> | | | | |
| - NDA 21-713 | ABILIFY® (aripiprazole) Oral Solution | Schizophrenia | Nov 20, 2003 | Dec 10, 2004 |
| - NDA 21-729 | ABILIFY DISCMELT® (aripiprazole) Orally Disintegrating Tablet | Schizophrenia | Dec 22, 2003 | June 7, 2006 |
| - NDA 21-866 | ABILIFY® (aripiprazole) Injection for intramuscular use | Schizophrenia and Bipolar Disorder | Nov 29, 2005 | Sept 20, 2006 |

The primary NDA for aripiprazole, NDA 21-436 (schizophrenia, oral tablet; submitted 31 Oct 2001, approved 15 Nov, 2002), contained the majority of the nonclinical studies that were conducted to support the use of this drug as an oral formulation for schizophrenia.

The present NDA, submitted under section 505(b), seeks approval for Aripiprazole monohydrate extended-release suspension for injection (Intramuscular Depot) for the maintenance treatment of schizophrenia. This IM Depot product is an extended-release injectable suspension designed to provide effective drug release for 4 weeks and to deliver 300 mg and 400 mg of aripiprazole in 300- and 400-mg/vial strength, respectively. The sponsor's clinical rationale for the development of this IM Depot formulation is the efficacy and safety/tolerability of a once-monthly treatment. It was developed under IND 67380 (13 May 2003).

3 Studies Submitted

The submitted studies, specifically conducted with aripiprazole extended release suspension for IM injection, include PK, general toxicology studies [single-dose in dogs, and repeat-dose toxicity in rats (4 and 26 weeks), dogs (4, 26, and 52 weeks) and monkeys (2- and 4 weeks)], and local tolerance studies (in rats, rabbits, dogs and monkeys), as listed in the following sponsor's table:

Submitted studies

PK

| Type of Study | Test System | Method of Administration | Test Facility | Report Number |
|---|----------------------------|--|--|---------------|
| Absorption | | | | |
| Plasma Concentrations of OPC-14597 and Its Metabolites and Muscular Concentration of OPC-14597 in Rats After Single Intramuscular Administrations of OPC-14597 Injectable Suspension at 12.5, 25 and 50 mg/kg or OPC-14597 Aqueous Solution at 3.75 mg/kg | Rat/Sprague Dawley M, F | Intramuscular | Tokushima Research Institute, Otsuka Pharmaceutical Co, Ltd, Japan | 022879 |
| Absorption, Distribution and Excretion of Radioactivity After Single Intramuscular Administration of ¹⁴ C-OPC-14597 to Rats | Rat/Sprague Dawley M, F | Intramuscular | Tokushima Research Institute, Otsuka Pharmaceutical Co, Ltd, Japan | 022847 |
| Pharmacokinetic Evaluation of Aripiprazole (BMS-337039) and Two Metabolites in Sinclair® Minipigs Following Intravenous, Oral, Intramuscular, and Subcutaneous Administration of Aripiprazole | Minipig/Sinclair M | Intravenous Oral (tablets) Intramuscular Subcutaneous | Bristol-Myers Squibb Research and Development, USA | 014124 |
| Organ Distribution | | | | |
| Plasma Concentrations of OPC-14597 and Its Metabolites and Muscular Concentration of OPC-14597 in Rats After Single Intramuscular Administrations of OPC-14597 Injectable Suspension at 12.5, 25 and 50 mg/kg or OPC-14597 Aqueous Solution at 3.75 mg/kg | Rat/Sprague Dawley M, F | Intramuscular | Tokushima Research Institute, Otsuka Pharmaceutical Co, Ltd, Japan | 022879 |
| High-performance Liquid Chromatographic Analysis of Radioactivity in Plasma and Injected Site After Single Intramuscular Administration of ¹⁴ C-OPC-14597 to Rats | Rat/Sprague Dawley M, F | Intramuscular | Tokushima Research Institute, Otsuka Pharmaceutical Co, Ltd, Japan | 025564 |
| Type of Study | | | | |
| Absorption, Distribution and Excretion of Radioactivity After Single Intramuscular Administration of ¹⁴ C-OPC-14597 to Rats | Rat/Sprague Dawley M, F | Intramuscular | Tokushima Research Institute, Otsuka Pharmaceutical Co, Ltd, Japan | 022847 |
| Metabolism in vivo | | | | |
| Plasma Concentrations of OPC-14597 and Its Metabolites and Muscular Concentration of OPC-14597 in Rats After Single Intramuscular Administrations of OPC-14597 Injectable Suspension at 12.5, 25 and 50 mg/kg or OPC-14597 Aqueous Solution at 3.75 mg/kg | Rat/Sprague Dawley M, F | Intramuscular | Tokushima Research Institute, Otsuka Pharmaceutical Co, Ltd, Japan | 022879 |
| Investigation of Metabolites of OPC-14597 in Injected Site After Single Intramuscular Administration of OPC-14597 Injectable Suspension to Rats | Rat muscle tissues M | In vitro | Tokushima Research Institute, Otsuka Pharmaceutical Co, Ltd, Japan | 024412 |
| Pharmacokinetic Evaluation of Aripiprazole (BMS-337039) and Two Metabolites in Sinclair® Minipigs Following Intravenous, Oral, Intramuscular, and Subcutaneous Administration of Aripiprazole | Minipig/Sinclair M | Intravenous Oral (tablets) Intramuscular Subcutaneous | Bristol-Myers Squibb Research and Development, USA | 014124 |
| Excretion | | | | |
| Absorption, Distribution and Excretion of Radioactivity After Single Intramuscular Administration of ¹⁴ C-OPC-14597 to Rats | Rat/Sprague Dawley M, F | Intramuscular | Tokushima Research Institute, Otsuka Pharmaceutical Co, Ltd, Japan | 022847 |

Continued on the next page

Toxicology studies

| Study | Species/Strain (Number/sex) | Route and Duration | Dose | Key Result | Report Number |
|--|--|---|---|---|------------------|
| Acute Toxicity | | | | | |
| Single Dose | Dog/Beagle 5/sex/dose | IM 1 Day | 100, 200 or 400 mg 100 mg/mL formulation | No mortality. Microscopic evidence of injection site inflammation at all doses with incomplete recovery. Plasma levels increased with dose. | 015504 |
| Single Dose | Dog/Beagle 5/sex/dose | IM 1 Day | 0 (saline), 150, 200, 300 or 400 mg 150 and 200 mg/mL formulation | No mortality. Clinical signs of exaggerated pharmacology and injection site trauma. Microscopic evidence of injection site inflammation at all doses with incomplete recovery. Plasma levels increased with dose. | 019933 |
| Repeat Dose Toxicity | | | | | |
| 4-Week Toxicity | Rat/Sprague Dawley 6/sex/dose | IM 4 Weeks | 0 (saline), 0 (placebo), 50 or 100 mg/kg; injections were given weekly or biweekly; total dose volume for each group was 0.5 mL/kg | Gross and microscopic evidence of injection site inflammation at all doses. Plasma levels increased with dose with higher plasma levels from seen with weekly treatment compared to biweekly treatment. | 018701 |
| 26-Week Toxicity with 4- Week Recovery | Rat/Sprague Dawley Main Study: 12/sex/dose Recovery: 5/sex/dose Saline, Placebo and 100 mg/kg groups) | IM 26 Weeks Recovery at 0 (saline), 0 (placebo) and 100 mg/kg | 0 (saline), 0 (placebo), 25, 50, 100; injections were given weekly (0.25 or 0.5 mL/kg) | NOAEL = 50 mg/kg (males), 100 mg/kg (females) Gross and microscopic evidence of injection site inflammation at all doses with incomplete recovery. Morphological changes in reproductive and mammary tissue considered a consequence of D ₂ partial agonist activity of aripiprazole. Plasma levels increased with dose. | 019345 |

Continued on the next page

Submitted studies (continued)

Toxicology (continued)

| Study | Species/Strain (Number/sex) | Route and Duration | Dose | Key Result | Report Number |
|---|--|---|---|---|--|
| 4-Week Toxicity | Dog/Beagle 3/sex/dose | IM 4 Weeks | 0 (saline), 0 (placebo), 20, 40 mg/kg; injections were given weekly or biweekly (0.1 or 0.2 mL/kg) | Gross and microscopic evidence of injection site inflammation at all doses. Plasma levels increased with dose with higher plasma levels from seen with weekly treatment compared to biweekly treatment. | 018702 |
| 26-Week Toxicity with 4- Week Recovery | Dog/Beagle Main Study: 3/sex/dose Recovery: 2/sex/dose | IM 26 Weeks | 0 (saline), 0 (placebo), 10, 20, 40 mg/kg; injections were given weekly for a total dose volume of 0.1 or 0.2 mL/kg | NOAEL = 40 mg/kg Gross and microscopic evidence of injection site inflammation observed at all doses with incomplete recovery. Plasma levels increased with dose. | 019346 |
| 52-Week Toxicity with 26- Week Recovery | Dog/Beagle Main Study: 4/sex/dose 39-Week Interim: 3/sex from the Saline and aripiprazole treated groups Recovery: 2/sex from the Saline and 40 mg/kg groups | IM 26 Weeks 39-Week Interim Evaluation 26-Week Recovery at 0 (saline) and 40 mg/kg | 0 (saline), 0 (placebo), 10, 20, 40 mg/kg; injections were given weekly for a total dose volume of 0.1 or 0.2 mL/kg | NOAEL = 40 mg/kg Gross and microscopic evidence of injection site inflammation observed at all doses periodically during the study with complete recovery. Plasma levels increased with dose. | 022262 022722 023492 |
| 2-Week Toxicity with 2- Week Recovery | Monkey/ cynomolgus Main Study: 3/sex/dose Recovery: | IM 2 Weeks | 0 (vehicle), 2, 4, 7.5 mg/kg at 1 mL/kg/day | Gross and microscopic evidence of injection site inflammation observed at all doses periodically during the study. Plasma levels increased with dose. | 013597 |

Continued on the next page

Submitted studies (continued)

Toxicology

| Study | Species/Strain (Number/sex) | Route and Duration | Dose | Key Result | Report Number |
|---|--|-----------------------|--|---|------------------|
| 4-Week Toxicity with 4- Week Recovery | Monkey/ cynomolgus Main Study: 3/sex/dose Recovery: 2/sex/dose | IM 4 Weeks | 0 (vehicle), 2, 4, 7.5 mg/kg at 1, 0.265, 0.53 or 1 mL/kg/day, respectively. | Clinical signs of exaggerated pharmacology. Increased AST observed at the high dose. Gross and microscopic evidence of injection site inflammation at all doses during the study. Plasma levels increased with dose. | 017831 |
| Local Tolerance | | | | | |
| Single Dose Irritation | Rat/Sprague Dawley Saline control: 6/sex Vehicle and aripiprazole groups: 15/sex | IM 1 Day | 0 (saline), 0 (vehicle), 12.5, 25 or 50 mg/kg at 0.125, 0.25 and 0.5 mL/kg, respectively. Controls (saline and vehicle) treated 0.5 mL/kg. | Gross and microscopic evidence of injection site inflammation at all doses with incomplete recovery. | 015505 |
| Single Dose Irritation | Rat/Sprague Dawley Saline control: 6/sex Vehicle and aripiprazole groups: 15/sex | IM 1 Day | 0.5 mL/kg of a 0 (saline), 75 or 100 mg/kg; 0.5 mL/kg. | Gross and microscopic evidence of injection site inflammation at all doses with incomplete recovery. | 019936 |
| 2-Week Repeat Dose with 2- Week Recovery | Rat/Sprague Dawley 12/sex/dose | IM 14 Days | 0.5 mL/kg of 0 (vehicle), 2 or 7.5 mg/mL solution | Gross and microscopic evidence of injection site inflammation at all doses with incomplete recovery. | 013598 |
| Single Dose Irritation | Rabbit/New Zealand White 12 males/dose | IM 1 Day | 0 (saline), 150 or 200 mg; 1 mL injection of a 150 or 200 mg/mL solution | Gross and microscopic evidence of injection site inflammation at all doses with incomplete recovery. | 019934 |
| Single Dose Irritation | Rabbit/New Zealand White 12 males/dose | IM 1 Day | 0 (saline), 0 (vehicle), 25, 50 or 100 mg at 1, 0.25, 0.5, 1 mL of a 100 mg/mL suspension | Gross and microscopic evidence of injection site inflammation at all doses with incomplete recovery. | 015567 |
| Single Dose Irritation | Rabbit/New Zealand White 6 females/dose | IM 1 Day | 1 mL of a 0 (saline), 0 (vehicle), 2, 4 or 7.5 mg/mL solution | Microscopic evidence of injection site inflammation at all doses with incomplete recovery at the high dose. | 013731 |

Continued on the next page

Submitted studies (continued)**Toxicology**

| Study | Species/Strain (Number/sex) | Route and Duration | Dose | Key Result | Report Number |
|------------------------------|---|-----------------------|---|--|------------------|
| Single Dose Irritation | Dog/ Beagle 1/sex/dose | IM 1 Day | 2 mL (200 mg), 3 mL (300 mg) or 4 mL (400 mg) of 100 mg/mL suspension as 2 injections | Gross or microscopic evidence of injection site inflammation at all doses. | 014893 |
| Single Dose Irritation | Monkey/ cynomolgus 3/sex/dose | IM 1 Day | 0.5 mL of 50 or 100 mg/mL suspension; 0.5 mL saline; 0.5 mL vehicle | Gross and microscopic evidence of injection site inflammation at all doses with incomplete recovery. | 015503 |

3.1 Studies Reviewed

All PK studies; pivotal toxicology studies (1 month rat, 52-week dog and 1 month monkey study); all local tolerance studies

3.2 Studies Not Reviewed

Studies cross-referenced from the relevant original NDA (see Regulatory History).

Waivers:

A waiver for conducting developmental and reproductive toxicity studies was granted to Otsuka by the FDA (e-mail from Keith Kiedrow to sponsor dated 23 June 2010, re IND 67,380 Serial Number 136, dated 5/25/2010) because the exposure after IM depot dosing was much lower than that after oral or intravenous dosing (as shown by repeated dose TK studies of the IM depot formulation), and reproductive and developmental toxicity evaluations had been previously conducted with the oral and intravenous administration.

A waiver for conducting nonclinical carcinogenicity studies was granted to OTSUKA by the FDA based on Executive CAC recommendation that the proposed 2-year carcinogenicity study was not necessary (Executive CAC Minutes of 9/26/2008).

3.3 Previous Reviews Referenced:

Lois M. Freed, Sonia Tabacova: NDA **21 436** Aripiprazole tablets (schizophrenia), Pharmacology /Toxicology Review, 8/29/2002;

Sonia Tabacova: sNDA **21 436** Aripiprazole tablets (bipolar disorder), Pharmacology /Toxicology Review, 4/6/2004

Sonia Tabacova: NDA **21713** Aripiprazole oral solution, Pharmacology/Toxicology Review, 9/18/2004;

Sonia Tabacova: NDA **21729** Aripiprazole oral disintegrating tablet, Pharmacology /Toxicology Memorandum, 10/12/2004

Sonia Tabacova: NDA **21866** Aripiprazole injectable IM formulation, Pharmacology /Toxicology Review, 9/6/2006

4 Pharmacology

4.1 Primary Pharmacology

No new studies submitted

4.2 Secondary Pharmacology

No new studies submitted

4.3 Safety Pharmacology

No new studies submitted

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The PK of aripiprazole intramuscular depot formulation was studied in rats and minipigs.

In rats, aripiprazole C_{max} and AUC increased with dose and there were no sex differences in plasma concentrations¹. Aripiprazole was stable at the injection site, without being metabolized or decomposed². As indicated by the residual amount of aripiprazole at the injection site at 168 and 1008 hrs post injection, absorption increased from approximately 39% to 84%, respectively, indicating a controlled release of the drug into systemic circulation.

In minipigs, the PK of aripiprazole (30 mg single dose) was compared following parenteral (intravenous, intramuscular, subcutaneous) and oral administration³. The mean AUC values were similar among the IM, IV and SC routes, while for the PO route the mean AUC was much lower. Aripiprazole was completely bioavailable after IM and SC administration (absolute bioavailability 111% and 102%, respectively) and incompletely bioavailable after PO administration (22.3%), suggestive of extensive first pass metabolism and/or incomplete absorption following PO administration.

Aripiprazole metabolites OPC-14857, DM-1451, DM-1452, OPC-3373 and 1-(2,3-dichlorophenyl)piperazine (DCPP) were analyzed in rats following single injections of aripiprazole IM depot formulation¹. Plasma concentrations of all metabolites except for DM-1451 were below the lower limit of quantification. The C_{max} and AUC_t of DM-1451 increased nonlinearly with the dose. There were no gender differences in the plasma concentrations of aripiprazole metabolites. The rank order of the C_{max} and AUC_t for aripiprazole and its metabolites was aripiprazole > DM-1451 > OPC-3373 > OPC-14857.

The biotransformation of aripiprazole was also evaluated in minipigs following IV, PO, IM, and SC administrations of a single 30 mg dose³. The plasma concentration of metabolite OPC-14857 was higher than that of metabolite DM-1451. DM-1451 was below LLQ in most plasma samples for IV and IM routes. The AUC_t ratios of metabolite OPC-14857 to parent drug were similar among IV, IM, and SC routes (2.67, 2.70, and 2.06%, respectively), but much higher after oral administration (11.4%). DM-1451 to parent drug AUC_t ratios were below LLQ for IV and IM routes, and 3.2% and 1.1 % for PO and SC ones.

Aripiprazole biotransformation at the injection site was determined in muscle tissue collected at 24, 168, 504 and 1008 hours after single IM administration of aripiprazole IM depot formulation to male rats⁴. There was no metabolite or degradation product of aripiprazole produced in the injection site up to 1008 hours (42 days) after administration, and aripiprazole administered as a depot formulation was stable at the injection site without being metabolized or decomposed during that period.

¹ Furukawa M. Plasma Concentrations of OPC-14597 and Its Metabolites and Muscular Concentration of OPC-14597 in Rats After Single Intramuscular Administrations of OPC-14597 Injectable Suspension at 12.5, 25 and 50 mg/kg or OPC-14597 Aqueous Solution at 3.75 mg/kg. Otsuka Study No. 028369, Otsuka Report No. 022879, 2009

² Koyama N. Investigation of Metabolites of OPC-14597 in Injected Site after Single Intramuscular Administration of OPC-14597 Injectable Suspension to Rats. Otsuka Study No. 030054, Otsuka Report No. 024412, 2010

³ Gao X, Patel R. Pharmacokinetic Evaluation of Aripiprazole (BMS-337039) and Two Metabolites in Sinclair® Minipigs Following Intravenous, Oral, Intramuscular, and Subcutaneous Administration of Aripiprazole. Otsuka Study No. 017371 (BMS Study No. MAP034/178/337039/005), Otsuka Report No. 014124, 2001

⁴ Koyama N. Investigation of Metabolites of OPC-14597 in Injected Site after Single Intramuscular Administration of OPC-14597 Injectable Suspension to Rats. Otsuka Study No. 030054, Otsuka Report No. 024412, 2010

In all TK studies, the systemic exposures to aripiprazole and metabolites following IM depot injection were dose-related and prolonged with parent compound still detectable at or over 29 days after a single injection. With repeat dosing, systemic levels of aripiprazole and metabolites increased with dose, but generally not dose-proportionally. There were no remarkable gender differences.

Absorption

Rats

The plasma concentrations of aripiprazole were determined following single IM injections of aripiprazole IM depot formulation at doses of 12.5, 25 and 50 mg/kg, and aripiprazole IM rapid formulation at a dose of 3.75 mg/kg to male rats. A single IM dose of 25 mg/kg of aripiprazole depot formulation was also administered to female rats to determine if there were sex differences in plasma exposure (Furukawa M. Plasma Concentrations of OPC-14597 and its Metabolites and Muscular Concentration of OPC-14597 in Rats After Single Intramuscular Administrations of OPC-14597 Injectable Suspension at 12.5, 25 and 50 mg/kg or OPC-14597 Aqueous Solution at 3.75 mg/kg. Otsuka Study No. 028369, Otsuka Report No. 022879, 2009).

The C_{max} and AUC of aripiprazole following administration of IM *depot* formulation at single doses of 12.5 to 50 mg/kg increased with dose and there was no sex difference in plasma concentrations of aripiprazole; C_{max} was reached in 7 days (as compared to 0.25 hours for aripiprazole IM *rapid* formulation at a single IM dose of 3.75 mg/kg), and the elimination half-time ($t_{1/2}$) was 5 to 18 days (as compared to 2.5 hours for aripiprazole IM *rapid* formulation at a single IM dose of 3.75 mg/kg). The pharmacokinetic data are summarized in the following sponsor's table.

Plasma Concentrations of Aripiprazole in Rats after Single IM doses of Aripiprazole Injectable Suspension

| Test Article: Aripiprazole IM Depot | | | | | |
|---|--|--|--|--|--|
| Species/Strain | Rat/Sprague Dawley |
| Gender (M/F)/Number of Animals | M/3 per time point | M/3 per time point | M/3 per time point | F/3 per time point | M/3 per time point |
| Feeding Condition | Fed | Fed | Fed | Fed | Fed |
| Vehicle/Formulation | Water for injection/IM rapid formulation | Water for injection/IM depot formulation |
| Method of Administration | Intramuscular | Intramuscular | Intramuscular | Intramuscular | Intramuscular |
| Dose (mg/kg as the free base) | 3.75 | 12.5 | 25 | 25 | 50 |
| Sample (whole blood, plasma, serum etc.) | Plasma | Plasma | Plasma | Plasma | Plasma |
| Analyte | OPC-14597 | OPC-14597 | OPC-14597 | OPC-14597 | OPC-14597 |
| Assay | LC-ESI-MS/MS | LC-ESI-MS/MS | LC-ESI-MS/MS | LC-ESI-MS/MS | LC-ESI-MS/MS |
| PK Parameters: | | | | | |
| t_{max} (h) | 0.25 | 168 | 168 | 168 | 168 |
| C_{max} (ng/mL) | 593.3 | 10.23 | 15.45 | 17.91 | 40.72 |
| AUC_t (ng·h/mL) (Time for calculation - h) | 1649 (0-24 h) | 2150 (0-672 h) | 5898 (0-840 h) | 5822 (0-840 h) | 12170 (0-1008 h) |
| AUC_{∞} (ng·h/mL) | 1651 | 2244 | 7123 | 7185 | 14750 |
| $t_{1/2,z}$ (h) (Time for calculation - h) | 2.487 | 126.7 (168-672 h) | 306.5 (168-840 h) | 386.1 (336-840 h) | 438.0 (336-1008 h) |
| MRT_{∞} (h) | 2.205 | 275.5 | 509.7 | 503.9 | 594.5 |
| Report No. | 022879 | 022879 | 022879 | 022879 | 022879 |

AUC_{∞} = area under the concentration-time curve from time zero to infinity; AUC_t = area under the concentration-time curve calculated to the last observable concentration at time t ; C_{max} = maximum (peak) plasma concentration of the drug; F = female; LC-ESI-MS/MS = liquid chromatography-electrospray ionization-tandem mass spectrometry; M = male; MRT_{∞} = mean residence time from time zero to infinity; $t_{1/2,z}$ = terminal-phase elimination half-life; t_{max} = time to maximum (peak) plasma concentration.

The time-courses of radioactive concentrations in the blood, plasma, tissues as well as urinary and fecal excretions of radioactivity were determined after a single intramuscular administration of ¹⁴C-OPC-14597 IM *rapid* formulation to rats at a dose of 3.75 mg/kg (see the sponsor's table below).

Absorption of Radioactivity after Single Intramuscular Administration of [¹⁴C]-Aripiprazole to Rats*

Test Article: ¹⁴C-OPC-14597 IM *rapid* formulation (single IM dose of 3.75 mg/kg)

| Species/Strain | Rat/Sprague Dawley | Rat/Sprague Dawley |
|---|---|---|
| Gender (M/F)/Number of Animals | M/3 per time point | F/3 per time point |
| Feeding Condition | Fed | Fed |
| Vehicle/Formulation | L-tartaric acid/Captisol/ Water for injection (rapid formulation) | L-tartaric acid/Captisol/ Water for injection (rapid formulation) |
| Method of Administration | Intramuscular | Intramuscular |
| Dose (mg/kg as the free base) | 3.75 | 3.75 |
| Sample (whole blood, plasma, serum etc.) | Blood | Blood |
| Analyte | Radioactivity | Radioactivity |
| Assay | LSC | LSC |
| PK Parameters: | | |
| t _{max} (h) | 0.1387 ± 0.0964 | 0.083 ± 0.000 |
| C _{max} (ng eq/mL) | 402.3 ± 83.0 | 465.2 ± 54.1 |
| AUC _t (ng eq·h/mL) (Time for calculation - h) | 1090 ± 56 (12 or 24 h) | 1409 ± 102 (24 h) |
| AUC _∞ (ng eq·h/mL) | 1297 ± 133 | 1614 ± 158 |
| t _{1/2,z} (h) (Time for calculation - h) | 7.431 ± 3.439 (4-12, 4-24, 0.083-12 h) | 10.03 ± 1.46 (8-24 h) |
| Report No. | 022847 | 022847 |

*Miyata K. Absorption, Distribution and Excretion of Radioactivity after Single Intramuscular Administration of ¹⁴C-OPC-14597 to Rats. Otsuka Study No. 028360, Otsuka Report No. 022847, 2009

Minipigs

The PK and absolute bioavailability of aripiprazole were also evaluated in male Sinclair minipigs (n=3) following IV, IM, SC and oral administrations ((b) (4) Pharmacokinetic Evaluation of Aripiprazole (BMS-337039) and two Metabolites in Sinclair® Minipigs Following Intravenous, Oral, Intramuscular, and Subcutaneous Administration of Aripiprazole. Otsuka Study No. 017371 (BMS Study No. MAP034/178/337039/005), Otsuka Report No. 014124, 2001. A single 30 mg dose was given by each route, according to a nonrandomized crossover design. An IM injection formulation of aripiprazole (7.5 mg/mL in 15% Captisol FM/0.05 M tartrate buffer) was used for IV, IM, and SC doses, and the 2 × 15 mg aripiprazole oral tablets were used for oral dose. The washout period between consecutive doses was at least 7 days. Serial blood samples were collected over 24 to 72 hours after dosing, and plasma concentrations of aripiprazole and two metabolites, DM-1451 and OPC- 14587, were determined by an LC/MS/MS method with a lower limit of quantitation (LLQ) of 1 ng/mL for all three analytes. The mean pharmacokinetic values for aripiprazole are summarized in the following sponsor's table.

PK of Aripiprazole in Sinclair® Minipigs Following Intravenous, Oral, Intramuscular, and Subcutaneous Single-dose Administration of Aripiprazole (Test Article: Aripiprazole IM Depot)

| Species/Strain ^a | Minipig/Sinclair | Minipig/Sinclair | Minipig/Sinclair | Minipig/Sinclair |
|--|--|---------------------|--|--|
| Gender (M/F)/Number of Animals | M/3 | M/3 | M/3 | M/3 |
| Feeding Condition | Fasted | Fasted | Fasted | Fasted |
| Vehicle/Formulation | 15% Captisol/0.05 M tartrate buffer (pH 4.3) | Tablet | 15% Captisol/0.05 M tartrate buffer (pH 4.3) | 15% Captisol/0.05 M tartrate buffer (pH 4.3) |
| Method of Administration | Intravenous (infusion) | Oral (Tablet) | Intramuscular | Subcutaneous |
| Dose | 30 mg (4 mL/minipig, 4 mL/5 minutes) | 30 mg | 30 mg (4 mL/minipig) | 30 mg (4 mL/minipig) |
| Sample (whole blood, plasma, serum etc.) | Plasma | Plasma | Plasma | Plasma |
| Analyte | OPC-14597 | OPC-14597 | OPC-14597 | OPC-14597 |
| Assay | LC/MS/MS | LC/MS/MS | LC/MS/MS | LC/MS/MS |
| PK Parameters: | | | | |
| t_{max} (h) ^b | 0.08 | 4.00 | 1.00 | 0.50 |
| C_{max} (ng/mL) | 684 ± 243 | 43.0 ± 32.0 | 316 ± 84 | 280 ± 58.3 |
| AUC _t (ng·h/mL) (Time for calculation - h) | 1547 ± 216 (12 to 24 h) | 370 ± 301 (24 h) | 1864 ± 739 (36 to 72 h) | 1642 ± 79.7 (36 to 60 h) |
| AUC _∞ (ng·h/mL) | 1658 ± 342 | 553 ^c | 1881 ± 745 | 1665 ± 85.9 |
| $t_{1/2,z}$ (h) (Time for calculation - h) | 5.47 (3.53) | 6.19 ^c | 8.13 (4.06) | 8.78 (5.07) |
| MRT _∞ (h) | 5.76 ± 3.65 | 8.74 ^c | 7.90 ± 3.10 | 9.53 ± 1.88 |
| CLT (mL/min/kg) | 21.1 ± 4.77 | ND | ND | ND |
| V _{ss} (L/kg) | 6.74 ± 2.98 | ND | ND | ND |
| Report No. | 014124 | 014124 | 014124 | 014124 |

BMS-337039 = OPC-14597; AUC_∞ = area under the concentration-time curve from time zero to infinity; AUC_t = area under the concentration-time curve calculated to the last observable concentration at time t; C_{max} = maximum (peak) plasma concentration of the drug; CLT = total body clearance; LC/MS/MS = liquid chromatography-electrospray ionization-tandem mass spectrometry; M = male; MRT_∞ = mean residence time from time zero to infinity; ND = not determined; $t_{1/2,z}$ = terminal-phase elimination half-life; t_{max} = time to maximum (peak) plasma concentration; V_{ss} = steady state volume of distribution.

^aThree Sinclair® male minipigs (approximately 5 months old and between 15.1-18.3 kg of weight at study initiation).

^bReported as median. ^cN = 2, the values were not obtained in one animal because the terminal phase of plasma concentration-time profile was not well defined in this animal.

The mean AUC values were similar among the IV, IM, and SC routes while the mean AUC value for the PO route was much lower. Aripiprazole was completely bioavailable from IM and SC routes (absolute bioavailability (F) = 111% and 102%, respectively) and incompletely bioavailable by PO route (F = 22.3%). The latter is suggestive of an extensive first pass metabolism and/or incomplete absorption of aripiprazole following PO administration.

Distribution

Following a single IV administration of aripiprazole IM depot formulation at a dose of 30 mg/kg to *minipigs*, aripiprazole was extensively distributed to extravascular tissues (V_{ss} = 6.74 L/kg); the estimated total body clearance was 21.1 mL/min/kg (see the table above).

In *rats*, the residual content of aripiprazole in the injection site was determined following a single IM injection of aripiprazole IM depot formulation to males and females at a dose of 25 mg/kg, and aripiprazole IM rapid formulation to females at a dose of 3.75 mg/kg (Furukawa M. Plasma Concentrations of OPC-14597 and Its Metabolites and Muscular Concentration of OPC-14597 in

Rats After Single Intramuscular Administrations of OPC-14597 Injectable Suspension at 12.5, 25 and 50 mg/kg or OPC-14597 Aqueous Solution at 3.75 mg/kg. Otsuka Study No.028369, Report No.022879, 2009). The residual contents of aripiprazole in the injection site after injection of aripiprazole IM *depot* formulation (25 mg/kg) were 60.8, 37.5 and 15.7% of the dose at 168, 504 and 1008 hours after dosing, respectively, indicating that the absorption of the depot formulation increased from 39% to 84% from 168 h to 1008 h after injection. The values were similar in males and females. The residual contents of aripiprazole after injection of aripiprazole IM *rapid* formulation (3.75 mg/kg) at 0.5 and 24 hours were 27.6 and 0.6% of the dose, respectively (see the following sponsor's table).

Muscular Concentration of Aripiprazole in Rats after Single IM Administrations of Aripiprazole Injectable Suspension (Aripiprazole IM Depot)

| Species/Strain | Rat/Sprague Dawley | Rat/Sprague Dawley | Rat/Sprague Dawley |
|--------------------------------|--|--|--|
| Gender (M/F)/Number of Animals | M/3 per time point | M/3 per time point | F/3 per time point |
| Feeding Condition | Fed | Fed | Fed |
| Vehicle/Formulation | Water for injection/IM rapid formulation | Water for injection/IM Depot formulation | Water for injection/IM Depot formulation |
| Method of Administration | Intramuscular | Intramuscular | Intramuscular |
| Dose (mg/kg as the free base) | 3.75 | 25 | 25 |
| Samples | Muscle | Muscle | Muscle |
| Analyte | OPC-14597 | OPC-14597 | OPC-14597 |
| Assay | HPLC | HPLC | HPLC |
| Time (h) | Mean Residual Content of OPC-14597 (% of Dose) | | |
| 0 | 101.5 ± 7.3 | 110.5 ± 5.8 | 102.0 ± 11.5 |
| 0.5 | 27.6 ± 7.7 | NA | NA |
| 24 | 0.6 ± 1.0 | 73.5 ± 16.7 | 82.5 ± 10.0 |
| 168 | NA | 60.8 ± 6.0 | 57.7 ± 10.6 |
| 504 | NA | 37.5 ± 3.1 | 37.1 ± 3.2 |
| 1008 | NA | 15.7 ± 5.1 | 7.8 ± 9.2 |
| Report No. | 022879 | 022879 | 022879 |

F = female; HPLC = high-performance liquid chromatography; M = male; NA = not available

The radioactivity of aripiprazole and its metabolites in plasma and injection site following single IM administration of ¹⁴C-aripiprazole (*rapid* formulation) at 3.75 mg/kg to male rats were measured by HPLC in samples collected at 0.25, 2, and 8 hours post dose (Itose M. High-performance Liquid Chromatographic Analysis of Radioactivity in Plasma and Injected Site after Single Intramuscular Administration of ¹⁴C-OPC-14597 to Rats. Otsuka Study No. 030447, Otsuka Report No. 025564, 2010). At 0.25 and 2 hrs post dose, unchanged aripiprazole was the most abundant substance in both plasma and muscle (injection site) samples; the percentage of unchanged aripiprazole to total radioactivity was more than 85%, as shown in the following sponsor's table. Metabolite OPC-3373 was detected in plasma at 0.25 hrs post dose. Analysis at 8 hrs post dose could not be conducted because the radioactivity concentrations in both plasma and muscle were insufficient to be measured by HPLC.

Radioactivity in Plasma and Injection Site after Single IM Administrations of [¹⁴C]-Aripiprazole to Rats

| | | | |
|---------------------------------|---|--------------------------|-------------|
| Species/Strain: | Rat/Sprague Dawley | | |
| Gender (M/F)/Number of Animals: | M/3 per time point | | |
| Feeding Condition: | Fed | | |
| Vehicle/Formulation: | L-tartaric acid/Captisol/Water for injection (rapid formulation) | | |
| Method of Administration: | Intramuscular | | |
| Dose (mg/kg of the free base): | 3.75 | | |
| Radionuclide: | ¹⁴ C-OPC-14597 | | |
| Specific Activity: | 57.9 mCi/mmol | | |
| Sampling Time: | 0.25 and 2 hours for both plasma and muscle samples | | |
| Analyte: | OPC-14597, OPC-3373, and total radioactivity | | |
| Assay: | HPLC flow scintillation analyzer and liquid scintillation counter | | |
| | | % of Total Radioactivity | |
| | | OPC-14597 | OPC-3373 |
| Plasma | 0.25 | 85.5 ± 12.6 | 14.5 ± 12.6 |
| | 2 | 100.0 ± 0.0 | 0.0 ± 0.0 |
| Muscle | 0.25 | 100.0 ± 0.0 | 0.0 ± 0.0 |
| | 2 | 100.0 ± 0.0 | 0.0 ± 0.0 |

HPLC = high-performance liquid chromatography; M = male

These results showed that aripiprazole was the most abundant substance in both the systemic circulation and the muscle injection site after an intramuscular administration.

Tissue distribution of ¹⁴C-aripiprazole IM *rapid* formulation was determined in male rats after a single IM administration of 3.75 mg/kg (Miyata K. Absorption, Distribution and Excretion of Radioactivity after Single Intramuscular Administration of ¹⁴C-OPC-14597 to Rats. Otsuka Study No. 028360, Otsuka Report No.022847, 2009). The highest radioactive concentrations in blood, cerebrum, cerebellum, eyeball, lung, liver, adrenal gland, kidney, muscle and plasma were measured at 0.25 hrs after administration and decreased thereafter. At 168 hours, radioactive concentrations were still detected in harderian and submaxillary glands, liver, adrenal gland and kidney, but not detected in blood, cerebrum, cerebellum, eyeball, lung, fat, muscle tissues and plasma. The residual radioactivity in the femoral muscle (injection site) was 35.2% of the dose at 0.25 hrs and 0.68% of the dose at 168 hrs, showing that almost all dosed radioactivity was completely eliminated from the injection site by 168 h post injection (see the following sponsor's table).

Distribution of Radioactivity after Single Intramuscular Administration of [14C]-Aripiprazole to Rats

| | |
|---------------------------------|--|
| Species/Strain: | Rat/Sprague Dawley |
| Gender (M/F)/Number of Animals: | M/3 per time point |
| Feeding Condition: | Fed |
| Vehicle/Formulation: | L-tartaric acid/Captisol/Water for injection (rapid formulation) |
| Method of Administration: | Intramuscular |
| Dose (mg/kg of the free base): | 3.75 |
| Radionuclide: | ¹⁴ C-OPC-14597 |
| Specific Activity: | 57.9 mCi/mmol |
| Sampling Time: | 0.25, 2, 8, 24, 72, and 168 hours postdose |
| Analyte: | Radioactivity |
| Assay: | Liquid scintillation counter |

| Tissues/Organs | Concentration (ug eq/g or mL) | | | | | |
|--------------------|-------------------------------|---------------|---------------|---------------|---------------|---------------|
| | 0.25 hours | 2 hours | 8 hours | 24 hours | 72 hours | 168 hours |
| Blood | 778.3 ± 79.30 | 143.7 ± 46.53 | 22.42 ± 6.153 | ND | ND | ND |
| Cerebrum | 1669.2 ± 209.1 | 370.5 ± 99.44 | 81.90 ± 81.28 | 5.733 ± 9.930 | ND | ND |
| Cerebellum | 1755 ± 356.7 | 383.7 ± 122.6 | 34.99 ± 5.215 | 4.850 ± 8.400 | ND | ND |
| Eyeball | 671.5 ± 150.9 | 186.4 ± 45.81 | 52.64 ± 22.89 | 16.71 ± 2.973 | ND | ND |
| Harderian gland | 4927 ± 1202 | 9831 ± 1974 | 3957 ± 1525 | 1725 ± 231.8 | 146.0 ± 36.29 | 20.26 ± 20.03 |
| Submaxillary gland | 4137 ± 741.4 | 4955 ± 949.3 | 1257 ± 308.9 | 904.1 ± 516.8 | 474.2 ± 367.2 | 178.4 ± 202.2 |
| Lung | 20480 ± 3093 | 5016 ± 2084 | 256.4 ± 68.23 | 76.07 ± 8.396 | ND | ND |
| Liver | 7327 ± 545.2 | 4312 ± 990.6 | 1853 ± 194.3 | 490.8 ± 44.11 | 69.29 ± 18.30 | 14.53 ± 12.59 |
| Adrenal gland | 14880 ± 1378 | 4700 ± 115.2 | 1477 ± 260.1 | 1208 ± 7.000 | 386.6 ± 214.7 | 156.2 ± 65.83 |
| Kidney | 8410 ± 292.7 | 1855 ± 456.8 | 410.1 ± 83.53 | 151.1 ± 23.47 | 60.04 ± 12.49 | 25.52 ± 9.466 |
| Fat | 915.5 ± 160.8 | 1671 ± 548.2 | 334.7 ± 320.8 | 29.24 ± 5.445 | ND | ND |
| Muscle | 1904 ± 299.5 | 701.2 ± 220.9 | 161.8 ± 192.7 | 13.54 ± 12.04 | ND | ND |
| Plasma | 1021 ± 119.7 | 192.7 ± 55.21 | 42.45 ± 5.167 | 15.69 ± 1.057 | ND | ND |

| Tissues/Organs | Ratio of Tissue to Plasma | | | | | |
|--------------------|---------------------------|---------------|---------------|---------------|----------|-----------|
| | 0.25 hours | 2 hours | 8 hours | 24 hours | 72 hours | 168 hours |
| Blood | 0.764 ± 0.044 | 0.742 ± 0.035 | 0.522 ± 0.083 | ND | NC | NC |
| Cerebrum | 1.634 ± 0.017 | 1.952 ± 0.421 | 1.987 ± 2.049 | 0.344 ± 0.596 | NC | NC |
| Cerebellum | 1.708 ± 0.143 | 2.011 ± 0.531 | 0.824 ± 0.052 | 0.291 ± 0.504 | NC | NC |
| Eyeball | 0.653 ± 0.084 | 0.976 ± 0.112 | 1.279 ± 0.628 | 1.066 ± 0.178 | NC | NC |
| Harderian gland | 4.780 ± 0.579 | 53.08 ± 15.36 | 93.59 ± 37.85 | 110.1 ± 14.77 | NC | NC |
| Submaxillary gland | 4.070 ± 0.777 | 26.84 ± 8.102 | 29.44 ± 5.009 | 57.46 ± 32.25 | NC | NC |
| Lung | 20.01 ± 0.838 | 26.15 ± 9.908 | 6.007 ± 1.204 | 4.855 ± 0.516 | NC | NC |
| Liver | 7.209 ± 0.573 | 22.69 ± 2.248 | 43.84 ± 4.358 | 31.35 ± 3.213 | NC | NC |
| Adrenal gland | 14.65 ± 1.609 | 25.74 ± 7.318 | 34.73 ± 4.036 | 77.23 ± 5.152 | NC | NC |
| Kidney | 8.288 ± 0.678 | 10.08 ± 3.193 | 9.618 ± 1.079 | 9.592 ± 0.856 | NC | NC |
| Fat | 0.895 ± 0.102 | 8.673 ± 1.949 | 8.097 ± 8.095 | 1.855 ± 0.254 | NC | NC |
| Muscle | 1.885 ± 0.400 | 3.649 ± 0.786 | 3.931 ± 4.835 | 0.863 ± 0.753 | NC | NC |

n = 3

M = male; NC = not calculated; ND = not detected; No. = number.

Metabolism

Aripiprazole metabolites OPC-14857, DM-1451, DM-1452, OPC-3373 and DCPD were measured in plasma following single intramuscular injections of aripiprazole IM *depot* formulation at doses of 12.5, 25 and 50 mg/kg to male rats; aripiprazole IM *rapid* formulation at a dose of 3.75 mg/kg was injected to male rats for comparison (Furukawa M. Plasma Concentrations of OPC-14597 and Its Metabolites and Muscular Concentration of OPC-14597 in Rats after Single Intramuscular Administrations of OPC-14597 Injectable Suspension at 12.5, 25 and 50 mg/kg or OPC-14597 Aqueous Solution at 3.75 mg/kg. Otsuka Study No. 028369,

Otsuka Report No.022879, 2009). The plasma concentrations of metabolites OPC-14857, DM-1452, OPC- 3373 and DCPD were lower than the lower limit of quantification in all dose groups. Metabolite DM-1451 was detected in rat plasma at 25 and 50 mg/kg, but not at the 12.5 mg/kg dose level (see the following sponsor's table). The plasma exposure parameters of DM-1451 increased nonlinearly with the dose increment: C_{max} 0.9 to 4.8 ng/mL and AUC_t 44.2 to 748.6 ng. h/mL at 25 and 50 mg/kg, respectively. The t_{max} was 168 hrs. There were no sex differences in the plasma concentrations of aripiprazole metabolites after IM depot formulation injection (25 mg/kg) to female rats. The rank order of the C_{max} and AUC_t for aripiprazole and its metabolites was aripiprazole > DM-1451 > OPC-3373 > OPC-14857.

**Plasma Concentrations of Aripiprazole Metabolite DM-1451 in Rats
After Single IM Administrations of Aripiprazole Injectable Suspension**

| Species/Strain | Rat/Sprague Dawley |
|---|--|--|--|--|--|
| Gender (M/F)/Number of Animals | M/3 per time point | M/3 per time point | M/3 per time point | F/3 per time point | M/3 per time point |
| Feeding Condition | Fed | Fed | Fed | Fed | Fed |
| Vehicle/Formulation | Water for injection/IM rapid formulation | Water for injection/IM depot formulation |
| Method of Administration | Intramuscular | Intramuscular | Intramuscular | Intramuscular | Intramuscular |
| Dose (mg/kg as the free base) | 3.75 | 12.5 | 25 | 25 | 50 |
| Sample (whole blood, plasma, serum etc.) | Plasma | Plasma | Plasma | Plasma | Plasma |
| Analyte | DM-1451 | DM-1451 | DM-1451 | DM-1451 | DM-1451 |
| Assay | LC-ESI-MS/MS | LC-ESI-MS/MS | LC-ESI-MS/MS | LC-ESI-MS/MS | LC-ESI-MS/MS |
| PK Parameters: | | | | | |
| t_{max} (h) | 0.5 | ND | 168 | 168 | 168 |
| C_{max} (ng/mL) | 16.52 | ND | 0.921 | 1.079 | 4.834 |
| AUC_t (ng·h/mL) (Time for calculation - h) | 95.74 (0-8 h) | ND | 44.19 (0-168 h) | 51.79 (0-168 h) | 748.6 (0-336 h) |
| AUC_{∞} (ng·h/mL) | 150.0 | NC | NC | NC | NC |
| $t_{1/2,z}$ (h) (Time for calculation - h) | 5.512 (0.5-8 h) | NC | NC | NC | NC |
| MRT_{∞} (h) | 7.64 | NC | NC | NC | NC |
| Report No. | 022879 | 022879 | 022879 | 022879 | 022879 |

AUC_{∞} = area under the concentration-time curve from time zero to infinity; AUC_t = area under the concentration-time curve calculated to the last observable concentration at time t; C_{max} = maximum (peak) plasma concentration of the drug; DCPD = a metabolite of OPC-14597; F = female; LC-ESI-MS/MS = liquid chromatography-electrospray ionization-tandem mass spectrometry; M = male; MRT_{∞} = mean residence time from time zero to infinity; NC = not calculated; ND = not determined; $t_{1/2,z}$ = terminal-phase elimination half-life; t_{max} = time to maximum (peak) plasma concentration.

The biotransformation of aripiprazole was also evaluated following IV, PO, IM, and SC administrations of a single 30 mg dose in three male Sinclair minipigs ((b) (4)). Pharmacokinetic Evaluation of Aripiprazole (BMS-337039) and Two Metabolites in Sinclair® Minipigs Following Intravenous, Oral, Intramuscular, and Subcutaneous Administration of Aripiprazole. Otsuka Study No. 017371 (BMS Study No. MAP034/178/337039/005), Otsuka Report No.014124, 2001). An intramuscular injection formulation of aripiprazole (7.5 mg/mL in 15% CaptisolFM/0.05 M tartrate buffer) was used for IV, IM, and SC doses, and the 2 × 15 mg aripiprazole oral tablets were used for oral dose. A washout period of at least 7 days was observed between consecutive doses. Serial blood samples were collected over 24 to 72 hours after dosing, and plasma concentrations of two metabolites, DM-1451 and OPC-14857, were determined using an LC/MS/MS method with a lower limit of quantitation (LLQ) of 1 ng/mL for

the analytes. Plasma concentrations of metabolite OPC-14857 were higher than the plasma concentrations of DM-1451 for all routes of administration. The OPC-14857 to parent drug AUC_t ratios were essentially similar among IV, IM, and SC routes (2.67, 2.70, and 2.06%, respectively); after PO dose, the mean OPC-14857 to parent drug AUC_t ratio (11.4%) was much higher compared to IV, IM, and SC doses (see the sponsor's table below). The mean DM-1451 to parent drug AUC_t ratios were 3.2% and 1.1 % for PO and SC doses; for IV and IM doses, DM-1451 concentrations in most plasma samples were below LLQ. The metabolite data support the finding that aripiprazole undergoes extensive first pass metabolism after PO administration.

PK Evaluation of Aripiprazole (BMS-337039) Metabolites in Sinclair® Minipigs Following Intravenous, Oral, Intramuscular, and Subcutaneous Administration of Aripiprazole

| Species/Strain ^a | Minipig/Sinclair | Minipig/Sinclair | Minipig/Sinclair | Minipig/Sinclair |
|--|--|---------------------------------|--|--|
| Gender (M/F)/Number of Animals | M/3 | M/3 | M/3 | M/3 |
| Feeding Condition | Fasted | Fasted | Fasted | Fasted |
| Vehicle/Formulation | 15% Captisol/0.05 M tartrate buffer (pH 4.3) | Tablet | 15% Captisol/0.05 M tartrate buffer (pH 4.3) | 15% Captisol/0.05 M tartrate buffer (pH 4.3) |
| Method of Administration | Intravenous (infusion) | Oral (Tablet) | Intramuscular | Subcutaneous |
| Dose | 30 mg (4 mL/minipig, 4 mL/5 minutes) | 30 mg | 30 mg (4 mL/minipig) | 30 mg (4 mL/minipig) |
| Sample (whole blood, plasma, serum etc.) | Plasma | Plasma | Plasma | Plasma |
| Analyte | OPC-14857 | OPC-14857 | OPC-14857 | OPC-14857 |
| Assay | LC/MS/MS | LC/MS/MS | LC/MS/MS | LC/MS/MS |
| PK Parameters: | | | | |
| t _{max} (h) | 4.00 ^b (2.00, 8.00) | 4.00 ^b (4.00, 24.00) | 8.00 ^b (4.00, 12.00) | 8.00 ^b (8.00, 8.00) |
| C _{max} (ng/mL) | 4.11 ± 1.18 | 4.41 ± 2.84 | 6.17 ± 2.55 | 2.99 ± 0.26 |
| AUC _t (ng·h/mL) (Time for calculation - h) | 41.6 ± 8.37 | 60.5 ^c | 54.8 ± 42.11 | 34.3 ± 13.96 |
| Ratio of AUC(metabolite) to AUC(parent) (%) | 2.67 ± 0.16 | 11.4 ^c | 2.70 ± 1.05 | 2.06 ± 0.74 |
| Report No. | 014124 | 014124 | 014124 | 014124 |

| Species/Strain ^a | Minipig/Sinclair | Minipig/Sinclair | Minipig/Sinclair | Minipig/Sinclair |
|--|--|--------------------------------|--|--|
| Gender (M/F)/Number of Animals | M/3 | M/3 | M/3 | M/3 |
| Feeding Condition | Fasted | Fasted | Fasted | Fasted |
| Vehicle/Formulation | 15% Captisol/0.05 M tartrate buffer (pH 4.3) | Tablet | 15% Captisol/0.05 M tartrate buffer (pH 4.3) | 15% Captisol/0.05 M tartrate buffer (pH 4.3) |
| Method of Administration | Intravenous (infusion) | Oral (Tablet) | Intramuscular | Subcutaneous |
| Dose | 30 mg (4 mL/minipig, 4 mL/5 minutes) | 30 mg | 30 mg (4 mL/minipig) | 30 mg (4 mL/minipig) |
| Sample (whole blood, plasma, serum etc.) | Plasma | Plasma | Plasma | Plasma |
| Analyte | DM-1451 | DM-1451 | DM-1451 | DM-1451 |
| Assay | LC/MS/MS | LC/MS/MS | LC/MS/MS | LC/MS/MS |
| PK Parameters: | | | | |
| t _{max} (h) | ND ^b | 4.00 (4.00, 4.00) ^c | 8.00 ^d | 8.00 (8.00, 8.00) ^c |
| C _{max} (ng/mL) | ND ^b | 2.23 ^c | 3.46 ^d | 2.58 ^c |
| AUC _t (ng·h/mL) (Time for calculation - h) | ND ^b | 17.1 ^c | NR ^e | 18.4 ^c |
| Ratio of AUC(metabolite) to AUC(parent) (%) | ND ^b | 3.19 ^c | NR ^e | 1.10 ^c |
| Report No. | 014124 | 014124 | 014124 | 014124 |

BMS-337039 = OPC-14597; AUC_t = area under the concentration-time curve calculated to the last observable concentration at time t; C_{max} = maximum (peak) plasma concentration of the drug; LC/MS/MS = liquid chromatography-

electrospray ionization-tandem mass spectrometry; LLQ = lower limit of quantitation; M = male; ND = not determined; NR = not reported; t_{max} = time to maximum (peak) plasma concentration;

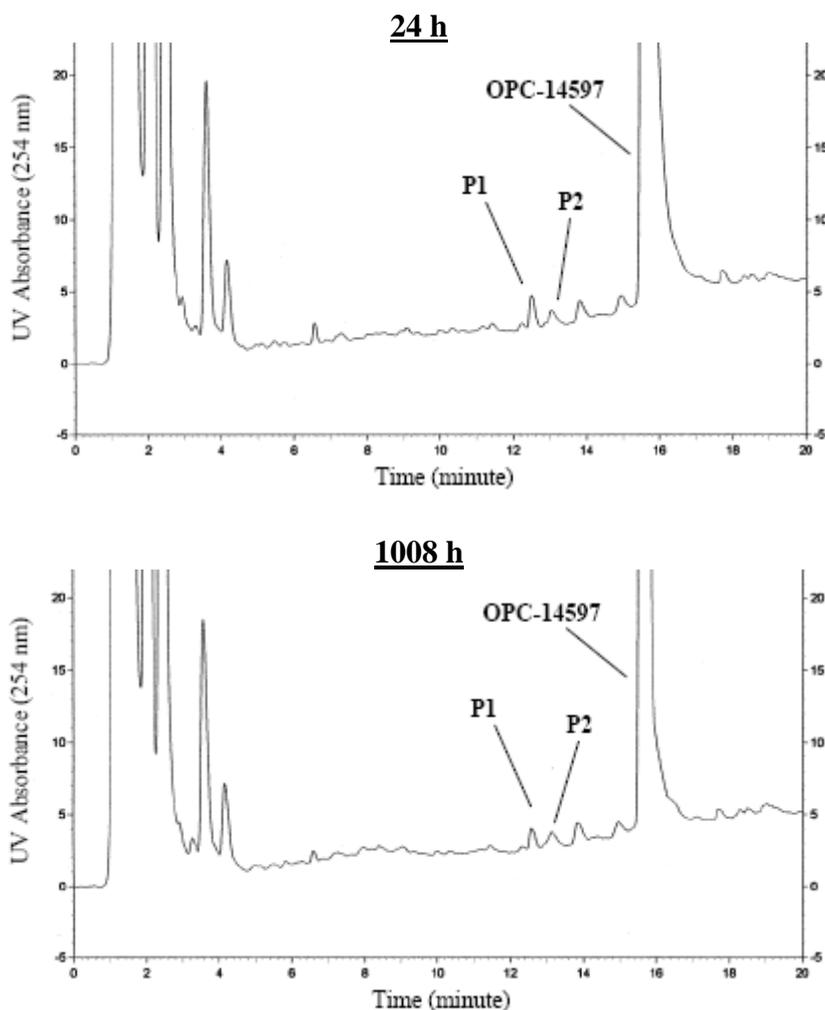
^aThree Sinclair® male minipigs (approximately 5 months old and between 15.1-18.3 kg of weight at study initiation).

^bAll plasma concentrations in all individual animals were below LLQ. C n = 2. d n = 1. ^e Only two or less than two plasma concentrations were above LLQ in all individual animals.

Aripiprazole metabolites in the injection site were determined in muscle tissue collected at 24, 168, 504 and 1008 hours after single IM administration of aripiprazole IM depot formulation to male rats ^{(b) (4)} Investigation of Metabolites of OPC-14597 in Injected Site after Single Intramuscular Administration of OPC-14597 Injectable Suspension to Rats. Otsuka Study No. 030054, Otsuka Report No.024412, 2010). The samples were analyzed by liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS); two peaks related to aripiprazole were detected in the HPLC and mass chromatograms of the dosed rat muscle homogenate samples, however, the peak area ratios of these to aripiprazole in the chromatograms were less than 0.6%. One of the peaks was identified as DM-1452. The other peak could not be identified. The peaks detected in the dosed samples did not increase with time (as shown in the following figures from the study report). These peaks were also detected in the muscle homogenate samples of the control group immediately after injection of aripiprazole IM depot formulation.

Figure 1

HPLC Chromatogram of Dosed Rat Muscle Homogenate Sample
(Male, 25 mg/0.25 mL/kg)



These findings suggested that these two peaks were not of biotransformed metabolite and support the conclusion that there was no metabolite or degradation product of aripiprazole produced in the injection site at 1008 hours (42 days) after dosing, and aripiprazole administered

as a depot formulation remained at the injection site without being metabolized or decomposed during that period.

Excretion

Urinary and fecal excretion was measured in rats after single intramuscular administration of ^{14}C -aripiprazole IM *rapid* formulation at a dose of 3.75 mg/kg (b) (4). Absorption, Distribution and Excretion of Radioactivity after Single Intramuscular Administration of ^{14}C -OPC-14597 to Rats. Otsuka Study No. 028360, Otsuka Report No.022847, 2009). The excretion data are summarized in the following sponsor's tables.

Excretion of Radioactivity after Single Intramuscular Administration of ^{14}C -Aripiprazole to Rats

Males

| Test Article: Aripiprazole IM Depot | | | |
|-------------------------------------|--|--------------|--------------------|
| Species/Strain | Rat/Sprague Dawley | | |
| Gender (M/F)/Number of Animals | M/3 | | |
| Feeding Condition | Fed | | |
| Vehicle/Formulation | L-tartaric acid/Captisol/Water for injection (rapid formulation) | | |
| Method of Administration | Intramuscular | | |
| Dose (mg/kg as the free base) | 3.75 | | |
| Radionuclide | ^{14}C -OPC-14597 | | |
| Specific Activity | 57.9 mCi/mmol | | |
| Analyte | Radioactivity ^a | | |
| Assay | Liquid scintillation counter | | |
| Excretion Route | Cumulative Excreted Radioactivity (% of Dose) | | |
| | Urine | Feces | Total ^b |
| Time | | | |
| 0 - 24 (h) | 4.52 ± 1.11 | 72.08 ± 1.97 | 76.60 ± 2.68 |
| 0 - 48 (h) | 4.73 ± 1.09 | 85.23 ± 0.57 | 89.96 ± 1.61 |
| 0 - 72 (h) | 4.83 ± 1.09 | 88.19 ± 0.77 | 93.02 ± 1.22 |
| 0 - 96 (h) | 4.88 ± 1.14 | 89.77 ± 1.30 | 94.65 ± 1.31 |
| 0 - 120 (h) | 4.88 ± 1.14 | 90.72 ± 1.66 | 95.60 ± 1.43 |
| 0 - 144 (h) | 4.88 ± 1.14 | 91.46 ± 2.08 | 96.34 ± 1.65 |
| 0 - 168 (h) | 4.88 ± 1.14 | 92.05 ± 2.48 | 96.93 ± 1.93 |
| Report Number | 022847 | | |

^aData expressed as the mean ± standard deviation of 3 animals.

Females

| Test Article: Aripiprazole IM Depot | | | |
|-------------------------------------|--|---------------|--------------------|
| Species/Strain | Rat/Sprague Dawley | | |
| Gender (M/F)/Number of Animals | F/3 | | |
| Feeding Condition | Fed | | |
| Vehicle/Formulation | L-tartaric acid/Captisol/Water for injection (rapid formulation) | | |
| Method of Administration | Intramuscular | | |
| Dose (mg/kg as the free base) | 3.75 | | |
| Radionuclide | ^{14}C -OPC-14597 | | |
| Specific Activity | 57.9 mCi/mmol | | |
| Analyte | Radioactivity ^a | | |
| Assay | Liquid scintillation counter | | |
| Excretion Route | Cumulative Excreted Radioactivity (% of Dose) | | |
| | Urine | Feces | Total ^b |
| Time | | | |
| 0 - 24 (h) | 5.76 ± 1.50 | 68.79 ± 13.83 | 74.54 ± 12.35 |
| 0 - 48 (h) | 6.25 ± 1.61 | 85.08 ± 8.79 | 91.33 ± 7.20 |
| 0 - 72 (h) | 6.41 ± 1.68 | 88.36 ± 7.19 | 94.77 ± 5.60 |
| 0 - 96 (h) | 6.52 ± 1.70 | 89.35 ± 6.66 | 95.86 ± 5.08 |
| 0 - 120 (h) | 6.57 ± 1.74 | 90.15 ± 6.19 | 96.72 ± 4.60 |
| 0 - 144 (h) | 6.57 ± 1.74 | 90.71 ± 5.90 | 97.28 ± 4.33 |
| 0 - 168 (h) | 6.57 ± 1.74 | 91.09 ± 5.70 | 97.69 ± 4.11 |
| Report Number | 022847 | | |

^aData expressed as the mean ± standard deviation of 3 animals.

^bThe value of cage washing was added to the total value from 0 to 168 hours.

The cumulative urinary and fecal excretion of radioactivity within 168 hrs after administration was 4.88% and 92.05% in males, and 6.57% and 91.09% in females, respectively. The excretion of total radioactivity, including cage washings, within 168 hrs accounted for 96.93% and 97.69% for male and female rats respectively, indicating that the excretion was complete.

Pharmacokinetic Drug Interaction

Pharmacokinetic drug interaction studies are cross-referenced from the previously approved NDA 21-436 (schizophrenia, oral tablet; submitted October 31, 2001).

5.2 Toxicokinetics

(Included in toxicity studies)

6 General Toxicology

Background: The nonclinical testing strategy for intramuscular aripiprazole was abbreviated since it was supported, in part, by results from previous in vitro and in vivo nonclinical studies conducted to support other formulations and indications for aripiprazole.

The toxicity and local irritation profile of intramuscular aripiprazole was characterized in single- and repeat-dose toxicity studies in rats, dogs, monkeys and rabbits. The carcinogenicity as well as reproductive and developmental toxicity and juvenile toxicity studies of aripiprazole in mice, rats, rabbits and dogs was previously evaluated following oral and/or intravenous administration described in the primary and supplemental NDAs. Since the systemic exposure to aripiprazole in toxicity studies was found to exceed the human exposure at the oral MRHD of 30 mg/day, and the human plasma exposure at MRHD (400 mg; 200 mg BID) for the intramuscular depot formulation did not exceed the systemic exposure at the oral MRHD, carcinogenicity and reproductive and developmental toxicity studies using the intramuscular depot formulation of aripiprazole would not provide any further meaningful information to assess the potential hazard in humans. Therefore, a carcinogenicity study of the intramuscular depot formulation was not conducted in accordance with the Executive CAC recommendation (Executive CAC Minutes, September 26, 2008). A waiver for undertaking developmental and reproductive toxicity studies was granted by the FDA (e-mail from K. Kiedrow (FDA) to Otsuka, June 23, 2010).

6.1 Single-Dose Toxicity

| Study | Species/Strain (Number/sex) | Route and Duration | Dose | Key Result | Report Number |
|-------------|--------------------------------|--------------------------|---|---|------------------|
| Single Dose | Dog/Beagle 5/sex/dose | IM 1 Day | 100, 200 or 400 mg | No mortality. Microscopic evidence of injection site inflammation at all doses with incomplete recovery. Plasma levels increased with dose. | 015504 |
| Single Dose | Dog/Beagle 5/sex/dose | IM 1 Day | 0 (saline), 150, 200, 300 or 400 mg | No mortality. Clinical signs of exaggerated pharmacology and injection site trauma. Microscopic evidence of injection site inflammation at all doses with incomplete recovery. Plasma levels increased with dose. | 019933 |

In an initial single-dose toxicity study [BMS-337039: Single-dose Intramuscular Depot Toxicity Study in Dogs. Otsuka Study No. 019797 (BMS Study No. DM02014), Otsuka Report No. 015504, 2003], aripiprazole (100 mg/mL) was administered IM to dogs (5/sex/dose) at doses of 100, 200 or 400 mg (dose volumes of 1, 2, or 4, 1-mL injections, respectively). A vehicle control group received two 1-mL injections of the carrier formulation (CMC, ^(b)₍₄₎mannitol, sodium monobasic phosphate and sterile water for injection, pH ~7; mean particle size ^(b)₍₄₎). Swelling at the injection sites was noted on Days 1 to 4 at all doses in females and at MD and HD in males. Changes in hematology parameters (RBC, WBC, leukocytes) attributable to inflammatory response at the injection sites, were found at MD and HD on Day 2. There were no

toxicologically significant changes in clinical chemistry parameters. At necropsy, white discoloration was observed in the muscle at the injection site at all doses.

In a second single-dose study [BMS-337039: Single-Dose Intramuscular Depot Toxicity Study in Dogs (II). Otsuka Study No. 025802, (BMS Study No. DM04016), Otsuka Report No. 019933, 2006], aripiprazole in the CMC formulation as described above was administered to dogs (5/sex/dose) at doses of 0 (saline control), 150, 200, 300 or 400 mg (150 and 200 mg/mL formulations as one or two 1-mL injections) into the right hind limb thigh. Tremors were observed at all doses; transient decreased activity at ≥ 200 mg on the day of dosing (not present on the next day). These effects were likely an exaggerated pharmacological effect of aripiprazole. Clinical observations at the injection site included pain, transient hind limb lameness/limping (all doses, on the day of dosing), and hindlimb muscle fasciculations in 1 male at 300 mg and 1 female at 400 mg (on Day 2, resolved by Day 3). Changes in hematology parameters (WBC, neutrophils) registered at all doses on Day 2 were likely related to local inflammation at the injection site. At necropsy, white foci at the injection site were seen in all treated animals at Week 5 and Days 43/44 post-dosing.

In both single dose studies, a dose-related granulomatous inflammation was observed characterized by numerous epithelioid macrophages with multinucleated (foreign body) giant cells and lymphocytes that were localized primarily in the interstitial tissue of skeletal muscle. The macrophages were associated with deposits of birefringent crystalline material (interpreted as deposited drug), and represented a foreign body reaction to the deposited drug. Injection site findings diminished, but did not completely resolve up to Days 43/44 post-dosing in either study. Toxicokinetic evaluations from each of these studies revealed a dose-related plasma exposure to aripiprazole and metabolites with no gender differences. Plasma levels of the parent drug were measurable up to 27 days after injection.

Plasma Toxicokinetics of Aripiprazole and Metabolites Following a Single Intramuscular Injection of Aripiprazole to Dogs

Otsuka Study No. 019797; Otsuka Report No. 015504

| Dose (mg) | Aripiprazole | | DM-1451 | | OPC-14857 | |
|--|--------------|--------|-----------------|-----------------|-----------------|--------|
| | Male | Female | Male | Female | Male | Female |
| C_{max} (ng/mL) | | | | | | |
| 100 | 12.3 | 34.7 | <1 | 1.31 (n = 2) | 3.00 | 10.1 |
| 200 | 55.7 | 79.5 | 1.80 (n = 3) | 1.67 (n = 4) | 18.0 | 37.8 |
| 400 | 140 | 122 | 5.13 (n = 3) | 2.57 (n = 4) | 52.4 | 61.5 |
| $AUC_{0-\infty}$ (ng·day/mL)^a | | | | | | |
| 100 | 112 | 254 | NC | NC | 25.4 (n = 3) | 60.1 |
| 200 | 474 | 683 | NC | NC | 133 | 308 |
| 400 | 936 | 1027 | 2.93 (n = 1) | 7.23 (n = 2) | 348 | 354 |

NC = Not calculated

^aThe AUC values were calculated from time 0 to infinity for aripiprazole and from 0 to the last measurable concentration for the metabolites. The lower limit of quantification was 1 ng/mL.

Plasma Toxicokinetics of Aripiprazole and Metabolites Following a Single Intramuscular Injection of Aripiprazole to Dogs

Otsuka Study No. 025802, Otsuka Report No. 019933

| | Dose (mg) | | | | | | | |
|--------------------------|-----------|--------|--------|--------|--------|--------|--------|--------|
| | 150 | | 200 | | 300 | | 400 | |
| | Male | Female | Male | Female | Male | Female | Male | Female |
| Aripiprazole | | | | | | | | |
| C_{max} (ng/mL) | 42.6 | 38.7 | 77.0 | 55.2 | 87.4 | 77.2 | 139 | 134 |
| AUC_{0-648h} (ng·h/mL) | 12,600 | 9670 | 20,000 | 16,200 | 28,100 | 23,100 | 44,500 | 45,800 |
| OPC-14857 | | | | | | | | |
| C_{max} (ng/mL) | 22.9 | 13.2 | 32.3 | 24.5 | 36.8 | 30.4 | 61.9 | 51.0 |
| AUC_{0-648h} (ng·h/mL) | 5890 | 2660 | 9000 | 6740 | 11,400 | 8510 | 19,800 | 17,600 |

6.2 Repeat-Dose Toxicity

The pivotal repeat dose toxicity studies supporting the clinical development of intramuscular aripiprazole IM depot formulation were performed in rats (26-weeks), dogs (26 and 52 weeks) and monkeys (4 weeks). Aripiprazole was administered weekly in the in rat and dog, whereas daily administrations were used for the monkey study. All pivotal toxicity studies were conducted in compliance with GLP regulations. Dose selection for pivotal studies was based on results from preceding intramuscular exploratory or range-finding studies. Toxicokinetic evaluations were also conducted in all of these pivotal studies.

Pivotal repeat dose toxicity studies**Study title: Twenty-six-week Intermittent Repeated Intramuscular Depot Toxicity Study of OPC-14597 in Rats with Four-week Recovery Test**

Study no.: 024105
Study report no.: 019345, 2007
Conducting laboratory and location: Tokushima Research Institute
Otsuka Pharmaceutical Co., Tokushima, Japan
Date of study initiation: 9 May 2006 (Completion: 27 Feb 2007)
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: OPC-14597 for injectable suspension,
Lot Nos.: 2J64454 and 4M63442, purity 101.2% and
103.8 % for 200 and 400 mg /vial, respectively

Key Study Findings

Aripiprazole depot formulation administered by weekly IM injections to SD rats for 26 weeks at doses of 25, 50 and 100 mg resulted in decreased body weights and food consumption at HD vs. control, more expressed and non-recoverable in males. Subcutaneous nodule and swelling at the injection site were observed after injection, but resolved either between injections or as the study progressed. Injection site findings included “white foci” of dose-dependent size, not fully reversible after the recovery period, and microscopically characterized by granulomatous inflammation (accumulation of macrophages, eosinophilic deposits and proliferation of capillaries with swollen endothelial cells) associated with deposits of birefringent crystal-like material (interpreted as drug). The following findings could be extension of the drug pharmacology: mammary gland hyperplasia in females at all doses (irreversible at HD at the end of recovery period), lobular hyperplasia and milk secretion in female mammary gland, hypertrophy of corpora lutea in the ovary, mucification of epithelium in the uterine cervix and vagina and atrophy of pars intermedia of the pituitary gland were observed. The NOAEL was 50 mg/kg in males and 100 mg/kg in females with mean C_{max} and AUC_{7d} of aripiprazole at week 26 of 98.1ng/mL and 598.6 ng.d/mL respectively for males and 1135.3 ng/mL and 4336.2 ng.d/mL, respectively for females.

Methods

Doses: 0 (saline), 25, 50 and 100 mg/kg of OPC-14597

Frequency of dosing: Weekly

Route of administration: Intramuscular injection in the hindlimbs (sura, thigh and rump) once weekly for 26 weeks in the morning

Dose volume: 0.5 mL/kg in control, MD and HD groups; 0.25 mL/kg for LD group

Formulation/Vehicle: OPC-14597 for injectable suspension (a white cake constituted with about (b) (4) water for injection)/ 0% OPC-14597 solution for injection, Lot No.: 06C00P000

Species/Strain: Rat/Sprague-Dawley [CrI: CD(SD)]

Number/Sex/Group: 12

Age: 8-9 weeks at initiation of dosing

Weight: 304-363 g (M) 197-246 g (F)

Satellite groups: TK of aripiprazole and its metabolites in all dose groups in additional 3/sex/group rats

Deviation from study protocol: Occasional short interruptions of air ventilation and air conditioning due to maintenance work. As these events were transient and none of the animals showed any abnormalities at the end of quarantine period, it is unlikely that these events have affected the study results.

Study design: OPC-14597 depot formulation of was administered by a weekly intramuscular injection to groups of 12 rats/sex for 26 weeks at 0 (saline), 25, 50 and 100 mg/kg of OPC-14597. Two control groups of 12 rats/sex each were used: a saline and a placebo control (0% OPC-14597 solution for injection, Lot No.: 06C00P000). The control or test article was administered by weekly rotation to 5 specified injection sites of each hindlimb (sura, thigh and rump) once weekly in the morning, at a dose volume of 0.25 mL/kg (LD) or 0.5 (2x0.25) mL/kg for all other groups. The choice of the total dose volume of 0.5 mL/kg as 2 injections (0.25mL/kg/site) was based on the preceding 4-week rat toxicity study.

The reversibility of toxicity was examined after a subsequent 4-week treatment-free recovery period using additional 5 animals of each sex at 0(saline), 0 (placebo control) and HD group. A satellite group was set for each dose group to determine the plasma concentrations of OPC-14597 and its metabolites to assess the systemic exposure.

Observations: General condition including the injection site (once daily), body weight (once weekly from Day 1 to Week 13, fortnightly thereafter until Week 25, and finally at Week 26), food consumption, hematology and blood chemistry (on the day of necropsy), urinalysis (weeks 14 and 26), ophthalmoscopy (at pre-dose and in weeks 14 and 26), necropsy, organ weights (brain, pituitary gland, submaxillary glands (including the sublingual glands), thyroid glands (including the parathyroid glands), thymus, heart, lungs, liver, spleen, adrenal glands, kidneys, testes, seminal vesicle, prostate, ovaries, and uterus), gross pathology and histopathology (liver, kidneys, ureters, thymus, mesenteric lymph nodes, mandibular lymph nodes, popliteal lymph nodes, spleen, heart, aorta, lungs, bronchi, trachea, larynx, tongue, esophagus, submaxillary glands, sublingual glands, parotid glands, stomach, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, seminal vesicles, prostate, ovaries, oviducts, uterus, uterine cervix, vagina, urinary bladder, pituitary gland, adrenal glands, thyroid glands, parathyroid glands, skin, mammary glands, skeletal (triceps branchii) muscle, sciatic nerve, brain, spinal cord (cervical, thoracic and lumbar), optic nerve, harderian glands, sternum with marrow, femur with marrow, stifle joint, and injection site).

Toxicokinetics: Plasma concentrations of OPC-14597 and its metabolites: OPC-14857, DM-1451, DM-1452, OPC-3373 and DCPD were determined in the satellite dose groups. Approximately 1 mL blood was collected from 3 animals/sex of each treated group on Days 1, 2, 4 and 7 post-dosing in Week 1, and at pre-dosing, 0.25, 2, 4 and 7 days post-dosing in Weeks 4, 13, and 26, as well as in recovery Weeks 1, 2, 3 and 4. The plasma concentrations of OPC-14597 and the metabolites were determined by high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) 2, 4 and 7 days post-dosing in Weeks 4, 13 and 26, as well as in

recovery Weeks 1, 2, 3 and 4. C_{max} , t_{max} and area under the plasma concentration curve from 0 to 7 days (AUC_{7d}) were determined. The AUC_{7d} was calculated based on the plasma concentrations at each time point using the linear trapezoidal rule.

Results

Mortality: There was no drug-related mortality. One control female was sacrificed moribund on study day 72. **Clinical Signs:** Subcutaneous nodules and swelling at the injection sites after dosing were occasionally observed in all dosed groups during the dosing and recovery period, but diminished with time .

Body Weights: Dose-dependent decrease in body weight was seen in males at MD and HD, statistically significant at HD. At the end of the dosing period, the mean body weight of HD males was 91% of control values; the decrease in body weight persisted (though not statistically significant) after discontinuation of treatment, so that at the end of recovery period, the mean body weight of HD males was 87% of the control group. In contrast, increase in body weight gain was seen in treated females compared to saline and placebo controls, but this effect was not dose-dependent as it was most expressed at LD (see the following sponsor's tables).

| Sex : Male | | Body Weight | | | | |
|----------------|------------------|------------------|------------------|------------------|---------------------|--|
| | | Unit : g | | | | |
| Dose | S0 | A0 | A25 | A50 | A100 | |
| No. of Animals | 17 | 17 | 12 | 12 | 17 | |
| Week | Mean±S.D. n | Mean±S.D. n | Mean±S.D. n | Mean±S.D. n | Mean±S.D. n | |
| 25 | 637.5±64.0 17 | 660.6±64.0 17 | 634.2±56.2 12 | 618.3±71.9 12 | 584.3±42.8*## 17 | |
| 26 | 646.3±69.3 17 | 669.4±65.8 17 | 641.8±58.4 12 | 623.8±73.7 12 | 590.6±42.6## 17 | |
| R1 | 651.2±64.1 5 | 655.6±95.0 5 | | | 570.6±61.4 5 | |
| R3 | 668.0±66.4 5 | 674.6±100.0 5 | | | 587.0±66.3 5 | |
| R4 | 675.2±69.3 5 | 676.2±97.3 5 | | | 589.2±67.0 5 | |

| Sex : Female | | Body Weight | | | | |
|----------------|------------------|------------------|------------------|------------------|------------------|--|
| | | Unit : g | | | | |
| Dose | S0 | A0 | A25 | A50 | A100 | |
| No. of Animals | 17 | 17 | 12 | 12 | 17 | |
| Week | Mean±S.D. n | Mean±S.D. n | Mean±S.D. n | Mean±S.D. n | Mean±S.D. n | |
| 25 | 347.3±22.5 16 | 336.6±24.9 17 | 378.0±40.2 12 | 354.6±32.5 12 | 357.8±26.0 17 | |
| 26 | 354.1±25.4 16 | 341.5±26.1 17 | 385.5±43.9 12 | 359.3±34.8 12 | 362.2±27.0 17 | |
| R1 | 329.0±13.8 4 | 354.0±20.8 5 | | | 369.2±39.4 5 | |
| R3 | 334.0±15.3 4 | 355.2±24.0 5 | | | 370.4±36.9 5 | |
| R4 | 337.5±9.4 4 | 363.2±23.0 5 | | | 373.8±32.8 5 | |

S0 : 0mg/kg A0 : 0mg/kg A25 : 25mg/kg A50 : 50mg/kg A100 : 100mg/kg
Week : Rn;Recovery Week
* ; P<0.05 , ** ; P<0.01 : Significant difference from the control (S0 vs A25, A50, A100 or S0 vs A100)
\$; P<0.05 , \$\$; P<0.01 : Significant difference from the control (S0 vs A0)

Feed Consumption

Decreased (11%) food consumption was observed in HD males compared to control and placebo that correlated with the decrease in body weight in this group; this change persisted (although not statistically significant) during the recovery period. A smaller (4%) and transient decrease in food consumption was also observed in MD males. A decrease in food consumption was occasionally observed in females at MD but not at HD.

Ophthalmoscopy

No drug-related changes.

Hematology

Increases in platelet counts were observed at MD (11% in females) and HD (12% and 18% in males and females, respectively). However, no other hematology changes suggesting hemorrhage were observed, and most of the individual values were within the range of the control and placebo values. A relationship of this finding to the IM injection of OPC-14597 was unlikely since there were no corresponding hemorrhagic changes at the injection site. It thus appears that the change in the platelet counts was not of toxicological concern.

Minimal decreases in MCH were observed at HD (females), however this change was likely incidental since there were no changes in the other erythrocyte indices.

Clinical Chemistry

A small increase in the mean AST value was observed in HDM, but most of the individual values were within the range of the control. Two HD males had AST values (111 and 126 IU/L) in excess of the upper limit of the control males (104 IU/L). At the end of the recovery period, increased AST was still observed in HD males (see the following sponsor's table). Since the differences were small and there were no drug-related histopathology changes in the liver, this small increase in AST was not of toxicological concern. A relationship to the IM injection of OPC-14597 was unlikely since no corresponding muscular degeneration or necrosis was observed at the injection site in any of the treated animals.

Clinical Chemistry: AST and ALT in males

Sex : Male

| Dose | Item (unit) Stage | S0 Mean±S.D. n | A0 Mean±S.D. n | A25 Mean±S.D. n | A50 Mean±S.D. n | A100 Mean±S.D. n |
|--------------------|-------------------|----------------------|----------------------|-----------------------|-----------------------|------------------------|
| AST(GOT) (IU/L) | Week26 | 62.0±14.4 12 | 140.6±252.1 12 | 68.3±10.4 12 | 67.2±11.7 12 | 85.6±18.1** 12 |
| | R.Week4 | 63.8±7.7 5 | 76.8±16.6 5 | | | 99.0±22.5* 4 |
| ALT(GPT) (IU/L) | Week26 | 28.2±4.4 12 | 140.5±377.8 12 | 33.8±9.7 12 | 28.5±6.6 12 | 35.3±12.1 12 |
| | R.Week4 | 31.0±4.2 5 | 37.6±7.5 5 | | | 39.3±11.5 4 |

S0 : 0mg/kg A0 : 0mg/kg A25 : 25mg/kg A50 : 50mg/kg A100 : 100mg/kg

* ; P<0.05 , ** ; P<0.01 : Significant difference from the control (S0 vs A25, A50, A100 or S0 vs A100)

§ ; P<0.05 , §§ ; P<0.01 : Significant difference from the control (S0 vs A0)

+) # ; P<0.05 , ## ; P<0.01 : Significant difference from the control (A0 vs A25, A50, A100 or A0 vs A100)

In females, decreased cholesterol (at HD) and phospholipids (at MD and HD) were observed. These changes were still observed in HD females at the end of the recovery period (as shown in the following sponsor's table). However, since most of the individual values were within the range of the saline and placebo controls, and there were no changes in the body weight and food consumption in HDF, it is unlikely that these small decreases were toxicologically significant.

Clinical Chemistry – Lipid indices in females

Sex : Female

| Dose | | S0 | A0 | A25 | A50 | A100 |
|-----------------|---------|------------------|------------------|------------------|-------------------|----------------------|
| Item (unit) | Stage | Mean±S.D. | Mean±S.D. | Mean±S.D. | Mean±S.D. | Mean±S.D. |
| No of animals | | n | n | n | n | n |
| CHO+ (mg/dL) | Week26 | 93.5±16.1 12 | 85.1±10.7 12 | 90.2±20.4 12 | 77.3±14.6 12 | 75.5±17.9* 12 |
| | R.Week4 | 113.5±23.6 4 | 99.8±11.0 5 | | | 62.2±9.9***## 5 |
| PL+ (mg/dL) | Week26 | 166.7±24.0 12 | 158.3±20.9 12 | 164.2±34.8 12 | 137.8±20.6* 12 | 136.2±22.8* 12 |
| | R.Week4 | 195.8±43.3 4 | 184.4±18.7 5 | | | 119.8±17.7***## 5 |
| TG (mg/dL) | Week26 | 33.7±14.1 12 | 43.7±41.0 12 | 41.6±23.7 12 | 26.1±6.3 12 | 22.6±9.8 12 |
| | R.Week4 | 23.5±9.0 4 | 38.4±14.4 5 | | | 21.2±8.0 5 |

S0 : 0mg/kg A0 : 0mg/kg A25 : 25mg/kg A50 : 50mg/kg A100 : 100mg/kg
 * ; P<0.05 , ** ; P<0.01 : Significant difference from the control (S0 vs A25, A50, A100 or S0 vs A100)
 \$; P<0.05 , \$\$; P<0.01 : Significant difference from the control (S0 vs A0)
 +) # ; P<0.05 , ## ; P<0.01 : Significant difference from the control (A0 vs A25, A50, A100 or A0 vs A100)

Decreased total protein was found in males at all dose levels (not dose-dependent), and small decreases in total protein and A/G ratio due to decreased albumin content were seen in females at HD and MD. These changes were too slight to be toxicologically meaningful.

Urinalysis

Mean urinary sodium excretion was increased (up to 2-fold vs. control at wk 26) in MD and HD female groups; creatinine excretion was also increased (up to 1.3x vs. control at wk 26) in females at MD and HD. However, most of the individual urinary sodium values in treated females were within the range of the control groups in Week 26, and no related changes in the plasma sodium concentration were present. The increase in creatinine excretion was small, and there were no related changes in plasma creatinine concentration in the treated females.

Gross Pathology

The following changes were observed at the end of the dosing period (see the following sponsor's table):

Injection site: White foci (corresponding to the subcutaneous nodules observed in vivo) were present at the injection sites in all animals dosed with OPC-14597 suspension. Their size was generally dose-related and ranged from 1 mm linear to 8 × 8 × 20 mm. These changes were not reversible at HD, and at the end of the recovery period white foci were still observed in all HD animals.

Mammary gland: A dose-dependent enlargement of mammary glands was observed in dosed females (1 LDF, 5 MDF, and 8 HDF) and in 1 placebo control female; galactoceles were seen in

1LDF, 1 MDF and 1 placebo control female. These changes were not fully reversible after discontinuation of treatment; at the end of the recovery period, mammary enlargement was still present in 3 HD females.

Other changes, such as discolored foci in the lung, bronchus, glandular stomach mucosa or the eye were sporadically seen in single animals at MD and HD. These low incidence findings were either not dose dependent, or usually experienced in intact rats.

| Item : Macropathological Findings - Summary | | Stage : Week26 | | | | |
|---|----------|-----------------|----|------|-----|------|
| Sex : Male | | | | | | |
| Dose | | S0 | A0 | A25 | A50 | A100 |
| No. of Animals | | 12 | 12 | 12 | 12 | 12 |
| Injection site | Examined | 12 | 12 | 12 | 12 | 12 |
| White focus | | 0 | 0 | 12 | 12 | 12 |
| Sex : Female | | | | | | |
| Mammary gland | Examined | 12 | 12 | 12 | 12 | 12 |
| Development | | 0 | 1 | 1 | 5 | 8 |
| Galactoceles | | 0 | 1 | 1 | 1 | 0 |
| Mass | | 0 | 1 | 0 | 0 | 0 |
| Injection site | Examined | 12 | 12 | 12 | 12 | 12 |
| White focus | | 0 | 0 | 12 | 12 | 12 |
| S0 : 0mg/kg A0 : 0mg/kg A25 : 25mg/kg A50 : 50mg/kg A100 : 100mg/kg | | | | | | |
| Item : Macropathological Findings - Summary | | Stage : R.Week4 | | | | |
| Sex : Male | | | | | | |
| Dose | | S0 | A0 | A100 | | |
| No. of Animals | | 5 | 5 | 5 | | |
| Injection site | Examined | 5 | 5 | 5 | | |
| White focus | | 0 | 0 | 5 | | |
| Sex : Female | | | | | | |
| Mammary gland | Examined | 4 | 5 | 5 | | |
| Development | | 0 | 0 | 3 | | |
| Injection site | Examined | 4 | 5 | 5 | | |
| White focus | | 0 | 0 | 5 | | |
| S0 : 0mg/kg A0 : 0mg/kg A100 : 100mg/kg | | | | | | |

Organ Weights

At the end of the dosing period, the following changes were observed in both the absolute and relative weights: Males: a dose-dependent decrease (at MD and HD) in the weights of pituitary (up to 14%), liver (up to 18%) and kidneys (up to 8%); Females: decreased weights of the uterus (at MD and HD, up to 36%) and adrenal glands (all doses, up to 15% at HD), and increased weight of the spleen (at MD and HD, up to 19% at HD).

There were no corresponding histopathology changes in any of these organs, i.e., although decreased mean uterine weight was observed in HD females, all of the individual values were within the range of the control, and no atrophic changes were observed in the uterus.

At the end of the recovery period, the absolute and relative weights of the uterus in HD females were decreased, and the absolute weights of the ovaries were increased compared to control (it is of note that the latter changes were not significant at the end of the dosing period). In HD males, the absolute liver weight was decreased, but there were no changes in liver relative weight.

Histopathology**Adequate Battery:** Yes**Peer Review:** Yes**Histopathology Findings**

At the end of the dosing period, the following changes were observed:

Injection site: Granulomatous inflammation corresponding to the white foci was observed macroscopically at the injection site in all treated animals (12 of 12 per dose group), and consisted of accumulation of macrophages, eosinophilic deposits and proliferation of capillaries with swollen endothelial cells, in association with deposits of birefringent crystal-like material (interpreted as drug). Slight hemorrhage located within the granulomatous inflammation was noted in 2HD males.

Pituitary gland: Atrophy of pars intermedia was observed in all treated males and females except for 1 HD female.

Mammary gland: Lobular hyperplasia was observed in all treated females (12/12), as well as in 2 of 12 saline control females and in 3 of 12 placebo control females. Milk secretion was observed in 4 LD females, 4 MD females and in 7 HD females, as well as in 1 saline control and in 2 placebo control females.

Ovary: Hypertrophy of corpora lutea was observed in 5 LDF, 7 MDF, and 9 HDF.

Uterine cervix: Mucification of epithelium was observed in 2 LD and 3 HD females.

Vagina: Mucification of epithelium was observed in 3 LDF, 1MDF, and in 4 HD females.

Note: The findings in female reproductive and mammary tissues had been observed in previous oral toxicity studies in rats, and were considered a consequence of pharmacologically mediated hyperprolactinemia

Histopathology findings at end of dosing, Week 26**Sex: Male**

| Dose | | S0 | A0 | A25 | A50 | A100 |
|--------------------------------|----------|----|----|-----|-----|------|
| No. of Animals | | 12 | 12 | 12 | 12 | 12 |
| Pituitary | Examined | 12 | 12 | 12 | 12 | 12 |
| Atrophy of pars intermedia | ± | 0 | 0 | 0 | 0 | 0 |
| | + | 0 | 0 | 12 | 12 | 12 |
| | ++ | 0 | 0 | 0 | 0 | 0 |
| | +++ | 0 | 0 | 0 | 0 | 0 |
| Injection site | Examined | 12 | 12 | 12 | 12 | 12 |
| Granulomatous inflammation | ± | 0 | 0 | 0 | 0 | 0 |
| | + | 0 | 0 | 12 | 12 | 12 |
| | ++ | 0 | 0 | 0 | 0 | 0 |
| | +++ | 0 | 0 | 0 | 0 | 0 |
| Hemorrhage | ± | 0 | 0 | 0 | 0 | 2 |
| | + | 0 | 0 | 0 | 0 | 0 |
| | ++ | 0 | 0 | 0 | 0 | 0 |
| | +++ | 0 | 0 | 0 | 0 | 0 |
| Skeletal muscle | Examined | 12 | 12 | 0 | 0 | 12 |
| Focal necrosis of muscle fiber | ± | 1 | 0 | - | - | 0 |
| | + | 0 | 0 | - | - | 0 |
| | ++ | 0 | 0 | - | - | 0 |
| | +++ | 0 | 0 | - | - | 0 |

± : Very Slight , + : Slight , ++ : Moderate , +++ : Severe , - : Not Examined
 S0 : 0mg/kg A0 : 0mg/kg A25 : 25mg/kg A50 : 50mg/kg A100 : 100mg/kg

Histopathology findings at end of dosing, Week 26**Sex: Female**

| Dose No. of Animals | | S0 12 | A0 12 | A25 12 | A50 12 | A100 12 |
|------------------------------|----------|----------|----------|-----------|-----------|------------|
| Pituitary | Examined | 12 | 12 | 12 | 12 | 12 |
| Atrophy of pars intermedia | ± | 0 | 0 | 0 | 0 | 0 |
| | + | 0 | 0 | 12 | 12 | 11 |
| | ++ | 0 | 0 | 0 | 0 | 0 |
| | +++ | 0 | 0 | 0 | 0 | 0 |
| Mammary gland | Examined | 12 | 12 | 12 | 12 | 12 |
| Lobular hyperplasia | ± | 2 | 3 | 9 | 8 | 8 |
| | + | 0 | 0 | 3 | 4 | 4 |
| | ++ | 0 | 0 | 0 | 0 | 0 |
| | +++ | 0 | 0 | 0 | 0 | 0 |
| Milk secretion | ± | 1 | 2 | 4 | 4 | 7 |
| | + | 0 | 0 | 0 | 0 | 0 |
| | ++ | 0 | 0 | 0 | 0 | 0 |
| | +++ | 0 | 0 | 0 | 0 | 0 |
| Ovary | Examined | 12 | 12 | 12 | 12 | 12 |
| Hypertrophy of corpora lutea | ± | 0 | 0 | 0 | 0 | 0 |
| | + | 0 | 0 | 5 | 7 | 9 |
| | ++ | 0 | 0 | 0 | 0 | 0 |
| | +++ | 0 | 0 | 0 | 0 | 0 |
| Uterine cervix | Examined | 12 | 12 | 12 | 12 | 12 |
| Mucification of epithelium | ± | 0 | 0 | 0 | 0 | 0 |
| | + | 0 | 0 | 2 | 0 | 3 |
| | ++ | 0 | 0 | 0 | 0 | 0 |
| | +++ | 0 | 0 | 0 | 0 | 0 |
| Vagina | Examined | 12 | 12 | 12 | 12 | 11 |
| Mucification of epithelium | ± | 0 | 0 | 0 | 0 | 0 |
| | + | 0 | 0 | 3 | 1 | 4 |
| | ++ | 0 | 0 | 0 | 0 | 0 |
| | +++ | 0 | 0 | 0 | 0 | 0 |
| Injection site | Examined | 12 | 12 | 12 | 12 | 12 |
| Granulomatous inflammation | ± | 0 | 0 | 0 | 0 | 0 |
| | + | 0 | 0 | 12 | 12 | 12 |
| | ++ | 0 | 0 | 0 | 0 | 0 |
| | +++ | 0 | 0 | 0 | 0 | 0 |

± : Very Slight , + : Slight , ++ : Moderate , +++ : Severe
S0 : 0mg/kg A0 : 0mg/kg A25 : 25mg/kg A50 : 50mg/kg A100 : 100mg/kg

At the end of recovery period, granulomatous inflammation and birefringent crystal-like material within the foci of granulomatous inflammation, similar to that seen at the end of the dosing period, were observed in all HD animals, male and female; morphological changes in the reproductive and mammary tissues (lobular hyperplasia in 4 of 5 HD females) were still present, and atrophy of pars intermedia in the pituitary gland was still observed in all males and females at HD. Hypertrophy of corpora lutea in the ovary was observed in 2 of 5 examined HD females and mucification of vaginal epithelium was observed in 1 of 5 HD females.

Histopathology findings at the end of recovery period (Recovery Week4)**Male**

| Dose | | S0 | A0 | A100 |
|----------------------------|----------|----|----|------|
| No. of Animals | | 5 | 5 | 5 |
| Pituitary | Examined | 5 | 5 | 5 |
| Atrophy of pars intermedia | ± | 0 | 0 | 0 |
| | + | 0 | 0 | 5 |
| | ++ | 0 | 0 | 0 |
| | +++ | 0 | 0 | 0 |
| Injection site | Examined | 5 | 5 | 5 |
| Granulomatous inflammation | ± | 0 | 0 | 0 |
| | + | 0 | 0 | 5 |
| | ++ | 0 | 0 | 0 |
| | +++ | 0 | 0 | 0 |

Female

| | | | | |
|------------------------------|----------|---|---|---|
| Pituitary | Examined | 4 | 5 | 5 |
| Atrophy of pars intermedia | ± | 0 | 0 | 0 |
| | + | 0 | 0 | 5 |
| | ++ | 0 | 0 | 0 |
| | +++ | 0 | 0 | 0 |
| Mammary gland | Examined | 4 | 5 | 5 |
| Lobular hyperplasia | ± | 0 | 0 | 2 |
| | + | 0 | 0 | 2 |
| | ++ | 0 | 0 | 0 |
| | +++ | 0 | 0 | 0 |
| Ovary | Examined | 4 | 5 | 5 |
| Hypertrophy of corpora lutea | ± | 0 | 0 | 0 |
| | + | 0 | 0 | 2 |
| | ++ | 0 | 0 | 0 |
| | +++ | 0 | 0 | 0 |
| Vagina | Examined | 4 | 5 | 5 |
| Mucification of epithelium | ± | 0 | 0 | 0 |
| | + | 0 | 0 | 1 |
| | ++ | 0 | 0 | 0 |
| | +++ | 0 | 0 | 0 |
| Injection site | Examined | 4 | 5 | 5 |
| Granulomatous inflammation | ± | 0 | 0 | 0 |
| | + | 0 | 0 | 5 |
| | ++ | 0 | 0 | 0 |
| | +++ | 0 | 0 | 0 |

± : Very Slight , + : Slight , ++ : Moderate , +++ : Severe
 S0 : 0mg/kg A0 : 0mg/kg A100 : 100mg/kg

Toxicokinetics

The mean exposure values (C_{max} and AUC_{7d}) of OPC-14597 and its detectable metabolites (OPC-14857 and DM-1451) were elevated in a dose-related manner for both genders, except that they were slightly higher at LD than at MD in Week 26. The C_{max} and AUC_{7d} of OPC-14597 were higher than those of the metabolites. The C_{max} and AUC_{7d} of OPC-14597 and its metabolites in all dosed groups increased as the dosing period progressed. No remarkable sex

differences in the plasma concentrations of OPC-14597 and its metabolites were observed throughout the dosing period.

During the recovery period, the plasma concentrations of OPC-14597 and its metabolites decreased gradually in both sexes.

C_{max} (ng/mL)

| Substance-determined | Dose (mg/kg) | Week 1 | | Week 4 | | Week 26 | |
|----------------------|--------------|--------|--------|--------|--------|---------|--------|
| | | Male | Female | Male | Female | Male | Female |
| OPC-14597 | 25 | 18.8 | 12.6 | 42.1 | 31.1 | 118.5 | 121.7 |
| | 50 | 21.1 | 40.3 | 70.3 | 78.0 | 98.1 | 89.9 |
| | 100 | 58.8 | 70.0 | 161.0 | 138.0 | 978.4 | 1135.3 |
| OPC-14857 | 25 | < 2 | < 2 | < 2 | < 2 | 2.8 | 5.4 |
| | 50 | < 2 | 0.8 | 0.8 | 4.0 | 1.8 | 4.6 |
| | 100 | < 2 | 1.4 | 7.8 | 7.6 | 101.9 | 101.1 |
| DM-1451 | 25 | 3.6 | 1.8 | 4.9 | 3.1 | 9.0 | 5.6 |
| | 50 | 3.3 | 4.0 | 6.8 | 4.9 | 8.2 | 5.4 |
| | 100 | 7.1 | 5.2 | 10.9 | 7.5 | 11.5 | 8.2 |
| DM-1452 | 25 | < 2 | < 2 | < 2 | < 2 | < 2 | < 2 |
| | 50 | < 2 | < 2 | < 2 | 0.7 | < 2 | < 2 |
| | 100 | < 2 | < 2 | < 2 | 0.7 | 5.7 | 7.2 |
| OPC-3373 | 25 | < 2 | < 2 | < 2 | < 2 | 0.7 | < 2 |
| | 50 | < 2 | < 2 | < 2 | < 2 | < 2 | < 2 |
| | 100 | < 2 | < 2 | 2.4 | 2.4 | 13.8 | 14.8 |
| DCPP | 25 | < 2 | < 2 | < 2 | < 2 | < 2 | < 2 |
| | 50 | < 2 | < 2 | < 2 | < 2 | < 2 | < 2 |
| | 100 | < 2 | < 2 | < 2 | < 2 | 11.9 | 5.9 |

C_{max}, relative (%)

Males

| Item: C _{max} (%) /relative* | | | | | | | | | | |
|---------------------------------------|--------------|-------|-------|--------|-------|-------|---------|-------|-------|-------|
| Stage | Week 1 | | | Week 4 | | | Week 26 | | | |
| | Dose (mg/kg) | 25 | 50 | 100 | 25 | 50 | 100 | 25 | 50 | 100 |
| OPC-14597 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| OPC-14857 | NC | NC | NC | NC | 1.1 | 4.9 | 2.4 | 1.8 | 10.5 | |
| DM-1451 | 18.5 | 15.1 | 11.7 | 11.2 | 9.3 | 6.5 | 7.3 | 8.1 | 1.1 | |
| DM-1452 | NC | NC | NC | NC | NC | NC | NC | NC | 0.6 | |
| OPC-3373 | NC | NC | NC | NC | NC | 2.7 | 1.1 | NC | 2.5 | |
| DCPP | NC | NC | NC | NC | NC | NC | NC | NC | 2.4 | |

Females

| Item: C _{max} (%) /relative* | | | | | | | | | | |
|---------------------------------------|--------------|-------|-------|--------|-------|-------|---------|-------|-------|-------|
| Stage | Week 1 | | | Week 4 | | | Week 26 | | | |
| | Dose (mg/kg) | 25 | 50 | 100 | 25 | 50 | 100 | 25 | 50 | 100 |
| OPC-14597 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| OPC-14857 | NC | 2.0 | 2.0 | NC | 5.2 | 5.5 | 4.5 | 5.1 | 8.9 | |
| DM-1451 | 13.8 | 9.6 | 7.2 | 9.6 | 6.1 | 5.2 | 4.4 | 5.8 | 0.7 | |
| DM-1452 | NC | NC | NC | NC | 0.9 | 0.5 | NC | NC | 0.6 | |
| OPC-3373 | NC | NC | NC | NC | NC | 3.1 | NC | NC | 2.3 | |
| DCPP | NC | NC | NC | NC | NC | NC | NC | NC | 1.0 | |

*: C_{max} of OPC-14597 was set at 100%. Molar ratio.

NC: not calculated

AUC7d (ng.day/mL)

| Substance-determined | Dose (mg/kg) | Week 1 | | Week 4 | | Week 26 | |
|----------------------|--------------|--------|--------|--------|--------|---------|--------|
| | | Male | Female | Male | Female | Male | Female |
| OPC-14597 | 25 | 76.6 | 49.9 | 225.3 | 168.2 | 728.7 | 647.0 |
| | 50 | 96.6 | 130.4 | 406.8 | 431.8 | 598.6 | 536.0 |
| | 100 | 215.3 | 229.9 | 925.7 | 811.8 | 3840.8 | 4336.2 |
| OPC-14857 | 25 | NC | NC | NC | NC | 11.6 | 24.9 |
| | 50 | NC | 0.8 | 1.5 | 16.9 | 5.7 | 16.4 |
| | 100 | NC | 2.1 | 32.8 | 36.4 | 346.7 | 345.9 |
| DM-1451 | 25 | 11.2 | 3.7 | 26.0 | 15.7 | 51.9 | 31.1 |
| | 50 | 15.2 | 10.6 | 38.9 | 26.6 | 47.2 | 27.8 |
| | 100 | 30.7 | 18.9 | 62.6 | 41.3 | 69.7 | 50.9 |
| DM-1452 | 25 | NC | NC | NC | NC | NC | NC |
| | 50 | NC | NC | NC | 0.7 | NC | NC |
| | 100 | NC | NC | NC | 2.0 | 23.4 | 28.3 |
| OPC-3373 | 25 | NC | NC | NC | NC | 3.1 | NC |
| | 50 | NC | NC | NC | NC | NC | NC |
| | 100 | NC | NC | 11.1 | 6.3 | 69.3 | 48.4 |
| DCPP | 25 | NC | NC | NC | NC | NC | NC |
| | 50 | NC | NC | NC | NC | NC | NC |
| | 100 | NC | NC | NC | NC | 48.8 | 18.4 |

NC: not calculated

AUC7d, relative (%)

Males

| Item: AUC7d (%) /relative* | Stage | Week 1 | | | Week 4 | | | Week 26 | | |
|----------------------------|-------|--------|-------|-------|--------|-------|-------|---------|-------|-------|
| | | 25 | 50 | 100 | 25 | 50 | 100 | 25 | 50 | 100 |
| OPC-14597 | | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| OPC-14857 | | NC | NC | NC | NC | 0.4 | 3.6 | 1.6 | 1.0 | 9.1 |
| DM-1451 | | 14.1 | 15.2 | 13.8 | 11.1 | 9.2 | 6.5 | 6.9 | 7.6 | 1.8 |
| DM-1452 | | NC | NC | NC | NC | NC | NC | NC | NC | 0.6 |
| OPC-3373 | | NC | NC | NC | NC | NC | 2.2 | 0.8 | NC | 3.2 |
| DCPP | | NC | NC | NC | NC | NC | NC | NC | NC | 2.5 |

*: AUC7d of OPC-14597 was set at 100%. Molar ratio.

NC: not calculated

Females

| Item: AUC7d (%) /relative* | Stage | Week 1 | | | Week 4 | | | Week 26 | | |
|----------------------------|-------|--------|-------|-------|--------|-------|-------|---------|-------|-------|
| | | 25 | 50 | 100 | 25 | 50 | 100 | 25 | 50 | 100 |
| OPC-14597 | | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| OPC-14857 | | NC | 0.6 | 0.9 | NC | 3.9 | 4.5 | 3.9 | 3.1 | 8.0 |
| DM-1451 | | 7.2 | 7.8 | 7.9 | 9.0 | 5.9 | 4.9 | 4.6 | 5.0 | 1.1 |
| DM-1452 | | NC | NC | NC | NC | 0.2 | 0.2 | NC | NC | 0.6 |
| OPC-3373 | | NC | NC | NC | NC | NC | 1.4 | NC | NC | 2.0 |
| DCPP | | NC | NC | NC | NC | NC | NC | NC | NC | 0.8 |

*: AUC7d of OPC-14597 was set at 100%. Molar ratio.

NC: not calculated

Comments:

In hematology and blood chemistry, some drug-related changes were observed in males (at HD) and in females (at MD and HD). However, these effects were toxicologically insignificant since they were small and all or most of the individual values were within the range of the control and placebo control animals, and no corresponding histopathology abnormalities were present.

At the end of the recovery period, HD males still showed lower body weight, decreased food consumption, and slightly increased AST as compared to controls, while HD females showed small decreases in cholesterol and phospholipids. Morphological changes in the female reproductive and mammary tissues were still present at HD, and atrophy of pituitary pars intermedia was still found at HD in both genders. Similar findings in the female reproductive and mammary tissues had been observed in previous oral toxicity studies in rats (Hashimoto K, 1999; Bartek, Kelly 2001; Bartek, 2001 – as cited by the sponsor)⁵, and are likely a consequence of pharmacologically mediated hyperprolactinemia as those seen with other antipsychotics. Atrophy of pars intermedia in the pituitary had also been noted in previous rat studies of OPC-14597. This finding is likely pharmacologically mediated as a consequence of D₂ partial agonistic activity of OPC-14597.

Conclusion:

In conclusion, the weekly intramuscular injections of OPC-14597 depot formulation to rats at maximal dose of 100 mg/kg for a period of 26-weeks resulted in granulomatous inflammation to the deposited drug at the injection site. There was no morphologic evidence of drug-related skeletal muscle necrosis in any of the animals dosed with OPC-14597.

Morphological changes in female reproductive and mammary tissues and atrophy of pituitary pars intermedia in both genders were present at all dose levels, and were likely pharmacologically mediated as a consequence of D₂ partial agonistic activity of OPC-14597. The NOAEL was 50 mg/kg/week in the males and 100 mg/kg/week in the females since low body weight and decreased food consumption were observed in the males given 100 mg/kg, but not in the treated females under the conditions of the present study.

⁵ Hashimoto K. Four-week repeated oral dose toxicity study of OPC-14597 with 4-week recovery test in rats. Otsuka Study No. 014789, Otsuka Report No. 012974, 1999.

Bartek WJ, Kelly WA. BMS-337039: Twenty-six-week oral toxicity study in rats. Otsuka Study No. 017179, Otsuka Report No. 014030, 2001.

Bartek WJ. BMS-337039: Thirteen-week oral investigative study of hormone levels in rats. Otsuka Study No.017178. Otsuka Report No.014029, 2001.

Study title: Fifty-two-week Intermittent Repeated Intramuscular Depot Toxicity Study of OPC-14597 in Beagle Dogs with Twenty-six-week Recovery Test

Study no.: 026483
Study report no.: 023492
Conducting laboratory and location: Tokushima Research Institute
Otsuka Pharmaceutical Co., Tokushima, Japan
Date of study initiation: 10 Oct 2007 (Completion: 29 Sep 2009)
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: OPC-14597 for injectable suspension,
Lot Nos.: 07G94A200 and 07G88A400, purity 99.8% and
100.9 % for OPC-14597/200 and 400 mg /vial,
respectively

Key Study Findings

Administration of aripiprazole depot formulation to beagle dogs (4/sex/group) by weekly IM injections at doses of 0 (saline), 0 (base of depot formulation), 10, 20 and 40 mg/kg of aripiprazole for 52 weeks resulted in localized granulomatous inflammation at the injection site in the males at all dosages and in HD females, and in necrosis of muscle fibers involved in the granulomatous inflammation in 1 HD female at the end of the 52-week dosing period. The inflammation consisted of accumulation of macrophages, eosinophilic deposits, foreign body giant cells, lymphocytes and polymorphonuclear leukocytes, in association with deposits of birefringent crystal-like material (interpreted as drug). The gross- and histopathology changes at the injection site were reversible by the end of the 26-week recovery period. There were no clinical signs, and no drug-related changes in body weight, food consumption, hematology, blood chemistry, urinalysis, ophthalmology, audiology, electrocardiography, body temperature, no drug-related changes in organ weights or in gross- and histopathology of the systemic organs. The NOAEL was 40 mg/kg/week under the conditions of the present study, with mean C_{max} and AUC_{7d} of aripiprazole at Week 52 of 438ng/mL and 2460 ng.d/mL, respectively for males and 306 ng/mL and 1820 ng.d/mL, respectively for females.

Methods

Doses: 0 (saline), 0(0% OPC-14597 solution for injection), 10, 20 and 40 mg/kg of OPC-14597

Frequency of dosing: Weekly

Route of administration: Intramuscular injection in 6 specified areas of each hindlimb (thigh and rump) by weekly rotation

Dose volume:0.2 mL/kg

Formulation/Vehicle: OPC-14597 for injectable suspension (a whole white cake constituted with about 2 mL water for injection / 0% OPC-14597 solution for injection (Lot: 07H85P000) ; Physiological saline

Species/Strain: Dogs/Beagle

Number/Sex/Group: 7 (3 for interim sacrifice, wk 39 +4 for final sacrifice , wk 52)
+2 additional recovery animals at HD and saline control

Age: 8 months at initiation of dosing

Weight: 7.3 - 10.7 kg (M) and 6.2 - 9.7 kg (F)

Satellite groups: Recovery (2 animals/sex at HD and saline control)

Deviation from study protocol: Transient short deviations in animal room ventilation, humidity, air conditioning, and refrigerator temperature due to power failure were not likely to have affected the study results

Study design: (See the following sponsor's table)

| Sex | Group | Dose Level (mg/kg) | Conc. of OPC-14597 (mg/mL) | Dose Volume (mL/kg) | Number of Animals (Animal Nos.) | | |
|--------|-------|--------------------|----------------------------|---------------------|---------------------------------|----------------------|-------------------------------|
| | | | | | Week 39 ^a | Week 52 ^b | Recovery Week 26 ^c |
| Male | S0 | 0 | 0 | 0.2 | 3 (00001-00003) | 4 (00004-00007) | 2 (00008,00009) |
| | A0 | 0 | 0 | 0.2 | | 4 (00010-00013) | |
| | A10 | 10 | 100 | 0.1 | 3 (00014-00016) | 4 (00017-00020) | |
| | A20 | 20 | 100 | 0.2 | 3 (00021-00023) | 4 (00024-00027) | |
| | A40 | 40 | 200 | 0.2 | 3 (00028-00030) | 4 (00031-00034) | 2 (00035,00036) |
| Female | S0 | 0 | 0 | 0.2 | 3 (00037-00039) | 4 (00040-00043) | 2 (00044,00045) |
| | A0 | 0 | 0 | 0.2 | | 4 (00046-00049) | |
| | A10 | 10 | 100 | 0.1 | 3 (00050-00052) | 4 (00053-00056) | |
| | A20 | 20 | 100 | 0.2 | 3 (00057-00059) | 4 (00060-00063) | |
| | A40 | 40 | 200 | 0.2 | 3 (00064-00066) | 4 (00067-00070) | 2 (00071,00072) |

The animals of S0- and A0-groups were given physiological saline and 0% OPC-14597 solution, respectively.

^a The animals were terminated at the end of the 39-week dosing period.

^b The animals were terminated at the end of the 52-week dosing period.

^c The animals were terminated at the end of the 26-week recovery period.

Based on the results of a 26-week study (weekly doses of 10, 20 and 40 mg/kg), the 52-week dog study employed weekly IM injections of aripiprazole depot formulation at doses of 10, 20 and 40 mg/kg of aripiprazole, at a dose volume of 0.2 mL/kg (0.1 mL/kg for LD). Two control groups: saline and placebo (base of depot formulation, 0% OPC-14597 solution for injection) were used. Injection sites: the control or test articles were administered to 6 specified areas of each hindlimb (thigh and rump) by weekly rotation. Interim sacrifice was performed in Week 39. The reversibility of toxicity was examined after a 26-week treatment-free recovery period after 52 weeks of dosing, using an additional 2 animals of each sex (control and HD groups only). Plasma concentrations of OPC-14597 and its metabolites were determined at the end of the dosing period.

Rationale for Dose Selection

OPC-14597 injectable suspension was formulated to achieve final concentrations of 100 and 200 mg/mL. The maximal possible dose volume for the IM injection of OPC-14597 was 0.1 mL/kg/site, and weekly administration of a total dose volume of 0.2 mL/kg as 2 injections (0.1 mL/kg/site) into the right or left hindlimb was used successfully in the preliminary 4-week study. Since weekly IM administration of 0.2 mL/kg (0.1 mL/kg/site x 2 injections) of 200 mg/mL suspension at a maximal dose of 40 mg/kg was well tolerated in the 26-week study, 20 and 40 mg/kg of OPC-14597 were selected as MD and HD in the present study, injected weekly as 100 and 200 mg/mL suspensions at a total dose volume of 0.2 mL/kg (2 injections of 0.1 mL/kg/site). For the low dose of 10 mg/kg, 100 mg/mL suspension was administered weekly as a single injection of 0.1 mL/kg.

Observations: General condition including injection site (once daily), body weight (once weekly from Day 1 to Week 13, every 2 wks up to week 25, every 4 wks up to week 49, and also at weeks 39 and 52, and every 4 weeks during the recovery period. Food consumption (weekly); hematology and clinical chemistry (weeks 26, 39 and 51, and recovery week 26); urinalysis for pH, protein, glucose, ketones, bilirubin, occult blood and urobilinogen, and urine sediment (weeks 27, 38 and 50, and recovery week 26), ophthalmoscopy (weeks 27, 39 and 52, and recovery week 26), hearing tests (HD only) once before dosing, treatment weeks 27, 39 and 52, and recovery week 26.

Electrocardiography was performed on each animal under conscious conditions at predosing and at 24 hours post-dosing at Weeks 1, 26, 39 and 52, and at recovery week 26 (heart rate, P-, R-, and T-wave amplitudes, ST-segment amplitude, PR interval, QRS width, QT interval, QTc (correction by Van de Water, $QTc = QT - 87(RR - 1)$);

Necropsy, organ weights, gross pathology and histopathology: the following organs or tissues of all animals including a moribund sacrificed control female were fixed in 10% neutral buffered formalin: liver, gallbladder, kidneys, thymus, mandibular lymph nodes, medial retropharyngeal lymph nodes, mesenteric lymph nodes, popliteal lymph nodes, spleen, heart, aorta, lungs and bronchi, trachea, larynx, esophagus, submaxillary glands, sublingual glands, parotid glands, zygomatic glands, tongue, stomach, duodenum, jejunum, ileum (including Peyer's patch), cecum, colon, rectum, pancreas, urinary bladder, ureters, prostate, epididymides, ovaries, oviducts, uterus, uterine cervix, vagina, pituitary gland, thyroid glands, parathyroid glands, adrenal glands, skin, mammary glands, skeletal muscle (brachial biceps), brain, spinal cord (thoracic), sciatic nerve, optic nerve, lacrimal glands, injection site, sternum and femur (with marrow), and stifle joint (articular capsule, femoral trochlea). The treated tissues were stained with hematoxylin and eosin; Periodic acid-Schiff (PAS) staining was used to examine kidneys and testes of all dogs. Light microscopy was performed on all specimens. Additionally, unstained frozen sections were prepared from the formalin fixed injection site (R4), obtained from 2 males and 2 females of the treated groups at the end of the 39- and 52-week of dosing and of all HD animals at the end of the recovery period, and were examined microscopically with polarized light.

Toxicokinetics:

Plasma concentrations of OPC-14597 and its metabolites were determined in blood samples collected from dosed animals on weeks 1 and 26 (at 0.25 and 1 day after administration), weeks 39 and 52 (at pre-dosing and at 0.25, 1, 3 and 7 days after administration), and on recovery weeks 2, 4, 8, 12, 16, 20 and 26. In control and placebo control animals, only the concentration of OPC-14597 was determined.

The following toxicokinetic parameters were determined on Weeks 39 and 52: C_{max} , T_{max} , and area under the plasma concentration versus time curve from 0 to 7 days (AUC_{7d}). The AUC_{7d} was calculated based on the plasma concentrations at each time point using the trapezoidal rule.

Results

Mortality: No drug-related deaths occurred throughout the dosing period (1 control female receiving physiological saline was euthanized due to sudden deterioration associated with heart failure at Week 51).

Clinical Signs: There were no clinical signs associated with pain due to the IM administration of the depot formulation throughout the dosing period. The only drug-related finding at the injection site was the expected finding of a subcutaneous nodule (raised area containing deposits

of the test article) occasionally observed after injection in the males at all dosages and in the HD females.

There were no drug-related effects on body weight, food consumption, hematology, blood biochemistry, urinalysis, ophthalmology, audiology, electrocardiography, body temperature, organ weights, gross and microscopic examination of the systemic organs.

Body Weights: During the 52-week dosing period, there were no statistically significant changes in body weights or body weight gains of the treated groups in comparison to control for both sexes. A lower, though not statistically significant and non-dose-dependent, body weight gain was registered in treated females in all dosed groups, likely related to a corresponding (not statistically significant) decrease in food consumption. No statistically significant difference in body weights or body weight gains was observed between the placebo control and saline control groups, except for a lower body weight gain in the male placebo control group at Week 17.

Feed Consumption:

During the 52-week dosing period, statistically significant lower food consumption was occasionally observed in the treated groups when compared to control groups. The overall food intakes during the 39-week dosing period in the LD, MD and HD groups were respectively 85, 84 and 87% of the control group in males, and 80, 83 and 85% of the control group in the females, and those during the 52-week dosing period were respectively 84, 83 and 83% of the control group in males, and 75, 72 and 77% of the control group in females. No statistically significant difference was observed in the food consumption between the saline control and placebo control groups.

Ophthalmoscopy: No drug-related changes.

Conjunctival congestion was observed in control, placebo control and treated animals; but there was no increase in incidence in relation to either the dose level or the length of the dosing period. No changes in eyelids, nictating membrane, cornea, sclera, iris, medial portion, or fundus of both eyes were observed in any individuals of the control, placebo control, and treated groups.

Hematology: There were no toxicologically meaningful drug-related changes.

Decreased count of basophils was found in HD females at Week 51. However, the changes were small, and the individual values were almost within the variation observed in the control animals. A small decrease in MCHC was noted in HD males at Week 51 compared to control, but the comparison to the pre-value showed an increase. Some other parameters showed statistically significant changes in treated groups vs. control group and/or to the pre-values, but these changes were not related to either the dose level or the length of the dosing period.

Clinical Chemistry: There were no toxicologically significant drug-related changes.

Decreased aspartate aminotransferase (AST) was registered in both males and females at all dose levels on Weeks 26, 39 and 51; a decreased α 1-globulin content was noted at all dosages in the males (Week 51) and in females (Weeks 26, 39 and/or 51), but the individual decrease below the pre-value at Week 51 was almost within the range of the control animals. Decreased β -globulin content was seen in LD and HD females at Week 51, but all individual decreases vs. pre-values were within the fluctuation of the control animals, and the actual values were comparable to those observed in the control animals. Transiently increased Na was noted in both genders at HD in Week 39, but it was not observed at Week 51. Some other parameters showed statistically significant changes when compared to the values in the control group and/or to the pre-values,

but changes observed were not related to either the dose level or the length of the dosing period. The biological significance of the slight decreases in AST observed at all doses in the males and females was unclear, but without any other blood biochemical change it is likely to be of no toxicological importance

Urinalysis: No drug-related changes

EKG:

During the dosing period, some statistically significant differences were observed in the treated groups; however none of these changes were of toxicological significance.

Heart rate: there were statistically significant changes in the treated groups in comparison to control group and/or to the pre-value, but the changes observed were not related to either the dose level or the length of the dosing period. No difference was observed between the control and placebo control groups.

Heart rate (beats/min)

Sex: Male

| Dose Stage | Time | S0 Mean±S.D. n | A0 Mean±S.D. n | A10 Mean±S.D. n | A20 Mean±S.D. n | A40 Mean±S.D. n |
|------------|------|----------------------|----------------------|-----------------------|-----------------------|-----------------------|
| Week1 | Pre | 106.0±22.5 9 | 120.0±11.5 4 | 123.0±16.1 7 | 106.9±25.3 7 | 90.8±11.9 9 |
| | 24hr | 104.4±22.5 9 | 107.5±10.3 4 | 123.4±12.9 7 | 109.6±24.8 7 | 99.2±7.4 9 |
| Week26 | Pre | 94.6±12.3 9 | 95.5±6.4 4 | 127.1±11.6** 7 | 111.0±22.1 7 | 104.1±22.8 9 |
| | 24hr | 95.6±19.3 9 | 96.8±6.4 4 | 120.6±19.7 7 | 118.1±20.5 7 | 108.1±17.8 9 |
| Week39 | Pre | 94.4±19.1 9 | 95.8±13.1 4 | 124.0±19.6* 7 | 114.1±15.8 7 | 104.9±22.2 9 |
| | 24hr | 95.4±19.5 9 | 89.0±3.7 4 | 117.9±21.0 7 | 117.1±19.9 7 | 105.6±17.2 9 |
| Week52 | Pre | 103.2±12.8 6 | 108.0±20.4 4 | 124.3±15.1 7 | 113.8±16.0 4 | 105.7±8.7 6 |
| | 24hr | 100.3±19.1 6 | 88.3±10.0 4 | 122.3±16.7 4 | 119.5±17.5 4 | 108.7±18.3 6 |
| R. Week26 | | 94.0±18.4 2 | | | | 80.5±10.6 2 |

Sex: Female

| | | | | | | |
|-----------|------|-----------------|-----------------|------------------|-----------------|------------------|
| Week1 | Pre | 112.1±22.3 9 | 112.0±21.0 4 | 105.4±17.6 7 | 96.9±9.5 7 | 107.8±23.7 9 |
| | 24hr | 106.8±10.5 9 | 97.8±5.4 4 | 109.0±17.5 7 | 100.7±14.7 7 | 116.8±22.0 9 |
| Week26 | Pre | 107.9±20.5 9 | 98.5±2.1 4 | 125.3±11.9 7 | 103.6±19.4 7 | 115.1±22.6 9 |
| | 24hr | 102.0±19.0 9 | 95.5±15.9 4 | 117.4±12.6 7 | 107.1±19.9 7 | 120.3±21.2 9 |
| Week39 | Pre | 92.0±19.8 9 | 87.8±9.7 4 | 110.9±20.7 7 | 99.6±10.4 7 | 104.0±21.1 9 |
| | 24hr | 94.1±13.6 9 | 103.0±9.2 4 | 117.6±9.3** 7 | 108.9±13.9 7 | 113.6±16.1* 9 |
| Week52 | Pre | 101.6±25.0 5 | 92.0±17.9 4 | 100.5±15.5 4 | 102.5±17.2 4 | 107.2±21.6 6 |
| | 24hr | 99.8±14.6 5 | 91.0±9.5 4 | 109.0±7.7 4 | 107.8±14.2 4 | 114.0±18.2 6 |
| R. Week26 | | 109.0± 1 | | | | 132.0±4.2 2 |

S0 : 0mg/kg A0 : Placebo A10 : 10mg/kg A20 : 20mg/kg A40 : 40mg/kg
 * : P<0.05 , ** : P<0.01 : Significant difference from control
 @ : P<0.05 , @@ : P<0.01 : Significant difference from the pre-value (Week1)
 Statistical analysis were conducted between the control group (S0) and each treated group (A10, A20 and A40) and between the control (S0) and placebo control (A0) groups.

P-wave amplitude: some treated and placebo control animals showed increased P-wave amplitude (> 4.0 mV), but the changes over the pre-value at Week 1 were all within the range of the control animals.

R-wave amplitude: Individually, high R-wave amplitude (> 3.0 mV) was occasionally recorded in some treated animals, but similar high R-wave amplitude was noted before the initiation of administration or in the control animals.

ST-segment amplitude: There were no statistically significant changes in any treated group during the dosing period vs. control. In comparison to the pre-value, a statistically significant increase was seen in HD males but the changes were within the fluctuation observed in the placebo control group. Statistically significant increase was also noted in LD females at Week

52, but was not observed in either MD or HD females. No difference was observed between the control and placebo control groups.

QTc: There was no statistically significant change in any treated groups vs. control during the dosing period. In comparison to the pre-value, there was a statistically significant decrease in HD females at Week 39, but no change was observed at Week 52. Individually, the treated animals showed no abnormal changes. No difference was observed between the control and placebo control groups.

Cardiac rhythm: One HD male showed second degree atrio/ventricular block at all time points during the 39-week dosing period (including pre-dosing at Week 1). In addition, 2nd degree block was also noted in 1 HD male at Week 39, 1 HD female before dosing on Day1, and 1 control female at Week 52. At Recovery Week 26, there were no treatment-related changes in any parameters examined in any of the high dose animals.

Gross Pathology:

At necropsy, white foci in the muscles at the injection sites were observed at the end of both 39- and 52-week dosing periods in all animals dosed with OPC-14597 suspension at all dose levels. These foci were of variable shapes and sizes that ranged from 1 × 1 mm to 77 × 40 mm. They receded with time and at the end of the 26-week recovery period, there were no gross pathology changes at the injection site in any of the HD animals.

The gross examination of the systemic organs did not show any drug-related changes. Involution of the thymus and focal changes of the urinary bladder, lungs and bronchus, heart, jejunum, gallbladder, liver and pituitary were observed in several treated animals, but were of low incidences not dose-related, or usually observed in intact dogs. In 1 control female (00044), euthanized at Week 51, white foci in the left ventricle including the apex and papillary muscle and multiple scattered red foci on the endocardial surface of the left ventricle were evident.

Organ Weights

No drug-related changes were observed in any treated groups compared to control at the end of the 39-week dosing period and no treated animals showed any individual abnormal changes. At the end of the 52-week dosing period, decreased relative weight of the liver was noted in the HD males, but with no change in the absolute weight. In MD females, increases in the absolute and relative weights of the uterus were noted, but with no change in HD females.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histopathology Findings:

In all treated animals, the grossly observed white foci at the injection site were characterized microscopically by localized granulomatous inflammation consisting of accumulation of macrophages, eosinophilic deposits, foreign body giant cells, lymphocytes and polymorphonuclear leukocytes, in association with deposits of birefringent crystal-like material. Examination with polarized light of selected unstained frozen sections, obtained from 2 male and 2 female animals of the treated groups revealed the presence of birefringent crystal-like material (interpreted as drug) within the foci of granulomatous inflammation at the injection sites.

Necrosis of muscle fibers was noted in 1 HD female at the end of the 52-week dosing period; it was limited to the muscle fibers involved in the granulomatous inflammation, possibly as a consequence of focal ischemia secondary to granulomatous inflammation since interstitial tissues including vessels in the necrotic muscles also underwent necrosis. At the end of the 26-week recovery period, there were no gross- and histopathology changes at the injection site in any of the HD animals.

Other findings at the injection site included regeneration of muscle fibers in 1 male and 1 female of the placebo control group, 2 males and 2 females of LD group, 3 MD females 1 HD male. The microscopic examination of the systemic organs did not show any drug-related changes.

Toxicokinetics

The toxicokinetic parameters of plasma concentrations of OPC-14597 and its metabolites are summarized in the following sponsor's tables.

C_{max} (ng/mL)

| Substance-determined | Dose Level (mg/kg) | Week 39 | | Week 52 | |
|----------------------|--------------------|---------|--------|---------|--------|
| | | Male | Female | Male | Female |
| OPC-14597 | 10 | 61.7 | 58.6 | 66.8 | 60.6 |
| | 20 | 135 | 102 | 155 | 109 |
| | 40 | 377 | 336 | 438 | 306 |
| OPC-14857 | 10 | 15.6 | 13.1 | 18.4 | 14.0 |
| | 20 | 39.4 | 33.7 | 59.6 | 35.9 |
| | 40 | 123 | 137 | 163 | 122 |
| DM-1451 | 10 | < 1.00 | < 1.00 | < 1.00 | < 1.00 |
| | 20 | < 1.00 | 1.65 | 0.838 | 1.22 |
| | 40 | 3.27 | 2.29 | 2.86 | 1.84 |
| DM-1452 | 10 | 6.07 | 5.40 | 7.70 | 6.20 |
| | 20 | 15.4 | 13.2 | 25.1 | 15.2 |
| | 40 | 50.4 | 57.4 | 64.4 | 47.6 |
| OPC-3373 | 10 | 1.36 | 1.85 | 1.53 | 1.59 |
| | 20 | 3.21 | 4.92 | 3.36 | 5.10 |
| | 40 | 10.4 | 11.0 | 13.8 | 11.4 |
| DCPP | 10 | 0.285 | < 1.00 | < 1.00 | < 1.00 |
| | 20 | 1.41 | 1.44 | 1.99 | 1.46 |
| | 40 | 6.76 | 5.16 | 8.71 | 5.33 |

AUC_{7d} (ng · d/mL)

| Substance-determined | Dose Level (mg/kg) | Week 39 | | Week 52 | |
|----------------------|--------------------|---------|--------|---------|--------|
| | | Male | Female | Male | Female |
| OPC-14597 | 10 | 388 | 354 | 400 | 357 |
| | 20 | 815 | 607 | 833 | 693 |
| | 40 | 2300 | 2010 | 2460 | 1820 |
| OPC-14857 | 10 | 97.3 | 73.0 | 107 | 83.5 |
| | 20 | 236 | 187 | 276 | 217 |
| | 40 | 781 | 807 | 941 | 723 |
| DM-1451 | 10 | NC | NC | NC | NC |
| | 20 | NC | 6.93 | 1.71 | 5.77 |
| | 40 | 16.0 | 10.3 | 15.0 | 8.53 |
| DM-1452 | 10 | 37.0 | 28.7 | 43.3 | 37.3 |
| | 20 | 90.1 | 71.3 | 116 | 93.0 |
| | 40 | 317 | 339 | 349 | 290 |
| OPC-3373 | 10 | 6.62 | 8.69 | 7.54 | 9.11 |
| | 20 | 17.9 | 26.9 | 17.3 | 29.6 |
| | 40 | 63.6 | 65.8 | 70.7 | 60.1 |
| DCPP | 10 | 0.393 | NC | NC | NC |
| | 20 | 5.78 | 6.57 | 8.42 | 6.27 |
| | 40 | 38.1 | 30.7 | 47.0 | 26.2 |

NC: Not calculated

Changes in Plasma Concentrations of the High Dose Animals - Recovery Period

| Sex | Substance-determined | Recovery Week | | | | | | |
|--------|----------------------|---------------|--------|--------|--------|--------|--------|--------|
| | | 2 | 4 | 8 | 12 | 16 | 20 | 26 |
| Male | OPC-14597 | 115 | 71.0 | 36.3 | 12.8 | 4.53 | < 1.00 | < 1.00 |
| | OPC-14857 | 39.0 | 19.4 | 6.67 | 2.20 | < 1.00 | < 1.00 | < 1.00 |
| | DM-1451 | 0.885 | < 1.00 | < 1.00 | < 1.00 | < 1.00 | < 1.00 | < 1.00 |
| | DM-1452 | 14.7 | 7.23 | 2.36 | 0.51 | < 1.00 | < 1.00 | < 1.00 |
| | OPC-3373 | 3.34 | 2.03 | < 1.00 | < 1.00 | < 1.00 | < 1.00 | < 1.00 |
| | DCPP | 1.61 | < 1.00 | < 1.00 | < 1.00 | < 1.00 | < 1.00 | < 1.00 |
| Female | OPC-14597 | 126 | 94.5 | 40.3 | 16.0 | 6.65 | 0.78 | < 1.00 |
| | OPC-14857 | 42.2 | 19.3 | 4.22 | 1.74 | < 1.00 | < 1.00 | < 1.00 |
| | DM-1451 | < 1.00 | < 1.00 | < 1.00 | < 1.00 | < 1.00 | < 1.00 | < 1.00 |
| | DM-1452 | 19.2 | 9.05 | 1.92 | 0.66 | < 1.00 | < 1.00 | < 1.00 |
| | OPC-3373 | 2.92 | 1.41 | < 1.00 | < 1.00 | < 1.00 | < 1.00 | < 1.00 |
| | DCPP | 1.48 | < 1.00 | < 1.00 | < 1.00 | < 1.00 | < 1.00 | < 1.00 |

The mean C_{max} and AUC from 0 to 7 days (AUC_{7d}) of OPC-14597 and its metabolites at Week 52 were dose-related for both sexes, and were comparable to those at Week 39. The C_{max} and AUC_{7d} of OPC-14597 were higher than those of the metabolites. No remarkable sex differences were observed in the plasma concentrations of OPC-14597 and its metabolites. In the control and placebo groups, the results of all analyses for OPC-14597 were below the lower limit of quantification (< 1.00 ng/mL). During the recovery period, the plasma concentrations of OPC-14597 in the animals given 40 mg/kg slowly decreased, and were below the lower limit of quantification (< 1.00 ng/mL) at the end of the 26-recovery period.

Conclusion: The 52-week weekly IM administrations of the depot formulation of OPC-14597 at a maximal dose of 40 mg/kg were well tolerated and resulted in foreign-body type of granulomatous inflammation to deposited drug at the injection site with no significant muscle injury. No systemic toxicity was observed up to 40 mg/kg. Thus, the NOAEL was 40 mg/kg/week under the conditions of the present study, with mean C_{max} and AUC_{7d} of aripiprazole at Week 52 of 438 ng/mL and 2460 ng.d/mL, respectively for males and 306 ng/mL and 1820 ng.d/mL, respectively for females.

Study title: One-month intramuscular toxicity study in monkeys

Study no.: 023183
 Study report no.: 017831
 Conducting laboratory and location: Bristol-Myers Squibb Pharmaceutical Research Institute
 Departments of Toxicology and Pathology
 Syracuse, New York USA
 Date of study initiation: April 6, 2004 (first day of dosing)
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: Aripiprazole Lot No. R4217, purity 100.5 %

Key Study Findings

Intramuscular administration of aripiprazole at doses of 2, 4, or 7.5 mg/kg to Cynomolgus monkeys (5/sex/dose group) once daily for 29 days, resulted, at all doses, in CNS-related clinical signs (reduced activity, most likely pharmacologically mediated) and reversible skeletal muscle injury at the injection site. Small increases in serum aspartate aminotransferase at MD and HD

were likely a consequence of injection site injury. Microscopically, increased incidence and/or severity of skeletal muscle necrosis (minimal to mild), degeneration (mild to moderate), and regeneration (mild to moderate) were observed at injection sites at all doses, while injection site changes associated with the control article and/or intramuscular injection procedure were generally minimal in severity. All changes at the injection site showed evidence of reversibility. Following a 1-month postdose recovery period, there was no residual fibrosis at the injection sites of monkeys given either the control article or aripiprazole formulations. Exposures to aripiprazole and its pharmacologically active dehydro-metabolite (BMS-337044) were dose-proportional with no apparent sex differences. Small accumulation in systemic exposure occurred upon repeated dosing for 29 days. A NOAEL was not reached in this study (< the lowest tested IM dose of 2 mg/kg/day) since injection site skeletal muscle injury was present at all doses.

Methods

Doses: 0, 2, 4, and 7.5 mg/kg OPC-1459

Frequency of dosing: once daily for 29 days.

Route of administration: Intramuscular injection

Dose volume: 0.265, 0.53, or 1 ml/kg/day of a 7.5 mg/ml formulation for LD, MD and HD groups, respectively; 1 ml/kg/day of the control article for the control group.

Daily doses that exceeded 2 ml were divided and administered at separate sites in the muscle so that no more than 1.5 ml of formulation was delivered at any one site.

The injection sites were alternated daily between the right and left thighs.

Formulation/Vehicle: Test article carrier and control article (vehicle): 15% w/v Captisol (sulfobutylether- β -cyclo-dextrin) and 50 mM tartrate buffer (pH 4.5) in Sterile Water for Injection, USP

Species/Strain: Monkeys/Cynomolgus

Number/Sex/Group: 5

Age: 21- 39 months

Weight: 2.0 - 2.8 kg (males) and 2.0 - 2.6 kg (females)

Satellite groups: No

Deviation from study protocol: Transient short deviations were not likely to have affected study results

Experimental Design:

| Group Number | Daily Dose BMS-337039 (mg/kg) | Volume (ml/kg) | Concentration of BMS-337039 (mg/ml) | Number of Animals |
|--------------|-------------------------------|----------------|-------------------------------------|-------------------|
| 1 | 0 ^a | 1 | 0 | 5 M, 5 F |
| 2 | 2 | 0.265 | 7.5 | 5 M, 5 F |
| 3 | 4 | 0.53 | 7.5 | 5 M, 5 F |
| 4 | 7.5 | 1 | 7.5 | 5 M, 5 F |

a: 15% Captisol® and 50 mM tartrate buffer (~pH 4.5) in Sterile Water for Injection (USP).

Observations: survival, clinical signs (twice daily), body weight (pretest and at least once each week), food consumption (daily), physical examinations (including neurologic and respiratory function) and ophthalmology (prior to study inception and on days 23 and 57), hematology, clinical chemistry (blood samples collected prior to the first dose, during week 4, and during post-dose period), urine analysis (urine collected over an approximate 18-hour period pretest,

during week 4, and during post-dose period), and gross and histopathology examinations. Scheduled necropsies were conducted on day 30 for 2 or 3 animals/sex/group (depending on survival), and the remaining animals were necropsied on day 57 (at the end of a 1-month post-dose recovery period).

Electrocardiography: pre dosing and on days 23 and 49 or 50. Electrocardiogram tracings were assessed for heart rate, durations of the P wave, PR interval, QRS complex, QT, QTc interval (Fridericia method for correction), ST segment, and amplitudes of the P wave, R wave and T wave.

Necropsy, organ weights, gross pathology and histopathology: A complete necropsy was conducted on all animals and included gross examination and collection of standard tissues/organs. Bone-marrow smears of rib were collected from all animals at scheduled necropsies but were not examined.

Representative samples of each organ and tissue and all suspected gross lesions were collected and fixed in 10% neutral buffered formalin (except eyes which were fixed in 5% glutaraldehyde), sectioned, stained with hematoxylin and eosin, and examined by light microscopy. Gomori's iron reaction for hemosiderin and Schmorl's method for lipofuscin were used to determine the nature of the pigment present in the mesenteric lymph nodes in 2 animals. For post-dose recovery animals, only injection sites and gross lesions were examined.

Toxicokinetics: plasma concentrations of aripiprazole and its dehydro-metabolite BMS-337044 were determined at each dose level on days 1 and 29. Blood samples were collected from all drug-treated animals at approximately 10 and 30 min, and 1, 3, 8, and 24 hr after dosing. A comparable blood sample volume was collected from control animals and discarded. A validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) method was used with a lower limit of quantitation (LLQ) for aripiprazole and dehydro-aripiprazole of 1 ng/mL.

Results

Mortality: There was no drug-related mortality. A low-dose male had an emetic episode immediately after being given its daily oral dietary supplement; the monkey died shortly thereafter, and gross findings of white fluid in the trachea along with the histology finding of diffuse pulmonary fluid, confirmed that the death of this animal was likely due to aspiration of the dietary supplement.

Clinical Signs: Decreased activity and tremors at all doses (reversible and dose-related), attributable to pharmacology. Decreased activity occurred in all drug-treated animals with higher frequency at MD and HD; it was not observed in any monkeys during the 1-month recovery period. Tremors occurred with low incidence at LD, and with higher incidence and frequency at MD and HD; they decreased in frequency during the last 2 weeks of dosing, and were not observed in any animals during the 1-month recovery period); One MD male had a tonic convulsion on day 2. As no other convulsions were observed in the study, this single event was not likely to be drug-related in view of the lack of dose relationship and that it was observed prior to dosing.

Incidences of dehydration and "thin appearance" were seen at MD and HD, possibly secondary to decreased activity and food consumption. These signs were reversible.

Injection site: Scabbing, swelling and red discoloration at the injection site was seen at all doses and vehicle control; the number of days in which these findings were observed in affected animals was dose-dependent (see the sponsor's table below). These changes were completely reversible following the 1-month post-dose recovery period.

Injection-Site Findings in Monkeys

| Group | Red Discoloration | Swelling | Scabbing |
|---------------------|--------------------------|-----------------|-----------------|
| 1 (Vehicle Control) | 10 (6) | 10 (17) | 10 (4) |
| 2 (2 mg/kg/day) | 8 (8) | 10 (11) | 10 (7) |
| 3 (4 mg/kg/day) | 10 (7) | 10 (15) | 10 (12) |
| 4 (7.5 mg/kg/day) | 10 (11) | 10 (19) | 10 (19) |

a: Data are tabulated as the number of males and females affected (10/group) at least once during the dosing phase (days 1-29), with the average number of days in which the finding was observed in the affected animals, rounded to the nearest integer, in parentheses. Note: Values are not adjusted for survival.

Body Weight: There were no drug-related effects on body weight.

Feed Consumption: A reversible and dose-dependent decrease of food consumption occurred at all doses; mean food consumption during the dosing phase (days 1-29) was 22-28% lower than control at LD, and 51-59% lower than control at MD and HD. The drug-related decrease of food consumption was likely secondary to the pharmacologic activity of aripiprazole and occurred in all drug-treated animals except for 2 LD males. During the dosing phase (days 1-29), mean food consumption of males at LD, MD and HD was 22%, 59%, and 51% lower than control, respectively. Corresponding values in the females were 28%, 54%, and 58% lower than control, respectively.

Beginning on day 4 (females) or day 5 (males), each animal (including controls) was given a single daily 30 ml oral dose of Boost, a liquid nutritional supplement (dose increased to 45 ml 2 days later, until discontinuation during the first week of the recovery period), to prevent possible deterioration of the animals' condition secondary to the reduced food consumption.

Due to this nutritional supplementation, there was no adverse effect on body weight, despite the relatively large drug-related decreases of food consumption.

During the 1-month recovery period, food consumption recovered to pretest values,

Ophthalmoscopy: No drug-related changes.

Hematology:

There were no direct drug-related effects on hematology or coagulation parameters. Significant minimal increases in mean absolute (110%) and relative (124%) reticulocyte counts were seen in HD males and comparable increases in reticulocytes were evident in females at all doses, on an individual animal basis. These changes were likely a consequence of hemorrhage following the IM dosing procedure. A statistically significant minimal increase in the mean neutrophil count (128%) in HD males was not drug-related because the mean and individual values were comparable to pre-test. The statistically significant minimal decreases in mean prothrombin times in males at MD and HD (5 and 6%, respectively) and the statistically significant increased (30%) fibrinogen in females at HD were not of toxicology concern because both mean and individual values were within historical control reference ranges for the testing laboratory. The statistically significant minimal increases in platelet counts in females at LD and MD (49 and 43%, respectively) were also likely incidental since there was no similar change at HD.

Following a 1-month recovery period, the mean and individual values for hematology and coagulation parameters were comparable in animals from control and treated groups.

Clinical Chemistry:

Drug-related serum chemistry changes included statistically significant small decreases in serum gamma glutamyl transferase (GGT) in males at MD and HD (37 and 29%, respectively) and increases in serum aspartate aminotransferase (AST) in females at MD and HD (99 and 98%, respectively) and in 1 HD male (195%). These small increases in serum AST are attributable to skeletal muscle injury at the injection sites. Statistically significant minimal increases in serum sodium (2%) and alanine aminotransferase (53%) in LD males are attributable to biological variation because of the absence of a dose relationship and/or because values were comparable to pretest.

Following a 1-month recovery period, the mean and individual values of serum chemistry parameters were comparable in animals from control and treated groups.

Urine analysis: There were no drug-related changes. A minimal decrease in mean urine pH in HD females was not likely to be drug related as the mean and individual values were similar to pretest. Following a 1-month recovery period, mean and individual values for urinalysis parameters were comparable in animals from control and treated groups.

EKG: There were no significant abnormalities detected in any group compared to pre-dose electrocardiograms. Quantitative assessments did not identify any abnormalities in EKG parameters including QT interval.

Gross Pathology:

End-of-Dose Necropsy: Red or dark discoloration in the region of the injection site, interpreted to be hemorrhage, occurred in 1 MD male, 2 MD females and 2 HD females. Although these findings were not evident macroscopically in controls, microscopic evaluation demonstrated hemorrhage in the subcutis at the injection site in control and treated groups that was generally comparable in incidence and severity, suggesting that the red or dark injection site discoloration was likely a result of the IM injection procedure and not a direct drug-related effect.

Postdose Necropsy: There were no drug-related macroscopic findings.

Organ Weights:

End-of-Dose Necropsy: There were no drug-related organ weight changes. Statistically significant increases in mean absolute and relative heart weights (46% and 36%, respectively) were registered in LD females and in mean relative spleen weight (21%) in MD females, but similar changes were not observed at higher doses and, therefore were unrelated to treatment.

Post-dose Necropsy: There were no drug-related organ weight changes.

Histopathology

Adequate battery: Yes

Peer Review: Yes

Histopathology Findings:

End-of-Dose Necropsy: Drug-related findings were limited to the injection site and are described in the following sponsor's table.

| Incidence of Selected Microscopic Findings at the Injection Sites for End-of-Dose Animals | | | | |
|--|------------|------------|------------|------------|
| Dose (mg/kg/day): | 0 | 2 | 4 | 7.5 |
| No. of monkeys (M/F): | 3/3 | 2/3 | 3/3 | 3/3 |
| Sex: | M/F | M/F | M/F | M/F |
| <u>Necrosis, Skeletal Muscle:</u> | | | | |
| Minimal severity | 2/1 | -/- | 1/1 | 3/3 |
| Mild severity | -/- | -/2 | -/2 | -/- |
| <u>Degeneration, Skeletal Muscle:</u> | | | | |
| Minimal severity | 3/2 | 1/1 | 1/2 | 2/2 |
| Mild severity | -/1 | 1/2 | 2/1 | -/1 |
| Moderate severity | -/- | -/- | -/- | 1/- |
| <u>Regeneration, Skeletal Muscle:</u> | | | | |
| Minimal severity | 3/2 | -/2 | -/1 | -/- |
| Mild severity | -/- | -/1 | 2/1 | 3/3 |
| Moderate severity | -/- | -/- | -/1 | -/- |
| <u>Inflammation, Subacute:</u> | | | | |
| Minimal severity | 2/3 | 2/2 | 1/1 | 2/1 |
| Mild severity | -/- | -/- | 1/2 | 1/1 |
| <u>Fibroplasia/Fibrosis:</u> | | | | |
| Minimal severity | 2/2 | -/1 | -/1 | 1/3 |
| Mild severity | -/- | -/1 | -/- | 2/- |
| <u>Hemorrhage:</u> | | | | |
| Minimal severity | 2/2 | 1/3 | 2/3 | 3/3 |
| Mild severity | -/- | 1/- | -/- | -/- |
| Moderate severity | -/- | -/- | 1*/- | -/- |
| <u>Edema</u> | | | | |
| Minimal severity | -/2 | -/- | -/1 | 1/2 |

* Morphologic features of these findings were considered likely due to the anesthetic (Ketamine) administration at the injection site prior to necropsy.

The microscopic injection site changes were present in both control article- and aripiprazole-treated groups and included skeletal muscle necrosis, degeneration, and regeneration; subacute inflammation, fibroplasia/fibrosis, hemorrhage, and edema. These changes were generally minimal in severity in the control article group. In the dosed groups, there was evidence of drug-related exacerbation of these changes as indicated by: increased incidences and/or severity of minimal to mild skeletal muscle necrosis at all doses; degeneration (mild to moderate) and regeneration (mild to moderate). Mild subacute inflammation at the injection sites was observed at MD and HD, and an increased incidence and severity of minimal to mild fibroplasia/fibrosis, at HD. The hemorrhage tended to be in the subcutis and/or superficial layers of the skeletal muscle, indicating that it was likely due to the IM injection procedure. Acute and subacute inflammation and skeletal muscle degeneration and regeneration in the adjacent muscle and sciatic nerve were observed in a few animals in control and treated groups and were considered by the sponsor to be an extension of the injection site reaction. The injection site changes in this study were consistent with those observed at the same doses in a previous 2-week IM study in

monkeys with this formulation⁶ and showed no evidence in progression of severity with a longer duration of dosing. Other injection site lesions in a few animals in control and/or treated groups (edema, dermal scab formation with epithelial hyperplasia, macrophage infiltrates, ulceration, and foreign body granulomas) were attributable to the control article and/or IM dosing procedure. All other histologic lesions observed were naturally occurring and/or spontaneous lesions, which are commonly observed in laboratory housed monkeys.

Postdose Necropsy: In postdose animals, the most common lesions at the injection sites were minimal skeletal muscle regeneration and minimal subacute inflammation (see the following sponsor's table).

| Dose (mg/kg/day): | 0 | 2 | 4 | 7.5 |
|--|------------|------------|------------|------------|
| No. of monkeys (M/F): | 2/2 | 2/2 | 2/2 | 2/2 |
| Sex: | M/F | M/F | M/F | M/F |
| Regeneration, Skeletal Muscle, Minimal | 1/1 | 1/- | -/- | 1/- |
| Degeneration, Skeletal Muscle, Minimal | -/- | -/1 | 1/- | -/1 |
| Inflammation, Subacute, Minimal | -/- | -/2 | -/- | 1/- |

- Indicates absence of finding in group

The lower incidence of these alterations without a clear relationship to dose indicated that the injection site changes were reversible. There was no residual fibrosis in any group.

In summary, microscopic injection site changes were present in both control article- and aripiprazole-treated groups and included skeletal muscle necrosis, degeneration, and regeneration; subacute inflammation, fibroplasia/fibrosis, hemorrhage, and edema. The hemorrhage tended to be in the subcutis and/or superficial layers of the skeletal muscle, indicating that it was likely due to the IM injection procedure. In the dosed groups, there was evidence of drug-related exacerbation of these changes in comparison to control, as indicated by the increased incidences and/or severity of skeletal muscle necrosis (minimal to mild) at all doses; degeneration (mild to moderate) and regeneration (mild to moderate), and an increased incidence and severity of fibroplasia/fibrosis (minimal to mild), at HD. After a 1-month recovery period, the lower incidence of these alterations without a clear relationship to dose indicated that the injection site changes were reversible. There was no residual fibrosis in any group.

Toxicokinetics:

The toxicokinetic parameters of aripiprazole and its pharmacologically active dehydrometabolite, BMS-337044 are presented in the following sponsor's table:

⁶ (b) (4). BMS-337039: Two-week intramuscular toxicity study in monkeys (Study No. 99351), Report. Bristol-Myers Squibb Company, July 7, 2000. BMS Document Control Number 920007292

TK Parameters for aripiprazole and its dehydro-metabolite BMS-337044 in Monkeys

| Dose [mg/kg/day] | Study Day | Aripiprazole | | Dehydro- Aripiprazole | | Aripiprazole | | Dehydro- Aripiprazole | |
|-----------------------|-----------------|--------------------------|-----------|--------------------------|-----------|---------------------------------|-----------|--------------------------|-----------|
| | | Male | Female | Male | Female | Male | Female | Male | Female |
| | | C _{max} [ng/mL] | | | | AUC(0-T) [ng.h/mL] ^a | | | |
| 2 | 1 | 637 | 691 | 40 | 47 | 1746 | 1934 | 329 | 477 |
| | 29 ^b | 697 | 767 | 43 | 49 | 2263 | 2189 | 554 | 688 |
| 4 | 1 | 1096 | 2065 | 79 | 75 | 3678 | 4627 | 878 | 924 |
| | 29 | 1159 | 1586 | 121 | 91 | 5600 | 4809 | 1799 | 1445 |
| 7.5 | 1 | 2559 | 2931 | 167 | 135 | 9272 | 9471 | 2097 | 1716 |
| | 29 | 3733 | 3763 | 183 | 172 | 11070 | 9718 | 2703 | 2587 |
| | | C _{max} Ratios | | | | AUC(0-T) Ratios | | | |
| 1:2:3:8 Dose Ratio | 1 | 1:1.7:4.0 | 1:3.0:4.2 | 1:2.0:4.2 | 1:1.6:2.9 | 1:2.1:5.3 | 1:2.4:4.9 | 1:2.7:6.4 | 1:1.9:3.6 |
| | 29 | 1:1.7:5.4 | 1:2.1:4.9 | 1:2.8:4.3 | 1:1.8:3.5 | 1:2.5:4.9 | 1:2.2:4.4 | 1:3.3:4.9 | 1:2.1:3.8 |

^a Calculated from time zero to the time of the last quantifiable plasma concentration, ranging from 8 to 24 h post-dose.

^b N=4 due to the death of one animal on Day 15.

The systemic exposure to aripiprazole and dehydro-aripiprazole, following IM administration of 2- to 7.5-mg/kg/day doses of aripiprazole for 29 days, was dose-related; the C_{max} and AUC values appeared to increase dose-proportionally. The systemic exposure to aripiprazole and dehydro-aripiprazole appeared to be similar in females and males. The ratio of AUC values of aripiprazole on day 29 compared to day 1 were 1.2-1.5 (males) and 1.0-1.1 (females). In conclusion, the exposure of the monkeys to aripiprazole and dehydro-aripiprazole was dose-proportional without apparent sex-related differences and a small amount of accumulation in the systemic exposure was observed upon repeated daily dosing for 29 days.

Conclusion: Daily intramuscular administration of aripiprazole extended-release suspension to monkeys for 1 month at doses of 2, 4 and 7.5 mg/kg/day was associated with pharmacologically mediated CNS-related clinical signs and evidence of reversible skeletal muscle injury in injection sites at all doses. Small increases in serum aspartate aminotransferase at 4 and 7.5 mg/kg/day were likely a consequence of injection site injury. Microscopically, increased incidence and/or severity of skeletal muscle necrosis (minimal to mild), degeneration (mild to moderate), and regeneration (mild to moderate) were observed at injection sites at all doses, while injection site changes associated with the control article and/or intramuscular injection procedure were generally minimal in severity. A NOAEL was not reached in this study (< the lowest tested IM dose of 2 mg/kg/day) since injection site skeletal muscle injury was present at all doses.

7 Genetic Toxicology

Studies not performed

8 Carcinogenicity

Studies not performed

A waiver for conducting nonclinical carcinogenicity studies was granted to OTSUKA by the FDA based on Executive CAC recommendation that the proposed 2-year carcinogenicity study was not necessary (Executive CAC Minutes of 9/26/2008)

9 Reproductive and Developmental Toxicology

Studies not performed

A waiver for conducting developmental and reproductive toxicity studies was granted to Otsuka by the FDA (e-mail from Keith Kiedrow to sponsor dated 23 June 2010, re IND 67,380 Serial Number 136, dated 5/25/2010) because the exposure after IM depot administration was much lower than that after oral or intravenous dosing (as shown by repeated dose TK studies of the IM depot formulation), and reproductive and developmental toxicity evaluations had been previously conducted with oral and intravenous administration.

10 Special Toxicology Studies

Local Tolerance Studies

Eight definitive local tolerance studies were conducted with vehicles to be used in subsequent toxicity studies. Formulations of aripiprazole in carboxymethylcellulose (CMC) or in 15% Captisol were examined to determine a suitable formulation for use in toxicity studies.

The formulation proposed to be marketed for clinical use is based on CMC as suspending agent.

Study title: Single-dose Intramuscular Depot Irritation Study in Rats

Study no.: Otsuka Study No. 019798 (BMS Study No. DM02019)

Study report: Otsuka Report No. 015505, 2003 (BMS-337039)

Conducting laboratory and location: BMS, Pharmaceutical Research Institute, Departments of Toxicology and Pathology, Mt. Vernon, Indiana

Date of study initiation: May 7, 2002

GLP compliance: Yes

QA statement:

Drug, lot #, and % purity: Aripiprazole (OPC-31, OPC14597), Batch C00J99M

Key study findings: Administration of aripiprazole (100 mg/ml) in a CMC formulation ((b) (4) mannitol, monobasic sodium phosphate, sterile water for injection, pH 7; particle size (b) (4) to rats (15/sex/dose group) at single doses of 12.5, 25 or 50 mg/kg (corresponding dose volumes of 0.125, 0.25 or 0.5 mL/kg, respectively), resulted in a dose-related increase in the incidence of injection site swelling in all dosed groups (attributable to deposits of test article in the muscle), but not in control groups. At necropsy on Days 29 and 45, white discolored foci were observed macroscopically at the injection sites at all doses; the foci decreased in size over time but did not completely resolve by study termination (Day 45). Microscopically, the foci were characterized by a dose-related localized inflammatory response in association with deposits of birefringent crystals (interpreted as drug) in the muscle interstitium, consistent with a foreign body reaction to deposited drug. There was no evidence of muscle cell necrosis. Low incidence of “minimal fibrosis” was found in injection-site muscles at MD and HD. A partial resolution of the granulomatous inflammation and fibroplasia/fibrosis occurred by study termination on Day 45. The microscopic injection site changes in the vehicle control were comparable to those in the saline control. The exposure of the rats to aripiprazole was dose related with no apparent sex-related differences in the systemic exposure upon single IM depot dose administration.

Methods

| | |
|--------------------------|---|
| Doses: | 12.5, 25 or 50 mg/kg |
| Frequency of dosing: | Single |
| Route of administration: | IM into the right thigh muscle |
| Dose volume: | 0.125, 0.25 or 0.5 mL/kg, respectively |
| Formulation/Vehicle: | Suspension (100 mg/ml) CMC formulation/ (b) (4) mannitol, monobasic sodium phosphate, sterile water for injection, pH 7; particle size (b) (4) |
| Species/Strain: | Rat/ CrI:CD(SD)IGS BR |
| Number/Sex/Group: | 15 |
| Age: | 12-14 wks at study initiation |
| Weight: | 404-466 g (males) and 229-292 g (females) |
| Satellite groups: | TK |

Aripiprazole (100 mg/ml) in a CMC formulation ((b) (4) mannitol, monobasic sodium phosphate, sterile water for injection, pH 7; particle size (b) (4)) was administered to CrI:CD(SD)IGS BR rats (15/sex/dose group) at single doses of 12.5, 25 or 50 mg/kg (corresponding dose volumes of 0.125, 0.25 or 0.5 mL/kg, respectively). The high dose was selected based on volume considerations, as 0.5 mL/kg is an upper dose volume limit per injection site for an intramuscular depot injection into the thigh muscles of rats. These doses represented approximately 0.3-, 0.6-, and 1.2-fold multiples of the projected maximum clinical dose of 4 mL (400 mg) based on mg/m². An additional group of 6 rats/sex received IM injection of 0.5 mL/kg of 0.9% Sodium chloride for injection, USP and served as a saline control group; a second control group of 15 rats/sex received 0.5 mL/kg of the test article carrier formulation and served as a vehicle control group. On the day of dosing (day 1), rats were approximately 12-14 weeks old with body weight ranges of 404 to 466 g (males) and 229 to 292 g (females).

Experimental Design

| Group Number | Dose | | Concentration BMS-337039 (mg/ml) | Number of Animals |
|--------------|------------------------------------|----------------|----------------------------------|-------------------|
| | BMS-337039 (mg/kg) | Volume (ml/kg) | | |
| 1 | 0 (sterile saline) ^a | 0.5 | 0 | 6 M, 6 F |
| 2 | 0 (vehicle) ^b | 0.5 | 0 | 15 M, 15 F |
| 3 | 12.5 | 0.125 | 100 | 15 M, 15 F |
| 4 | 25 | 0.25 | 100 | 15 M, 15 F |
| 5 | 50 | 0.5 | 100 | 15 M, 15 F |

^a Control article
^b Comparator control

For the dosed and vehicle control groups, 9 animals/sex/group were euthanatized and necropsied on day 29; the remaining six animals/sex/group were euthanatized and necropsied on day 45. For

the saline control group, half of the animals (3/sex) were euthanatized and necropsied on both days 29 and 45.

Evaluations included injection site changes and plasma concentrations of aripiprazole and selected metabolites to assess systemic exposure. Blood samples for the evaluation of systemic exposure were collected on days 1 (6 h postdose), 2, 4, 7, 10, 15, 22, 29, 36, and 43 of the study. The plasma samples were analyzed for aripiprazole and metabolites DM-1451 and OPC-14857 by a validated LC/MS/MS method that had a lower limit of quantitation of 1 ng/mL

Results

There was no mortality or clinical signs.

A dose-related increase in the incidence of injection site swelling (attributable to deposits of test article in the muscle) was observed in all dosed groups with the total number of occurrences increasing with dose, but not in either of the control groups. The incidence was higher in males, which could be associated with the larger injection volume administered to males. The duration of injection site swelling was dose dependent and was longer in males.

Incidence, Duration, and Onset of Injection Site Swelling in Rats Administered Aripiprazole Depot Formulation

| Dose (mg/kg) | Males | | | Females | | |
|-------------------------|---------|---------|---------|---------|---------|----------|
| | 12.5 | 25 | 50 | 12.5 | 25 | 50 |
| No. of Animals Affected | 4 of 15 | 3 of 15 | 9 of 15 | 3 of 15 | 2 of 15 | 10 of 15 |
| Total Occurrences | 7 | 28 | 83 | 3 | 5 | 32 |
| Duration (days) | 1 - 3 | 2 - 19 | 1 - 25 | 1 | 2 - 3 | 1 - 7 |
| Average Duration (days) | 1.75 | 9.33 | 9.22 | 1.0 | 2.5 | 3.1 |
| Onset (days) | 2 - 5 | 2 - 6 | 1 - 2 | 2 - 4 | 2 - 3 | 2 - 4 |

Gross pathology: At necropsy on Days 29 and 45, white discolored foci were observed macroscopically at the injection sites at all doses; the foci generally decreased in size over time but did not completely resolve by study termination (Day 45).

Histopathology: Microscopically, the foci were characterized by a dose-related minimal to mild granulomatous inflammation and minimal fibroplasia/fibrosis on Day 29. The localized inflammatory response consisted of numerous epitheloid macrophages and foreign body giant cells in association with deposits of birefringent crystals (interpreted as drug) in the muscle interstitium. These findings were consistent with a foreign body reaction to deposited drug. No evidence of muscle cell necrosis was observed. By Day 45, partial resolution of the granulomatous inflammation and fibroplasia/fibrosis occurred. In the vehicle control animals, microscopic injection site changes were generally comparable to those seen with saline injection (see the following sponsor's table). The pathology evaluation was reviewed by a peer-review pathologist.

Histopathology Group Incidence

| | | | | | |
|--|-----|-------|-------|-------|-------|
| Dose (mg/kg): | SC | CC | 12.5 | 25 | 50 |
| No. of rats (M/F): | 6/6 | 15/15 | 15/15 | 15/15 | 15/15 |
| Sex: | M/F | M/F | M/F | M/F | M/F |
| Interim Necropsy (day 29)^a: | | | | | |
| Granulomatous Inflammation: | | | | | |
| Minimal severity | - | 0/1 | 7/8 | 6/9 | 3/6 |
| Mild severity | - | - | 1/0 | 3/0 | 6/3 |
| Fibroplasia/Fibrosis: | | | | | |
| Minimal severity | - | - | 2/0 | 1/1 | 1/3 |
| Terminal Necropsy (day 45)^b: | | | | | |
| Granulomatous Inflammation: | | | | | |
| Minimal severity | - | - | 5/4 | 5/6 | 6/6 |
| Fibroplasia/Fibrosis: | | | | | |
| Minimal severity | - | 1/0 | - | 1/1 | 1/0 |
| SC - saline control; CC - comparator (vehicle) control | | | | | |
| - Indicates absence of finding in group | | | | | |
| ^a N=9/sex/group except for SC (N=3/sex) | | | | | |
| ^b N=6/sex/group except for SC (N=3/sex) | | | | | |

Toxicokinetics**Plasma TK of Aripiprazole and Metabolites Following Single IM Injection of Aripiprazole to Rats**

| Dose (mg/kg) | Aripiprazole | | DM-1451 | | OPC-14587 | |
|----------------------------------|--------------|--------|---------|--------|-----------|--------|
| | Male | Female | Male | Female | Male | Female |
| C_{max} (ng/mL) | | | | | | |
| 12.5 | 13.8 | 12.3 | 2.12 | 1.10 | <1 | <1 |
| 25 | 18.0 | 25.3 | 2.53 | 2.03 | <1 | <1 |
| 50 | 41.9 | 52.6 | 4.43 | 3.36 | 1.23 | 4.63 |
| AUC^a (ng·d/mL) | | | | | | |
| 12.5 | 151 | 146 | ND | ND | ND | ND |
| 25 | 246 | 301 | 21.9 | ND | ND | ND |
| 50 | 480 | 484 | 42.0 | ND | ND | ND |

ND = Not determined.

^a Calculated from time zero to the last measurable plasma concentration; Day 42 for aripiprazole and between Days 14 and 21 for DM-1451. The lower limit of quantification was 1 ng/mL.

Systemic exposure to aripiprazole was dose-related with no remarkable gender differences, and was measurable up to Day 43 post-dose at all doses; AUC values increased similarly to the dose

increment in males and females; C_{max} values increased less than the dose increment in males but similar to the dose increment in females (see the above sponsor's table). T_{max} for aripiprazole was 6 days after dose administration. Plasma concentrations of metabolites DM-1451 and OPC-14857 were generally below the LLOQ (1 ng/mL) in most animals, although measurable DM-1451 plasma concentrations were found in some males at MD and HD on Days 14 and 21 post dose. For the metabolites DM-1451 and OPC-14587, the peak concentrations that were greater than the LLOQ usually occurred at 6 hours post-dose, although a few occurred at 6 days post-dose.

Conclusion:

Single intramuscular injections of 12.5, 25, or 50 mg/kg of a 100 mg/ml aripiprazole depot formulation in rats [representing approximately 0.3-, 0.6-, and 1.2-fold multiples of the projected maximum clinical dose of 4 ml (400 mg) based on body surface] were clinically well tolerated and resulted in a minimal to mild localized granulomatous inflammatory response (foreign body reaction) to deposited drug at the injection site. The inflammation diminished somewhat over time but did not resolve completely by day 45. There was no morphologic evidence of drug-related skeletal muscle necrosis at any dose level. Systemic exposure to aripiprazole was dose-proportional with no remarkable gender differences, and was measurable up to the end of study (Day 43 post-dose); plasma concentrations of metabolites DM-1451 and OPC-14857 were generally below the LLOQ (1 ng/mL) in most animals.

Study title: Single-dose Intramuscular Depot Irritation Study in Rats (II)

Study no.: Otsuka Study No. 025804 (BMS Study No. DM04018)

Study report: Otsuka Report No.019936, 2006

Conducting laboratory and location: BAS Evansville,
10424 Middle Mt. Vernon Road

Mt. Vernon, IN 47620

Date of study initiation:

GLP compliance: Yes

QA statement: Yes

Drug, lot #: Aripiprazole (OPC-31, OPC14597), Batch 2B52237

150 mg/mL formulation: Lot No. 52972-128-02,

200 mg/mL formulation: Lot No. 52972-122-02

Key Study Findings: A single IM injection of aripiprazole CMC formulation ^{(b)(4)}mannitol, monobasic sodium phosphate, sterile water for injection, pH 7; particle size ^{(w)(4)} to rats (15/sex/dose) at doses of 75 or 100 mg/kg; 150 and 200 mg/mL, dosig volume 0.5 mL/kg resulted in a localized, minimal to mild granulomatous inflammatory response to deposited drug at the injection site, consistent with a foreign-body reaction. This inflammation was not completely resolved by Day 45. No evidence of skeletal muscle injury at the injection sites was observed. There were no treatment-related clinical signs and grossly visible injection site findings. Aripiprazole plasma exposure was approximately dose-proportional in both genders; metabolite OPC-14857 was generally below the LLOQ at all sampling times.

Methods

| | |
|--------------------------|---|
| Doses: | 75 or 100 mg/kg; 150 and 200 mg/mL |
| Frequency of dosing: | Single dose |
| Route of administration: | IM injection |
| Dose volume: | 0.5 mL/kg |
| Formulation/Vehicle: | sodium carboxymethylcellulose-based depot formulation (b) (4) mannitol, monobasic sodium phosphate, sterile water for injection, pH 7; particle size (b) (4) (150 and 200 mg/mL) |
| Species/Strain: | Rats/ Sprague Dawley Crl:CD(SD)IGSBR |
| Number/Sex/Group: | 6 rats/sex in the control group and 15 rats/sex in the drug-treated groups |
| Age: | 11-13 weeks at study initiation |
| Weight: | Males – 367.7 - 479.3 g Females – 246.0 - 298.4 g |
| Satellite groups: | TK |

Experimental design: Three groups of Sprague Dawley rats were given a single IM dose of 0 (saline control, 0.9% Sodium Chloride for Injection, USP), 75, or 100 mg/kg of BMS-337039 (150 and 200 mg/mL formulations at 0.5 mL/kg).

Observations:

Parameters for evaluation included viability, daily clinical observations, and injection site scoring. At scheduled euthanasia, injection sites were examined from 3 rats/sex in the control group and 9 rats/sex in the drug-treated groups on Day 29; injection sites from the remaining animals (3/sex in the control group and 6/sex in the drug-treated groups) were examined on Day 45. Histopathology examination of injection sites from all animals was performed. Blood samples for systemic exposure were collected from treated animals on Days 1, 2, 4, 7, 10, 15, 22, 36 and 43 from the 3 animals/sex/group at each time point. On Day 1, blood samples were collected at approximately 6 hours after dosing; on all other days, samples were collected at approximately the same time of day as the animal was dosed on Day 1.

Results

There was no treatment-related mortality or clinical signs. Gross observations at necropsy were limited to white discoloration at the injection site in all treated animals on Days 29 and 45 that represented deposits of the test article.

Histopathology

Microscopically, the primary finding at the injection site was a localized, minimal to mild granulomatous inflammatory response to deposited drug (polymorphic birefringent crystalline material) that was consistent with a foreign-body reaction; the finding was slightly greater in severity at 100 mg/kg. This inflammation was not completely resolved by Day 45. Other drug-related injection site changes on Days 29 and/or 45 included minimal subacute inflammation at both dose levels and minimal fibroplasia/fibrosis at 100 mg/kg. No evidence of skeletal muscle injury at the injection sites was observed at any dose.

Incidence of Selected Drug-Related Microscopic Findings at the Injection Sites

| Dose (mg/kg): | 0 | 75 | 100 |
|------------------------------------|------------|------------|------------|
| Sex: | M/F | M/F | M/F |
| Interim Necropsy (Day 29): | | | |
| No. of Rats (M/F): | 3/3 | 9/9 | 9/9 |
| Inflammation, granulomatous | | | |
| Minimal | - | 6/5 | 2/4 |
| Mild | - | 3/4 | 7/5 |
| Inflammation, subacute | | | |
| Minimal | - | 4/3 | 2/2 |
| Fibroplasia/fibrosis | | | |
| Minimal | - | - | 1/3 |
| Terminal Necropsy (Day 45): | | | |
| No. of Rats (M/F): | 3/3 | 6/6 | 6/6 |
| Inflammation, granulomatous | | | |
| Minimal | - | 3/6 | 3/4 |
| Mild | - | 3/0 | 3/2 |
| Inflammation, subacute | | | |
| Minimal | - | - | 2/0 |
| Fibroplasia/fibrosis | | | |
| Minimal | - | - | 1/2 |

- Indicates absence of finding in group

Toxicokinetics

Aripiprazole C_{max} and AUC_{0-42d} were dose-related and approximately dose-proportional in both genders (see the following sponsor's table). The t_{max} (6 days) was not affected by gender or dose. Plasma concentrations for the metabolite OPC-14857 were less than the LLOQ at all sampling times in the 100 mg/kg dose group and were quantifiable only at the 6-hour sampling time for females and at the 216-hour sampling time for males in the 75 mg/kg dose group.

Plasma TK of Aripiprazole and Metabolite OPC-14857 Following Single IM Injection of Aripiprazole to Rats
Otsuka Study No. 025804

| Dose (mg/kg) | Aripiprazole | | OPC-14857 | |
|--------------------------------------|--------------|--------|-----------|--------|
| | Male | Female | Male | Female |
| C_{max} (ng/mL) | | | | |
| 75 | 62.5 | 45.7 | 1.03 | 1.50 |
| 100 | 66.5 | 50.1 | <1 | <1 |
| AUC_{0-42d} (ng·d/mL) | | | | |
| 75 | 16,500 | 11,200 | NC | NC |
| 100 | 21,100 | 13,300 | NC | NC |

NC = Not calculated. The lower limit of quantification was 1 ng/mL.

Conclusion:

Aripiprazole intramuscular doses of 75 and 100 mg/kg, administered as sodium carboxymethyl-cellulose-based depot formulations (150 and 200 mg/mL), produced no clinical evidence of irritation at the injection site. Microscopically, the primary finding at the injection site was a localized, minimal to mild granulomatous inflammatory response to deposited drug that was consistent with a foreign-body reaction. This inflammation was not completely resolved by Day 45. There was no evidence of skeletal muscle injury at the injection sites at either dose.

Study title: Single-dose Intramuscular Depot Irritation study in Male Rabbits

Study no.: Otsuka Study No. 019858 (BMS Study No. DM02020)

Study report: Otsuka Report No.015567, 2003

Conducting laboratory and location: BMS Pharmaceutical Research Institute

Departments of Toxicology and Pathology, Mt. Vernon, Indiana USA

Date of study initiation: 16 April, 2002

GLP compliance: Yes

QA statement: Yes

Drug, lot #: Aripiprazole (OPC-31, OPC14597), Batch C00J99M

Key Study Findings: Single intramuscular injections of up to 1.0 mL of a 100 mg/mL suspension of aripiprazole in a CMC formulation ((b) (4) mannitol, monobasic sodium phosphate, sterile water for injection, pH 7; particle size (b) (4)) to rabbits were well tolerated; there were no clinical signs (including signs associated with pain) or in-life injection site findings in any of the drug-treated groups. At necropsy, foci of white discoloration of dose-related size, consistent with deposits of test article and associated inflammation, were noted at injection sites in all drug-treated animals at all time points. The principal microscopic finding at the injection site was localized, dose-related granulomatous inflammation in the muscle interstitial tissue representing a foreign body reaction to deposited drug; the inflammation was not completely resolved by Day 57. Birefringent polymorphic crystalline material, interpreted as deposited drug, remained in areas of inflammation through Day 57 in all treated groups. There was no clinical chemistry or morphology evidence of drug-related skeletal muscle necrosis at any dose level. Injection site changes associated with the article carrier were limited to minimal muscle degeneration/regeneration in one rabbit in each group.

Methods

| | |
|--------------------------|---|
| Doses: | 25, 50 and 100 mg |
| Frequency of dosing: | Single dose |
| Route of administration: | IM |
| Dose volume: | 0.25, 0.5 and 1 mL, respectively |
| Formulation/Vehicle: | The formulation contained lyophilized 250 mg aripiprazole; (b) (4) sodium carboxymethyl-cellulose; (b) (4) mannitol, USP; and (b) (4) sodium phosphate, monobasic; adjusted to approximately pH 7 and (b) (4) |
| Species/Strain: | Rabbits/ New Zealand White Hra:(NZW) SPF |
| Number/Sex/Group: | 12 males/group |
| Age: | 4 to 5 months at study initiation |
| Weight: | 2.7 to 3.2 kg |
| Control groups: | saline control group (0.9% Sodium Chloride for Injection, USP) as; vehicle control group (1 mL of the test article carrier formulation) |

Study design: 100 mg/ml dosing formulation of BMS-337039, comparator control (test article carrier), or control article (sterile saline) were administered intramuscularly once in the right thigh muscle using a 21-gauge needle, according to the following sponsor's table.

| Group Number | Daily Dose | | Concentration BMS-337039 (mg/ml) | Number of Animals |
|--------------|---------------------------------|-------------|----------------------------------|-------------------|
| | BMS-337039 (mg) | Volume (ml) | | |
| 1 | 0 (sterile saline) ^a | 1 | 0 | 12 M |
| 2 | 0 (vehicle) ^b | 1 | 0 | 12 M |
| 3 | 25 | 0.25 | 100 | 12 M |
| 4 | 50 | 0.5 | 100 | 12 M |
| 5 | 100 | 1 | 100 | 12 M |

^a Control article

^b Comparator control

Dose selection: The high dose was selected based on volume considerations (1 ml considered the upper dose volume limit per injection site for an intramuscular injection in rabbits); in an exploratory single dose intramuscular irritation study in rabbits (Bristol-Myers Squibb Study No. DM01005), a 0.5 ml injection (50 mg) of this depot formulation was clinically well tolerated. The mid- and low doses were selected to allow for observation of dose-response.

Observations:

Clinical signs and injection site observations, serum AST and CK (Days 2, 4, and 7 post dose) and gross and microscopic pathology examinations of the injection sites on Days 4, 7, 15, 29 and 57 post dosing.

Results:

Single intramuscular injections of up to 1.0 mL of a 100 mg/mL suspension were clinically well tolerated, and there were no clinical signs (including signs associated with pain) or in-life injection site findings in any of the drug-treated groups.

At necropsy, white discoloration at the injection sites (consistent with deposits of the test article and associated inflammation) was noted in all drug-treated animals at all time points. The size of these discolored foci was generally dose related, and there was a reduction in size over time (see sponsor's table below). Rare observations of red discoloration (hemorrhage) seen grossly were considered to be due to the injection procedure as these were present in comparator control and treated animals.

Size Range of BMS-337039-Related White Discoloration

| Dose (mg): | 25 | | 50 | | 100 | |
|-------------------------------|--------------------------|------------|--------------------------|------------|--------------------------|------------|
| Volume (ml) | 0.25 | | 0.50 | | 1 | |
| No. of Rabbits: | 12 | | 12 | | 12 | |
| Sex: | M | | M | | M | |
| Lesion: | length (cm) X width (cm) | | length (cm) X width (cm) | | length (cm) X width (cm) | |
| Discoloration, white (day 4) | 1.8 to 2.5 | 0.4 to 0.5 | 3.5 | 0.5 | 2.5 to 3 | 1 |
| Discoloration, white (day 7) | 2.5 | 0.8 | 1.5 to 3.0 | 1.2 | 2 to 4 | 1.5 to 2 |
| Discoloration, white (day 15) | 2 to 2.5 | 0.8 to 1.2 | 1.8 to 3.0 | 0.6 to 1.0 | 1.8 to 4.0 | 0.5 to 1.5 |
| Discoloration, white (day 29) | 0.8 to 2.5 | 0.5 to 1.0 | 1.2 to 3.5 | 0.6 to 1.2 | 1.4 to 4.5 | 0.5 to 1.1 |
| Discoloration, white (day 57) | 1 to 1.5 | 0.4 to 1.0 | 0.5 | 0.5 | 1.5 | 1 |

Histopathology

The principal microscopic finding at the injection site was localized granulomatous inflammation in the muscle interstitial tissue, characterized by the presence of “epitheloid macrophages, heterophils and lymphocytes”, interpreted as a foreign body reaction to deposited drug. The inflammation was generally dose-related and most severe at Days 15 and 29, with incomplete resolution by Day 57. Birefringent polymorphic crystalline material, interpreted as deposited drug, remained in areas of inflammation through Day 57 for all treated groups. Additional drug-related histopathology included minimal to mild subacute inflammation through Day 15 in the LD and MD groups, and through Day 57 in the HD group; increased incidence of minimal muscle degeneration/regeneration, and minimal or mild fibroplasia that generally resolved by Day 57. Microscopic injection site changes associated with the CMC-based test article carrier were comparable to those seen with saline injection and were limited to minimal muscle degeneration/regeneration in one rabbit in each group on day 4. There were no drug-related changes in AST or CPK or morphology evidence of drug-related skeletal muscle necrosis at any dose level.

Incidence of Drug-Related Microscopic Findings

| Dose (mg): | 0 (Sterile Saline) ^a | | | | | 0 (Vehicle) ^b | | | | | 25 | | | | | 50 | | | | | 100 | | | | |
|--------------------------------|---------------------------------|---|----|----|-----------------|--------------------------|---|----|----|----|------|---|----|----|----|-----|---|----|----|----|-----|---|----|----|----|
| Concentration (mg/ml): | 0 | | | | | 0 | | | | | 100 | | | | | 100 | | | | | 100 | | | | |
| Volume (ml): | 1 | | | | | 1 | | | | | 0.25 | | | | | 0.5 | | | | | 1.0 | | | | |
| Sex: | M | | | | | M | | | | | M | | | | | M | | | | | M | | | | |
| Day | 4 | 7 | 15 | 29 | 57 ^c | 4 | 7 | 15 | 29 | 57 | 4 | 7 | 15 | 29 | 57 | 4 | 7 | 15 | 29 | 57 | 4 | 7 | 15 | 29 | 57 |
| No. of Rabbits: | 2 | 2 | 2 | 4 | 2 | 2 | 2 | 2 | 4 | 2 | 2 | 2 | 2 | 4 | 2 | 2 | 2 | 2 | 4 | 2 | 2 | 2 | 2 | 4 | 2 |
| <u>Inflammation</u> | | | | | | | | | | | | | | | | | | | | | | | | | |
| <u>granulomatous:</u> | | | | | | | | | | | | | | | | | | | | | | | | | |
| Minimal severity | - | - | - | - | - | - | - | - | - | - | 2 | 1 | - | 3 | 2 | 2 | - | - | - | 2 | 2 | - | - | - | 1 |
| Mild severity | - | - | - | - | - | - | - | - | - | - | - | 1 | 1 | 1 | - | - | 2 | 1 | 4 | - | - | 2 | 1 | 3 | 1 |
| Moderate severity | - | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | - | - | 1 | - | - | - | - | 1 | 1 | - |
| <u>Inflammation, subacute:</u> | | | | | | | | | | | | | | | | | | | | | | | | | |
| Minimal severity | - | - | - | - | - | - | - | - | - | - | 1 | 1 | 1 | - | - | 1 | 1 | 1 | - | - | 2 | 2 | 1 | 1 | 2 |
| Mild severity | - | - | - | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | 1 | 1 | - |
| <u>Degeneration, muscle:</u> | | | | | | | | | | | | | | | | | | | | | | | | | |
| Minimal severity | 1 | - | - | - | - | 1 | - | - | - | - | 1 | 1 | - | - | - | 2 | 2 | - | - | - | 2 | - | - | - | - |
| <u>Regeneration, muscle:</u> | | | | | | | | | | | | | | | | | | | | | | | | | |
| Minimal severity | 1 | - | - | - | - | 1 | - | - | - | - | 1 | 2 | 1 | - | - | 2 | 2 | 1 | - | - | 2 | - | - | 1 | - |
| <u>Fibroplasia/fibrosis:</u> | | | | | | | | | | | | | | | | | | | | | | | | | |
| Minimal severity | - | - | - | - | - | - | - | - | - | - | 2 | 1 | 1 | - | - | 2 | 2 | 1 | 1 | 1 | - | 2 | 1 | 2 | - |
| Mild severity | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | 2 | - |
| <u>Hemorrhage:</u> | | | | | | | | | | | | | | | | | | | | | | | | | |
| Minimal severity | - | - | - | 1 | - | - | - | - | - | - | 1 | 2 | - | - | - | 2 | 1 | - | - | - | 2 | 1 | 1 | - | - |
| <u>Birefringent material:</u> | | | | | | | | | | | | | | | | | | | | | | | | | |
| | - | - | - | - | - | - | - | - | - | - | 2 | 2 | 2 | 4 | 2 | 2 | 2 | 2 | 4 | 2 | 2 | 2 | 2 | 4 | 2 |

- Indicates absence of finding in group

^a Control article

^b Comparator control

^c Animal No. 1112 (day 52) included

Study title: BMS-337039: Single-Dose Intramuscular Depot Irritation Study in Male Rabbits (II)

Study no.: Otsuka Study No. 025803 (BMS Study No. DM04017)

Study report: Otsuka Report No.019934, 2006

Conducting laboratory and location: BAS Evansville, Mt. Vernon, IN 47620

Date of study initiation: 26 April 2004

GLP compliance: Yes

QA statement: Yes

Drug, lot #: Aripiprazole (OPC-31, OPC14597, BMS-337039-06) Batch 2B52237

Key Study Findings: In a second study in rabbits, IM administration of aripiprazole at single doses of 150 and 200 mg/kg (corresponding dose volume of 1 mL of a 150 or 200 mg/mL suspension in a CMC formulation), resulted in discoloration at the injection site in all treated rabbits at scheduled necropsies on Days 4, 7, 15, 29 and 57 at both doses. The foci of white/tan discoloration at the injection sites were deposits of test article (polymorphic, birefringent crystalline material), and decreased in size by Day 57 post dose. Microscopic findings at the injection site at all time points included mild granulomatous inflammation as a foreign-body reaction in response to deposited drug. The inflammatory reaction was most expressed on Day 15 and was partially reversible by Day 57 post injection. Drug-related findings indicative of skeletal muscle injury included minimal degeneration and necrosis (Days 4 and/or 7) and minimal regeneration (Day 15 post injection).

Methods

Doses: 150, 200 mg/kg

Frequency of dosing: single dose

Route of administration: IM

Dose volume: 1 mL of 150 or 200 mg/mL aripiprazole suspension

Formulation/Vehicle: CMC formulation (b) (4) mannitol, monobasic sodium phosphate, sterile water for injection, pH 7; particle size (b) (4)

Species/Strain: Rabbit/New Zealand White Hra:(NZW) SPF

Number/Sex/Group: 12/male

Age: 7 months

Weight: 2.8 – 3.4 kg

Control groups: Vehicle control (CMC formulation); Saline control (1 mL 0.9% Sodium Chloride for Injection (USP))

Observations: Clinical signs and injection site observations, periodic determination of serum AST and CK; gross and microscopic examinations of the injection sites on Days 4, 7, 15, 29 and 57.

Results:

Mild edema at the injection site was noted in 1 rabbit on Day 2 and persisted to Day 4 (day of necropsy). At necropsy, foci of white and/or red or tan discoloration at the injection sites were seen in all treated animals at scheduled sacrifices on Days 4, 7, 15, 29 and Day 57 at both doses. These foci of discoloration at the injection sites were assessed as deposited test article, and were visibly smaller on Day 57 as compared to Day 29. No differences in aspartate aminotransferase

(AST) and creatine kinase (CK) were observed between the treated and control rabbits (tested on Days 2, 4, and 7).

Histopathology

Microscopic finding at the injection site at all time points included generally mild granulomatous inflammation as a foreign-body reaction in response to deposited drug (polymorphic, birefringent crystalline material). The inflammatory reaction was most severe on Day 15 but declined in severity through Day 57. Drug-related findings indicative of skeletal muscle injury included minimal degeneration and necrosis (Days 4 and/or 7) and minimal regeneration (Day 15). Additionally, mild subacute inflammation was observed at the injection sites through Day 15.

Toxicokinetics:

The systemic exposures of male rabbits to aripiprazole following a single intramuscular depot dose of 150 or 200 mg were dose-related. C_{max} and AUC_{0-56d} after a single dose were generally proportional to the dose increment for aripiprazole. T_{max} for aripiprazole was 216 hours (9 days) after both doses. Most plasma concentrations of metabolite OPC-14857 were below the LLOQ (1 ng/mL). TK summary is presented in the following sponsor's table:

TK summary

Aripiprazole

| Parameter | Dose (mg) | |
|---------------------------|-----------|--------|
| | 150 | 200 |
| C_{max} (ng/mL) | 53.5 | 82.5 |
| $AUC_{0-1344h}$ (ng·h/mL) | 31,200 | 44,100 |
| T_{max} (h) | 216 | 216 |

Metabolite BMS-337044

| Parameter | Dose (mg) | |
|---------------------------|-----------|------|
| | 150 | 200 |
| C_{max} (ng/mL) | 1.44 | 2.03 |
| $AUC_{0-1344h}$ (ng·h/mL) | 336 | 419 |
| T_{max} (h) | 144* | 216 |

*One quantifiable concentration only

Study title: Single-Dose Intramuscular Depot Exploratory Irritation Study in Dogs

Study no.: Otsuka Study No. 018754 (BMS Study No. DM02015)

Study report: Otsuka Report No.014893, 2002

Conducting laboratory and location: BMS Departments of Toxicology and Pathology
Mt. Vernon, Indiana USA

Date of study initiation: 11 March 2002

GLP compliance: No

QA statement: No

Drug, lot #: Aripiprazole (OPC-31, OPC14597, BMS-337039) Batch C99G74M

Key Study Findings:

Aripiprazole formulation (100 mg/mL in 1% CMC) administered to beagle dogs (1/sex/dose) as two injections at dose volumes of 2, 3 or 4 mL to provide doses of 200, 300 or 400 mg, respectively, produced injection site changes (swelling and erythema) at MD and HD. At necropsy, white discoloration (deposits of test article) at the injection sites at all dose levels, and red discoloration of subcutaneous tissue (hemorrhage) were noted in MD and HD groups. Microscopically, the primary tissue response to deposited drug (birefringent crystalline material) was subacute inflammation at all doses (mild at LD, and moderate to marked at the higher doses). Other microscopic injection site changes were: edema, hemorrhage, degeneration of skeletal muscle, granulomatous inflammation, and fibroplasia/fibrosis. Systemic exposure to aripiprazole and metabolite OPC-14857 was dose related in females but not in males. The t_{max} ranged from 3 h to 72 h. C_{max} and AUC increased dose-proportionally in females, but showed no dose dependency in males.

Methods

| | |
|--------------------------|--|
| Doses: | 200, 300 or 400 mg |
| Frequency of dosing: | Single dose |
| Route of administration: | Intramuscularly, as two injections of equal volume in the right thigh muscle |
| Dose volume: | 2, 3 or 4 mL of aripiprazole suspension (100 mg/mL in 1% CMC) |
| Formulation/Vehicle: | Aripiprazole suspension (100 mg/mL) in (b) (4) CMC/ (b) (4) sodium carboxymethylcellulose (CMC), (b) (4) mannitol, and water (mean particle size of (b) (4)) |
| Species/Strain: | Dogs/Beagle |
| Number/Sex/Group: | 1/sex/dose |
| Age: | 10-12 months at study initiation |
| Weight: | 6-12 kg |

Deviation from study protocol:

Pharmacokinetics - Plasma concentrations of BMS-337040 were not determined.

Reason for amendment:

This metabolite of BMS-337039 was not detectable in a previous single-dose intramuscular exploratory study of prototype depot formulations and therefore was not analyzed.

Pharmacokinetics - The plasma samples were analyzed for BMS-337045, a metabolite of BMS-337039. The data will be included in the study records, but will not be reported.

Impact of Deviation:

Since this was an inadvertent measurement of a known metabolite of BMS-337039 that is not formed in any appreciable levels, it is the opinion of the study director that this deviation did not affect the validity or integrity of the study.

Experimental design: Aripiprazole formulation (100 mg/mL in ^{(b) (4)} CMC) was administered to dogs as two injections at dose volumes of 2, 3 or 4 mL to provide doses of 200, 300 or 400 mg, respectively (see the sponsor's table below)

| Group Number | Dose Volume (ml) | Number and Site ^a of Injections | Concentration BMS-337039 (mg/ml) | Dose (mg) | Number of Animals |
|--|------------------|--|----------------------------------|-----------|-------------------|
| 1 | 2 (2 x 1 ml) | 2 - right | 100 | 200 | 1 M, 1 F |
| 2 ^b | 3 (2 x 1.5 ml) | 2 - right | 100 | 300 | 1 M, 1 F |
| 3 ^b | 4 (2 x 2 ml) | 2 - right | 100 | 400 | 1 M, 1 F |
| ^a Thigh muscle | | | | | |
| ^b Group 2 and 3 animals will be dosed only if clinically acceptable tolerance in Group 1. | | | | | |

Observations: Clinical signs and injection site observations, periodic determination of serum aspartate aminotransferase (AST) and creatine kinase (CK), and gross and microscopic pathologic examinations of the injection sites on Day 4. Blood samples to determine systemic exposure of aripiprazole and the metabolite OPC-14857 (BMS-337044) were collected at 0.2, 0.5, 1, 3, and 8 hrs after injection on Day 1, and on days 2 and 4 of the study.

Results: Injection site swelling and erythema were present at MD and HD. There were no changes in AST or CPK compared to pre-test values. At necropsy, white discoloration (deposits of the test article) was apparent at the injection sites at all dose levels, and red discoloration of subcutaneous tissue (hemorrhage) and “minimal or mild” swelling were noted in the mid- and high dose groups.

Histopathology: Microscopically, the primary tissue response to deposited drug (birefringent crystalline material) was subacute inflammation (mild at LD, and moderate to marked at the higher doses). Other microscopic injection site changes observed were: edema, hemorrhage, degeneration of skeletal muscle, granulomatous inflammation, and fibroplasia/fibrosis. These changes were minimal to mild in severity.

Toxicokinetics:

Systemic exposure to aripiprazole and OPC-14857 was dose related in females but not in males. The t_{max} varied considerably across the dose groups and ranged from 3 h to 72 h. Between the low and mid dose, the C_{max} and AUC values increased approximately equally to the dose

increment in females, but displayed no dose dependency in males (see the following sponsor's table).

TK of aripiprazole and its metabolite OPC-14857 (BMS-337044)

| IM Dose [mg] | BMS-337039 | | BMS-337044 | |
|--|------------|-----------|------------|-----------|
| | Male | Female | Male | Female |
| C_{max} [ng/mL] (T_{max} [h]) | | | | |
| 200 | 83.1 (8) | 34.8 (8) | 43.1 (24) | 14.1 (24) |
| 300 | 28.1 (24) | 58.2 (24) | 9.5 (72) | 19.9 (24) |
| 400 | 58.6 (3) | 72.6 (72) | 20.1 (24) | 36.0 (72) |
| C_{max} Ratios | | | | |
| 1:1.5:2 Dose Ratio | 1:0.3:0.7 | 1:1.7:2.1 | 1:0.2:0.5 | 1:1.4:2.6 |
| IM Dose [mg] | BMS-337039 | | BMS-337044 | |
| | Male | Female | Male | Female |
| AUC^a [ng•days/mL] | | | | |
| 200 | 222.6 | 73.4 | 92.7 | 29.6 |
| 300 | 75.2 | 131.6 | 22.7 | 36.5 |
| 400 | 83.9 | 181.0 | 40.8 | 86.7 |
| AUC Ratios | | | | |
| 1:1.5:2 Dose Ratio | 1:0.3:0.4 | 1:1.8:2.5 | 1:0.2:0.4 | 1:1.2:2.9 |

^a Calculated from time zero to 72 h after dosing (study day 4).

Study title: Single-dose Intramuscular Exploratory Study of Prototype Depot Formulations in Monkeys

Study no.: Otsuka Study No. 019796 (BMS Study No. DM02005)

Study report: Otsuka Report No.015503, 2002

Conducting laboratory and location: BMS Departments of Toxicology and Pathology
Mt. Vernon, Indiana USA

Date of study initiation: February 5, 2002

GLP compliance: No (not necessary)

QA statement: No

Drug, lot #: Aripiprazole (OPC-31, OPC14597, BMS-337039) Batch

Key Study Findings: Intramuscular administration of 25 and 50 mg aripiprazole (as a single 0.5 ml dose of prototype depot formulation containing 50 or 100 mg/ml of aripiprazole in CMC) into the thigh muscle of cynomolgus monkeys produced significant acute local irritation, characterized by injection site swelling and discoloration. Grossly apparent white deposits of drug in the injection site muscles and granulomatous inflammation associated with deposited drug were the principal morphologic alterations at injection sites. Although the inflammation decreased over time neither the gross nor microscopic changes completely resolved by 29 days postdose. Drug-related increase in aspartate aminotransferase (1.4-fold to 6.3- fold) and creatine kinase (3-fold to 18.7-fold) vs. pretest values were registered at both tested doses on day 2 postdose, indicative of muscle injury in both sexes. Similar gross and microscopic injection site changes had been observed in rabbits and dogs at comparable or higher doses (0.5 ml/injection in rabbits and 1 ml/injection in dogs) of the same or similar prototype depot formulations. However, in contrast to monkeys, these prototype formulations did not produce increases in serum aspartate aminotransferase or creatine kinase values in other species. Because of the

“notable” acute local irritation associated with single doses of these prototype formulations, the investigators considered cynomolgus monkey “not an optimal non-rodent species for future evaluations of larger injection volumes or repeated dosing of aripiprazole intramuscular depot formulations”. Thus, the greater susceptibility of monkeys to local irritation by aripiprazole IM depot formulation would hinder administration of doses sufficiently high to achieve systemic exposure to aripiprazole adequate for safety assessment.

Methods

Doses: 25 and 50 mg

Frequency of dosing: Single dose

Route of administration: IM

Dose volume: 0.5 mL

Formulation/Vehicle: Aripiprazole suspension (50 and 100 mg/mL, mean particle size of (b) (4)) in (b) (4) CMC/ (b) (4) sodium carboxymethylcellulose, (b) (4) mannitol, and water

Species/Strain: Monkey/Cynomolgus

Number/Sex/Group: 3

Age: 2.5 to 4.3 years at study initiation

Weight: 4.0 to 6.6 kg for males and 3.2 to 4.3 kg for females

Satellite groups:

Study design:

| Experimental Design | | | | |
|-----------------------------|--------------------------|---|--|-------------------------|
| Group Number | Daily Dose | | Concentration BMS-337039 (mg/ml) | Number of Animals |
| | Volume (ml/injection) | Number and Site ^a of Injections | | |
| 1 (Test Article Carrier #3) | 0.5 | 1 - left | 0 | 3M, 3F |
| 2 (Formulation #4) | 0.5 | 1 - right | 50 | |
| 3 (Saline Control) | 0.5 | 1 - left | 0 | 3M, 3F |
| 4 (Formulation #5) | 0.5 | 1 - right | 100 | |

^a Thigh Muscle

Two aripiprazole prototype intramuscular depot formulations at concentrations of 50 or 100 mg/ml in (b) (4) sodium carboxymethylcellulose (CMC) were administered IM in the thigh muscle, as 0.5 ml single doses to two groups of 3 monkeys/sex. The corresponding test article carrier or saline was administered in the other thigh muscle of the same animals. Therefore, there was no control group included in this study.

Observations: survival, clinical signs and injection site observations, serum aspartate aminotransferase and creatine kinase, and gross and microscopic pathology (days 7, 15, and 29 postdose) of the injection sites. To evaluate systemic exposure, plasma concentrations of aripiprazole and its pharmacologically active metabolites OPC-14857 (BMS-337044) and DM-1451 (BMS-337040) were measured through day 29 postdose; only the results from the 100 mg/ml formulation were reported. Blood samples were collected on days 1, 2, 4, 7, 10, 15, 22, and 29. On day 1, samples were collected at 10 and 30 minutes, 1, 3, and 8 hours postdose. For all other days, samples were collected at approximately 24- hour intervals from the time of dosing. Necropsies were conducted on days 7, 15 and 29 post-dose.

Results: There was no mortality. Hypoactivity was noted in 1 female given the 100 mg/mL formulation; it was considered pharmacologically mediated and had been observed previously in oral studies with aripiprazole in monkeys.

Acute injection site swelling or discoloration was noted in 1 male or 1 female monkey at 100 mg/mL. Drug-related increase in aspartate aminotransferase (1.4-fold to 6.3- fold) and creatine kinase (3-fold to 18.7-fold) vs. pretest values occurred in both sexes on Day 2 post administration of the CMC/50 mg/mL formulation and the saline/100 mg/mL formulation. These changes affected from one to all three animals per group. Comparable changes were not seen in rabbits or dogs given the same or larger injection site volumes of these or comparable formulations. There were no indications of increased values of these parameters on day 4 post-dose. On day 7 postdose, a mild increase in AST (4.5x compared to the pretest value) and an increase in CK (21.8x compared to the pretest value) occurred in one LD female (50 mg/ml aripiprazole in (b)(4) sodium carboxymethylcellulose, Group 2). However, since there were no indications of increased values of these parameters on day 4 post-dose in this animal, or day 7 post-dose in the males or other females in this group or in animals given the higher concentration of drug (100 mg/ml aripiprazole in (b)(4) sodium carboxymethyl-cellulose), the relationship of these changes to treatment was uncertain.

Aspartate aminotransferase (AST) and creatine kinase (CK) in monkeys, Day 2 post-dose

| Males | | | | Females | | | |
|-------------------------------|------|------------|-----------|-------------------------------|------|------------|-----------|
| Day: 2 relative to Start Date | | | | Day: 2 relative to Start Date | | | |
| Group | Sex | AST U/L | CK U/L | Group | Sex | AST U/L | CK U/L |
| 1/2m | Mean | 92.0 | 515.7 | 1/2f | Mean | 89.3 | 863.3 |
| | S.D. | 34.1 | 67.5 | | S.D. | 54.4 | 909.9 |
| | N | 3 | 3 | | N | 3 | 3 |
| ----- | | | | ----- | | | |
| 3/4m | Mean | 126.3 | 5870.7 | 3/4f | Mean | 152.3 | 3024.3 |
| | S.D. | 92.2 | 9366.4 | | S.D. | 165.1 | 4616.2 |
| | N | 3 | 3 | | N | 3 | 3 |
| ----- | | | | ----- | | | |

Gross pathology

At necropsy, white discoloration, apparently test article deposits in muscle, was noted in the injection sites through Day 29 for both concentrations. The white foci were of variable shape and size and did not decrease with time.

Histopathology

Day 7 Necropsy: mild focal granulomatous inflammation, characterized primarily by the accumulation of neutrophils and macrophages with foamy cytoplasm around eosinophilic deposits of the test article. Examination of formalin-fixed, unstained frozen sections of muscle revealed the presence of birefringent crystals (consistent with drug) in the injection sites at both doses.

Minimal focal skeletal muscle regeneration occurred sporadically in the injection site muscles dosed with carrier and aripiprazole CMC formulations at both doses.

Day 15 Necropsy: mild focal granulomatous inflammation, characterized primarily by the accumulation of macrophages with foamy cytoplasm and lesser numbers of neutrophils around eosinophilic deposits of the test article and presence of birefringent crystals (consistent with drug) in the injection sites of both tested doses. Minimal fibroplasia/fibrosis in the injection site

muscles of one LD animal (F) and 2 HD animals (M and F) necropsied at this timepoint. Other changes were minimal skeletal muscle cell regeneration in the male and minimal hemorrhage in the female at HD.

Day 29 Necropsy: Focal granulomatous inflammation in the injection site muscles (minimal to mild at LD and mild at HD); minimal fibroplasia/fibrosis in the injection site muscle of the HD male. Presence of birefringent crystals (consistent with drug) in the injection sites of both doses.

The CMC carrier was associated with focal minimal chronic inflammation in the injection site muscle of a female, and a focus of perivascular necrosis and subacute inflammation as a consequence of needle damage in the injection site muscle of the male. At day 15, minimal skeletal muscle regeneration occurred in the injection site of one female dosed with CMC.

Saline Control

Day 7: Minimal focal skeletal muscle regeneration and minimal focal subacute inflammation occurred in the injection site muscles of the female dosed with saline.

Day 15: Minimal skeletal muscle regeneration and minimal subacute inflammation were apparent in the injection site muscles of both the male and female dosed with saline.

Day 29: A focus of minimal skeletal muscle cell regeneration, that correlated with the macroscopically apparent faint area of grey discoloration in the injection site muscle of the male dosed with saline.

Toxicokinetics

Plasma Toxicokinetics of Aripiprazole and Metabolites OPC-14857 and DM-1451 Following Single Intramuscular Injection of 50 mg (100 mg/mL) Aripiprazole to Monkeys

| Dose (mg) | Aripiprazole | | DM-1451 | | OPC-14857 | |
|-----------------------------------|--------------|--------|---------|-----------------|-----------|--------|
| | Male | Female | Male | Female | Male | Female |
| C _{max} (ng/mL) | 18.5 | 49.2 | <1 | 1.18 (n = 1) | 4.4 | 15.7 |
| AUC _{0-6d} (ng·d/mL) | 63.3 | 171.6 | NC | NC | 13.5 | 56.7 |
| AUC _{0-14d} (ng·d/mL) | 154.2 | 319.4 | NC | NC | 31.7 | 104.4 |
| AUC _{0-28d} (ng·d/mL) | 264.5 | 413.2 | NC | NC | 49.9 | 80.9 |

NC = Not calculated. The lower limit of quantification was 1 ng/mL.

The 100 mg/ml formulation produced systemic exposure to aripiprazole and metabolite OPC-14857 (BMS-337044) for up to 29 days postdose. Exposure was higher and more variable in female than in male monkeys. AUC values increased from day 7 through day 29 post-dose indicating continued absorption over this period. Analysis for metabolite DM-1451 (BMS-337040) showed that most concentrations were below the limit of quantitation (1 ng/mL).

In summary, acute injection site swelling or discoloration was observed in 1 male and 1 female monkey at 100 mg/mL and later observed in all 3 monkeys per group of each formulation. These findings were accompanied by a drug-related increase in aspartate aminotransferase (1.4-fold to 6.3- fold) and creatine kinase (3-fold to 18.7-fold) vs. pretest values recorded on Day 2 post administration of both formulations. Comparable changes were not seen in rabbits or dogs given the same or larger injection site volumes of these or comparable formulations. There were no indications of increased values of these parameters on day 4 post-dose. In general injection site findings included “minimal to mild” granulomatous inflammatory response to birefringent

crystalline drug deposits characterized by accumulation of macrophages with foamy cytoplasm and eosinophilic deposits of the test article. The severity of the microscopic changes tended to decrease with time, but complete resolution of the inflammatory process did not occur by Day 29. Focal inflammation and skeletal muscle regeneration was observed in the injections sites from CMC and saline administration as a consequence of needle damage from injection. This sensitivity to the local irritation potential of intramuscular depot formulations of aripiprazole probably reflects, in large part, the relatively small thigh-muscle mass in monkeys. The greater susceptibility of monkeys to local irritation by aripiprazole IM depot formulation would hinder administration of doses sufficiently high to achieve systemic exposure to aripiprazole adequate for safety assessment.

Different formulations of aripiprazole were examined to determine a suitable formulation for use in toxicity studies. A formulation using CMC was used to examine the local tolerance of aripiprazole in several studies as described above. Another formulation of aripiprazole in 15% Captisol was also examined in the rat and rabbit in the following studies.

Study title: Two-Week Intramuscular Tolerance Study in Rats

Study no.: Otsuka Study No. 016456 (BMS Study No. DM00005)

Study report: Otsuka Report No.013598, 2000

Conducting laboratory and location: BMS Departments of Toxicology and Pathology
Mt. Vernon, Indiana USA

Date of study initiation: January 11, 2000

GLP compliance: Yes

QA statement: Yes

Drug, lot #: Aripiprazole (OPC-31, OPC14597, BMS-337039) Batch No. C99G74M

Key study findings: IM administration of aripiprazole at doses 1 or 3.75 mg/kg/day for 2 weeks to rats (as a solution in 15% Captisol and 0.05 M tartrate buffer; dose volume 0.5 mL/kg at concentrations of 2 or 7.5 mg/mL), was associated with a transient AST elevation (HDF only) and morphologic evidence of reversible muscle irritation. A dose-related increased incidence of discoloration and swelling was observed at injection sites. Microscopically, degeneration, necrosis, and regeneration of muscle and subacute inflammation, hemorrhage, edema, and fibroplasia/fibrosis of muscle and surrounding connective tissue occurred at the injection sites, with generally minimal severity in the vehicle control and LD groups, and at a minimal to mild severity in HD group. In both dose groups, the fibroplasia was located primarily in connective tissue adjacent to the muscle, with lesser involvement of intramuscular connective tissue. The injection site changes were nearly completely reversible during the 2-week post-dose period. Only minimal, late stage muscle regeneration and fibroplasia/fibrosis were still present in vehicle control and dosed groups. Systemic exposure to aripiprazole was dose-related and exposure was higher in males. DM-1451 was the major circulating metabolite. Plasma concentrations for metabolites OPC-3373 and DCPD were below the LLOQ.

Methods

Doses: 0, 1 or 3.75 mg/kg/day

Frequency of dosing: Daily for 2 weeks

Route of administration: IM

Dose volume: 0.5 mL/kg (at concentrations of 2 or 7.5 mg/mL)

Formulation/Vehicle: Aripiprazole, as a solution in 15% (w/v) Captisol® (sulfobutyletherbeta-cyclodextrin) and 0.05 M tartrate buffer, in Sterile Water for injection, DSP, pH 4.3 to 4.5.

Species/Strain: Rat/ CrI:CD@(SD) IGS BR

Number/Sex/Group: 12

Age: 12 weeks at study initiation

Weight: 333.7 to 442.0 g (M) and 208.8 to 253.2 g (F)

Study design:

Dosed and vehicle control groups of 12 rats/sex were administered 0.5 mL/kg of the test

formulation or vehicle once daily for 2 weeks by intramuscular injection into the area of the biceps femoris of the hind leg, alternating daily between the right and left leg muscles. 8 rats/sex/group were necropsied and evaluated after 2 weeks of dosing and the rest at the end of a 2-week recovery period. Blood samples were collected at various time points on Day 10 to determine the systemic exposures to aripiprazole and selected metabolites.

Observations: Mortality and clinical observations (twice daily), injection sites (daily), body weight (baseline and weekly), food consumption (baseline and weekly); creatine kinase and aspartate aminotransferase (blood samples obtained at the scheduled necropsies); gross- and histopathology (representative samples of the injection sites from all animals were stained with hematoxylin and eosin, and examined by light microscopy)

Blood samples for toxicokinetic evaluation were collected at 10 and 30 min and 1, 3, 8, and 24 hrs after a daily dose on Day 10 from four animals/sex/group/time point for analysis of aripiprazole and its metabolites DM-1451, OPC-14857, DM-1452, OPC-3373 and 1-(2,3-dichlorophenyl) piperazine (DCPP).

Results

There was no mortality and no changes in body weight and food consumption. During the dosing phase, a dose-related increased incidence of discoloration and swelling at injection sites was observed. These findings resolved after 3 days of recovery. At the end of the dosing phase, a slight but statistically significant increase in the mean serum AST was observed in females at the high dose, but the mean and all individual values were within the range of historic controls. Mean AST values in males at all doses and females at 2 mg/ml did not differ from controls. There were no drug-related changes in mean serum creatine kinase (CK) at any dose (see the following sponsor's table).

**Serum AST and CPK in Rats Treated Intramuscularly
for 2 Weeks with Aripiprazole**

| Dose (mg/kg) | End of Dosing | | | | End of Recovery | | | |
|-----------------|----------------|-----------------|-----------------|-----------------|-----------------|---------------|-----------------|-----------------|
| | AST (IU/L) | | CPK (IU/L) | | AST (IU/L) | | CPK (IU/L) | |
| | Male | Female | Male | Female | Male | Female | Male | Female |
| Control | 81.1 ± 9.0 | 79.1 ± 10.2 | 176.1 ± 62.0 | 143.1 ± 40.8 | 72.3 ± 9.5 | 71.3 ± 4.3 | 115.5 ± 25.3 | 121.3 ± 31.9 |
| 1 | 80.8 ± 12.5 | 83.9 ± 14.2 | 162.0 ± 42.5 | 140.0 ± 22.0 | 73.0 ± 12.5 | 75.0 ± 7.6 | 131.3 ± 67.8 | 138.5 ± 22.3 |
| 3.75 | 85.1 ± 9.3 | 94.6 ± 10.8* | 147.5 ± 47.5 | 128.8 ± 21.6 | 73.0 ± 8.8 | 69.3 ± 5.9 | 169.5 ± 82.0 | 114.5 ± 37.6 |

* $p \leq 0.05$; Values are the mean \pm standard deviation

Gross pathology

At the end-of-dosing necropsy, increased incidence of dark and/or red discoloration of injection sites was present.

Histopathology

Peer review: Yes

At the injection sites, degeneration, necrosis, and regeneration of muscle and subacute inflammation, hemorrhage, edema, and fibroplasia/fibrosis of muscle and surrounding connective tissue occurred with a "minimal severity" in the vehicle and LD groups and at a

“minimal to mild” severity in the HD group. In all dose groups, the fibroplasia was located primarily in connective tissue adjacent to the muscle, with lesser involvement of intramuscular connective tissue in most animals. During the 2-week recovery period, there was nearly complete reversibility of injection site changes. Only minimal, late stage muscle regeneration and fibroplasia/fibrosis were still present in both control and dosed animals. The injection site histopathology changes in vehicle control and LD groups were generally minimal in severity and attributed to the vehicle and/or dosing procedure, while changes at the high dose were minimal to mild and were in part drug related. Overall, intramuscular administration of a 15% Captisol formulation of aripiprazole to rats for 2 weeks was associated with morphologic evidence of reversible muscle irritation.

Incidence of Findings at the Injection Sites at End-of-Dose

| Dose (mg/ml/day): | 0 | 2 | 7.5 |
|---------------------------------------|-----|-----|-----|
| No. of Rats (M/F): | 8/8 | 8/8 | 8/8 |
| Sex: | M/F | M/F | M/F |
| <u>Necrosis, muscle:</u> | | | |
| Minimal severity | 7/7 | 7/7 | 4/2 |
| Mild severity | - | - | 4/5 |
| <u>Degeneration, muscle:</u> | | | |
| Minimal severity | 8/7 | 7/7 | 8/8 |
| <u>Inflammation, subacute:</u> | | | |
| Minimal severity | 7/8 | 7/7 | 1/0 |
| Mild severity | - | 1/1 | 7/8 |
| <u>Hemorrhage:</u> | | | |
| Minimal severity | 5/3 | 5/6 | 5/2 |
| Mild severity | 0/2 | 0/1 | 2/5 |
| <u>Edema:</u> | | | |
| Minimal severity | 0/1 | 2/1 | 8/4 |
| Mild severity | - | - | 0/4 |
| <u>Regeneration, muscle:</u> | | | |
| Minimal severity | 6/8 | 7/8 | 0/2 |
| Mild severity | 2/0 | 1/0 | 8/6 |
| <u>Fibroplasia/fibrosis:</u> | | | |
| Minimal severity | 6/8 | 6/8 | - |
| Mild severity | - | 1/0 | 8/8 |

- Indicates absence of finding in group

Toxicokinetics

Systemic exposure to aripiprazole was dose-related and higher in males than in females. DM-1451 was the major circulating metabolite; it was measurable up to 3 h. and 8 h. after dosing at

LD and HD, respectively. Plasma concentrations for metabolites OPC-14857 and DM-1452 were below the LLOQ at LD, but at HD they were measurable up to 3 hrs post dose. The rest of metabolites (OPC-3373 and DCPD) were below the LLOQ at all time points for both tested dose levels.

**Plasma Toxicokinetics of Aripiprazole and Metabolites Following Daily IM Injection of Aripiprazole
for 2 Weeks to Rats**

| Dose (mg/kg) | Aripiprazole | | OPC-14857 | | DM-1451 | | DM-1452 | |
|---|--------------|--------|-----------|--------|---------|--------|---------|--------|
| | Male | Female | Male | Female | Male | Female | Male | Female |
| C_{max} (ng/mL) | | | | | | | | |
| 1 | 198 | 182 | <1 | <1 | 5.50 | 3.05 | <1 | <1 |
| 3.75 | 911 | 719 | 2.21 | 2.62 | 12.3 | 5.92 | 1.76 | 1.86 |
| AUC_{0-t} (ng · hr/mL)^a | | | | | | | | |
| 1 | 365 | 272 | NC | NC | 10.61 | NC | NC | NC |
| 3.75 | 18.50 | 11.31 | 5.48 | 6.60 | 55.5 | 15.27 | 4.52 | 4.35 |

NC = Not calculated. The lower limit of quantification was 1 ng/mL.

^aFor aripiprazole, t = 8 to 24 hours. For OPC-14857, DM-1451 and DM-1452, t = 3 to 8 hours

Study title: Single-Dose Intramuscular Irritation Study in RabbitsStudy no.: Otsuka Study No. 016719 (BMS Study No. 99356)

Study report: Otsuka Report No.013731, 2000

Conducting laboratory and location: (b) (4)

Date of study initiation: 20 December 1999

GLP compliance: Yes

QA statement: Yes

Drug, lot #: Aripiprazole (OPC-31, OPC14597, BMS-337039) Lot No. C99G74M

Key study findings: Administration of a single IM dose (1 mL) of aripiprazole, at concentrations of 2, 4 or 7.5 mg/mL (as a solution in 15% Captisol and 0.05 M tartrate buffer), to female rabbits resulted in dose-related increased creatin kinase in all dosed groups on Day 2 compared to saline or vehicle controls. Injection site edema (“slight”) was observed across all groups including controls. At necropsy, focal tan areas associated with and/or surrounded by hemorrhage were observed at injection sites at MD and HD. Microscopically, muscle degeneration/regeneration and inflammation were observed on Day 4 post dose with “minimal” severity in the saline control group, “minimal to mild” severity in the vehicle control, LD and MD groups, and “mild to moderate” severity in the HD group. “Minimal to mild” hemorrhage and mineralization of degenerative muscle fibers were noted in vehicle- and drug-treated groups. By Day 18 post dose, there was nearly complete reversibility of injection site effects: minimal muscle regeneration and mineralization were still present in one animal at 2 mg/mL, and slight edema and “minimal” muscle regeneration, inflammation, mineralization, and/or fibrosis were apparent in the HD group. In conclusion, evidence of muscle irritation was observed in rabbits given a single intramuscular injection of 15% Captisol®/0.05M tartrate buffer and 2, 4 and 7.5 mg/mL aripiprazole formulations. The microscopic injection site changes observed at LD appeared to be primarily related to the carrier and the dosing procedure since the incidence and severity of microscopic changes were generally comparable to those observed in the vehicle control group.

Methods

| | |
|--------------------------|---|
| Doses: | 2, 4 or 7.5 mg |
| Frequency of dosing: | Daily for 2 weeks |
| Route of administration: | IM |
| Dose volume: | 1 mL |
| Formulation/Vehicle: | Aripiprazole solution in 15% Captisol® and 0.05M Tartrate buffer (pH 4.5) |
| Species/Strain: | Rabbits/ New Zealand White SPF |
| Number/Sex/Group: | 6 (females only) |
| Age: | 15 wks at study initiation |
| Weight: | 3.1 to 3.6 kg. |
| Satellite groups: | No |

Study design: Aripiprazole concentrations selected spanned those to be administered clinically (2 and 7.5 mg/mL) (higher concentrations of aripiprazole in 15% Captisol® resulted in precipitation of test article at the site of injection). Two control groups, a saline control (0.9% sodium chloride) and a vehicle control, were used in this study. A post-dose recovery period of

18 days was used. Four animals per group were necropsied on Day 4, and 2 animals/group were necropsied on Day 18.

Observations:

Body weight, clinical and injection site observations, serum creatine phosphokinase and aspartate aminotransferase [blood for AST and CK determination was collected from all animals prior to in-life initiation (day -4), on day 2 (24 hrs after IM injection) and on day 18 (recovery phase)], and gross and histopathology evaluations of injection sites.

Results

There was no mortality, clinical signs, or effect on body weights. “Slight” injection site edema was observed across all groups including controls. Increased creatine kinase relative to pre-dose values was observed in all groups, including controls. A dose-related increase in creatine kinase compared to saline or vehicle controls was observed in all dosed groups on Day 2; this change was reversible by Day 18 (see the sponsor’s table below).

Serum AST and CK in Rabbits Treated Intramuscularly with a Single Dose of Aripiprazole

| Study Day | 0 (saline) | 0 (vehicle) | 2 mg/mL | 4 mg/mL | 7.5 mg/mL |
|---------------------|-------------|----------------|---------------|---------------------------|-----------------------------|
| AST (IU/L) | | | | | |
| Day -4 | 37 ± 7.1 | 35 ± 9.2 | 31 ± 8.3 | 36 ± 10.5 | 37 ± 7.6 |
| Day 2 | 29 ± 4.5 | 29 ± 9.8 | 35 ± 11.6 | 31 ± 7.3 | 30 ± 8.4 |
| Day 18 ^a | 36 | 30 | 29 | 21 | 36 |
| CPK (IU/L) | | | | | |
| Day -4 | 427 ± 141.6 | 479 ± 123.7 | 379 ± 33.9 | 310 ± 110.1 | 402 ± 105.7 |
| Day 2 | 623 ± 159.7 | 2388 ± 649.1** | 2167 ± 960.8* | 3149 ± 582.8 [#] | 6042 ± 1139.3 ^{#Φ} |
| Day 18 ^a | 757 | 429 | 460 | 173 | 360 |

^aOn Day 18, n = 2 rabbits.

* p ≤ 0.05; ** p ≤ 0.01; # p ≤ 0.001 compared to saline control. Φ p ≤ 0.001 compared to vehicle control. Values are the mean ± standard deviation.

Gross pathology

Focal tan-colored areas associated with and/or surrounded by hemorrhage were observed at injection sites of some animals at MD and HD on Day 4 necropsy.

Histopathology

The saline control produced muscular changes compatible with the physical trauma of needle puncture and fluid instillation. The vehicle control produced changes in the muscle that were slightly more severe than the saline control group. Subjective microscopic assessment of the test article-treated injection sites suggested a drug-related increase in the severity of degenerative/regenerative changes, inflammation, hemorrhage and mineralization in the high-dose group. These changes appeared reversible as noted by the near total resolution of lesions by the end of the recovery period (only minimal residual muscle regeneration and mineralization in one LD and one HD animal and minimal inflammation in two HD animals remained on day 18). In addition, fibrosis was noted in the high-dose group at the end of the recovery period,

11 Integrated Summary and Safety Evaluation

The nonclinical testing strategy for intramuscular aripiprazole was abbreviated since it was supported, in part, by results from previous in vitro and in vivo nonclinical studies conducted to support other formulations and indications for aripiprazole. No genetic toxicity, carcinogenicity, reproductive/developmental toxicity, or juvenile toxicity studies were performed with intramuscular aripiprazole because these specific toxicities had been previously evaluated following oral and/or intravenous administration described in the primary and supplemental NDAs. Since the systemic human plasma exposure to aripiprazole at MRHD for the intramuscular depot formulation (400 mg; 200 mg BID) did not exceed the systemic exposure at the oral MRHD (30 mg/day), carcinogenicity and reproductive and developmental toxicity studies using the intramuscular depot formulation of aripiprazole would not have provided any further information to assess the potential hazard in humans. Therefore, a carcinogenicity study of the intramuscular depot formulation was not conducted in accordance with the Executive CAC recommendation (Executive CAC Minutes, September 26, 2008) and a waiver for undertaking developmental and reproductive toxicity studies was granted by the FDA to Otsuka, (June 23, 2010).

Studies conducted specifically with the IM depot formulation include PK/TK, general toxicity and local irritation profile in single- and repeat-dose regimens in rats, dogs, monkeys and rabbits. Pharmacokinetics: After injection of aripiprazole IM depot formulation in rats, the C_{max} and AUC of aripiprazole increased with the dose increment, and there was no gender difference in plasma concentrations. Aripiprazole injected as a depot formulation was stable at the injection site without being metabolized or decomposed. Based on the residual amount of aripiprazole in the injection site, the absorption of aripiprazole increased approximately from 39% up to 84% from 168 hours to 1008 hrs post injection, indicating a controlled release of the drug into systemic circulation. The absolute bioavailability (assessed in mini pigs) indicated that aripiprazole was completely bioavailable from IM and SC routes (111 % and 102%, respectively) and incompletely bioavailable by oral route (22.3%), which is suggestive of the extensive first pass metabolism and/or incomplete absorption of aripiprazole following PO administration.

Assessment of aripiprazole metabolites OPC-14857, DM-1451, DM-1452, OPC-3373 and 1-(2, 3 dichlorophenyl)piperazine (DCPP) following single IM injections of aripiprazole IM depot formulation to rats, showed that the plasma concentrations of DM-1451 increased nonlinearly with the dose increment while the rest of the assessed metabolites (OPC-14857, DM-1452, OPC-3373 and DCPP) were below the lower limit of quantification (LLQ). The rank order of the C_{max} and AUC_t for aripiprazole and its metabolites was aripiprazole > DM-1451 > OPC-3373 > OPC-14857.

The pivotal repeat dose general toxicity studies supporting the clinical development of intramuscular aripiprazole IM depot formulation were performed in rats (26-weeks), dogs (52 weeks) and monkeys (4 weeks). Aripiprazole was administered weekly in the 26-week study in rats and the 52-week study in dogs, whereas daily administrations were used for the 4-week monkey study. All pivotal toxicity studies were conducted in compliance with GLP regulations. Dose selection for pivotal studies was based on results from preceding intramuscular exploratory or range-finding studies.

Rats: Weekly intramuscular injections of OPC-14597 depot formulation to rats at maximal dose of 100 mg/kg for a period of 26-weeks resulted in granulomatous inflammation to the deposited drug at the injection site. There was no morphologic evidence of drug-related skeletal muscle necrosis in any of the dosed animals. Morphological changes in female reproductive and mammary tissues and atrophy of pituitary pars intermedia in both genders were present at all dose levels, and were likely pharmacologically mediated as a consequence of D₂ partial agonistic activity of OPC-14597. The NOAEL was 50 mg/kg/week in the males and 100 mg/kg/week in the females since low body weight and decreased food consumption were observed in the males given 100 mg/kg, but not in the treated females under the conditions of the present study. At the NOAEL, the C_{max} and AUC_{7d} of aripiprazole at week 26 were 98.1ng/mL and 598.6 ng.d/mL respectively for males and 1135.3 ng/mL and 4336.2 ng.d/mL, respectively for females.

Dogs: Administration of aripiprazole depot formulation to beagle dogs by weekly IM injections at doses of 10, 20 and 40 mg/kg of aripiprazole for 52 weeks resulted in localized granulomatous inflammation at the injection site in the males at all dosages and in HD females, and in necrosis of muscle fibers involved in the granulomatous inflammation in 1 HD female at the end of the 52-week dosing period. The inflammation consisted of accumulation of macrophages, eosinophilic deposits, foreign body giant cells, lymphocytes and polymorphonuclear leukocytes, in association with deposits of birefringent crystal-like material (interpreted as drug). The gross- and histopathology changes at the injection site were reversible by the end of the 26-week recovery period. There were no clinical signs, and no drug-related changes in body weight, food consumption, hematology, blood chemistry, urinalysis, ophthalmology, audiology, electrocardiography, body temperature, no drug-related changes in organ weights or in gross- and histopathology of the systemic organs. The NOAEL was 40 mg/kg/week. At the NOAEL, the C_{max} and AUC_{7d} of aripiprazole at Week 52 were 438ng/mL and 2460 ng.d/mL, respectively for males and 306 ng/mL and 1820 ng.d/mL, respectively for females.

Monkeys: Intramuscular daily administration of aripiprazole depot formulation at doses of 2, 4, or 7.5 mg/kg to Cynomolgus monkeys for 29 days, resulted, at all doses, in CNS-related clinical signs (reduced activity, most likely pharmacologically mediated) and reversible skeletal muscle injury at the injection site. Small increases in serum aspartate aminotransferase at mid- and high dose were likely a consequence of injection site injury. Microscopically, increased incidence and/or severity of skeletal muscle necrosis (minimal to mild), degeneration (mild to moderate), and regeneration (mild to moderate) were observed at injection sites at all doses, while injection site changes associated with the control article and/or intramuscular injection procedure were generally minimal in severity. All changes at the injection site showed evidence of reversibility. Following a 1-month post-dose recovery period, there was no residual fibrosis at the injection sites of monkeys given either the control article or aripiprazole formulations. Exposures to aripiprazole and its pharmacologically active dehydro-metabolite (BMS-337044) were dose-proportional with no apparent sex differences. Small accumulation in systemic exposure occurred upon repeated dosing for 29 days. A NOAEL was not reached in this study (lowest tested dose of 2 mg/kg/day) since injection site skeletal muscle injury was present at all doses.

Local Tolerance: Local tolerance studies were conducted in rats, rabbits, dogs and monkeys with different IM depot formulations to determine a suitable formulation for use in toxicity studies. A formulation based on carboxymethyl cellulose (CMC) was found to be well tolerated in the rat, rabbit and dog, but not in the monkey. Another formulation of aripiprazole (in 15% Captisol) was examined in the rat and rabbit and was also found to be well tolerated in these 2 species. Microscopically, the primary finding at the injection site from these studies was a localized,

granulomatous inflammatory response to deposited drug consistent with a foreign-body reaction in response to deposited drug (polymorphic, birefringent crystalline material). This inflammation was not completely resolved by termination of the studies.

- CMC formulation local tolerance studies: Aripiprazole in a CMC formulation ((b) (4) mannitol, monobasic sodium phosphate, sterile water for injection, pH 7; particle size (b) (4)) was administered to rats in two local tolerance studies, at single IM doses of 12.5, 25 and 50 mg/kg, with corresponding dose volumes of 0.12, 0.25 and 0.5 mL/kg, respectively. At all doses, there was a dose-related increase in the incidence of injection site swelling that represented deposits of test article in the muscle; white discolored foci were observed at necropsy. Microscopically, the primary finding at the injection site from both studies was a localized, granulomatous inflammatory response to deposited drug that was consistent with a foreign-body reaction. This inflammation was not completely resolved by Day 45 post-dose.

Local tolerance studies using the CMC-based formulation were also conducted in rabbits. In the first study aripiprazole was administered as single IM doses of 25, 50 or 100 mg (corresponding dose volumes of 0.25, 0.5 or 1 mL, respectively, of a 100 mg/mL suspension). A second study employed single IM doses of 150 or 200 mg (corresponding dose volume of 1 mL of a 150 or 200 mg/mL suspension). At the lower doses of the first study (up to 100 mg/injection), there were no clinical signs or injection site findings in any of the drug-treated groups. At the higher doses of the second study, mild edema at the injection site was noted and white or tan discoloration at the injection sites (consistent with deposits of the test article and associated inflammation) was noted in all drug-treated animals. The size of these discolored foci was generally dose related, and there was a reduction in size over time. The microscopic findings at the injection site were similar to those seen in rats and consisted of granulomatous inflammation as a foreign-body reaction in response to deposited drug (polymorphic, birefringent crystalline material). The inflammatory reaction was most severe early in the study (e.g., Day 15) and declined in severity towards the end of recovery period (Day 57 post-dose). Other drug-related findings indicative of skeletal muscle injury included “minimal” degeneration and necrosis (Days 4 and/or 7 post-dose) and “minimal regeneration” (Day 15 post-dose).

Local tolerance to the CMC-based formulation was studied in dogs administered aripiprazole (100 mg/mL in (b) (4) CMC) as two injections at total dose volumes of 2, 3 or 4 mL to provide doses of 200, 300 or 400 mg, respectively. At the mid- and high dose, swelling and erythema were evident at the injection sites, most likely related to the large dosing volume; at necropsy, white discoloration (deposits of the test article), red discoloration of subcutaneous tissue (hemorrhage) and swelling were noted at the injection sites in mid- and high dose groups. Microscopically, the primary tissue response to deposited drug was subacute inflammation at all doses with a dose-related severity. Other microscopic injection site changes included: edema, hemorrhage, degeneration of skeletal muscle, granulomatous inflammation, and fibroplasia/fibrosis.

Intramuscular administration of aripiprazole in CMC formulation to monkeys resulted in greater toxicity (primarily at the injection site) compared to other tested species. In monkeys, 0.5 mL of aripiprazole formulation (50 or 100 mg/mL in (b) (4) CMC), administered IM at total dose volume of 0.5 mL to provide doses of 25 or 50 mg, respectively, resulted in hypoactivity at HD and acute injection site swelling and discoloration at both doses. Compared to pretest values, administration of the 50 mg/mL and 100 mg/mL formulation resulted in a drug-related increase in aspartate aminotransferase and creatine phosphokinase in both sexes on Day 2 postdose, partially reversible by Days 4 and 7 postdose. White discoloration (apparent test article deposits

in muscle), was noted in the injection sites through Day 29 for both concentrations and did not decrease with time. Microscopically, granulomatous inflammatory response to birefringent crystalline drug deposits was noted that was characterized by accumulation of macrophages with foamy cytoplasm and eosinophils. Although the severity of the microscopic changes tended to decrease with time, a complete resolution of the inflammatory process did not occur by study termination (Day 29 post dose).

- Captisol formulation local tolerance studies: Two studies were conducted using a formulation based on 15% Captisol and 0.05 M tartrate buffer (pH 4.3 to 4.5) vehicle. In the first study, rats were treated once daily for 2 weeks with IM doses of 1 or 3.75 mg/kg (0.5 mL/kg at concentrations of 2 or 7.5 mg/mL). A dose-related increased incidence of discoloration and swelling at injection sites was observed that resolved 3 days after discontinuation of treatment. At the end of the dosing phase, a slight but statistically significant increase in the mean serum AST was observed in females at the high dose. At the end-of-dosing necropsy, degeneration, necrosis, and regeneration of muscle and subacute inflammation, hemorrhage, edema, and fibroplasia/fibrosis of muscle and surrounding connective tissue were observed microscopically in the high dose group, as well as in the vehicle and low dose groups with lesser severity. During the 2-week postdose period, there was nearly complete reversibility of injection site changes.

In rabbits, single 1 mL doses of aripiprazole in 15% Captisol and 0.05 M tartrate buffer (pH 4.5) were administered using concentrations of 2, 4, or 7.5 mg/mL. "Slight" injection site edema was observed across all groups including controls. A dose-related increase in creatinine phosphokinase on Day 2 was observed compared to saline or vehicle controls, reversible by Day 18 post dose. At Day 4 necropsy, focal tan areas associated with and/or surrounded by hemorrhage were observed at injection sites of some animals at mid- and high dose. Microscopically, muscle degeneration/regeneration and inflammation were observed with minimal severity in the saline control group, minimal to mild severity in the vehicle control, LD and MD groups and greater severity in the HD group. By Day 18, minimal muscle regeneration and mineralization were still present in one animal at 2 mg/mL, and very slight edema and minimal muscle regeneration, inflammation, mineralization, and/or fibrosis were apparent at HD.

In summary, local irritation was assessed in studies using single intramuscular injections of aripiprazole in a CMC-based formulation to rats, dog and rabbits resulted in similar gross and histopathology changes at the injection site. The changes observed consisted of white or discolored foci, interpreted as deposition of the drug, and associated granulomatous inflammation. Cellular infiltrates, e.g., macrophages, were occasionally seen in association with the local tissue inflammatory reaction. A comparable inflammatory response was also observed in rats and rabbits administered aripiprazole in a Captisol formulation. During the postdose recovery period, the injection site changes diminished in size and severity but the recovery was incomplete even with a prolonged (up to 57 days) post-dose recovery period.

The toxicity observed in the monkey, in contrast to the rat, dog and rabbit, was generally more severe with either single or repeat dosing. In addition to clinical signs related to the pharmacological action of aripiprazole, injection site changes included the expected local inflammatory reaction as well as skeletal muscle degeneration and/or necrosis with regeneration, subacute inflammation, hemorrhage, edema and fibroplasia/fibrosis with associated changes in clinical pathology changes. These findings were considered to some extent to the vehicle (Captisol) and trauma associated with injection with a slight exacerbation of the injection site injury attributed to aripiprazole. In addition, the greater sensitivity to injection site reaction

observed in the monkey was most likely due to the more frequent (daily) dosing regimen, in contrast to weekly dosing in other species, as well as the smaller thigh tissue mass in the monkey. Systemic exposure to aripiprazole and metabolites following intramuscular injection was dose-related in all of the toxicity studies described above. Exposure to aripiprazole with intramuscular injection was prolonged with the parent compound detected at 29 days or more following a single injection. With repeat dosing, systemic levels of aripiprazole and metabolites increased with dose and the increase was not generally dose proportional. There were no remarkable differences between genders (sponsor's summary table below).

Comparison of TK Parameters for Aripiprazole (IM Depot) and Metabolites across Species at the NOAEL
(TK data represent repeat-dose exposure at the end of the dosing period)

| Species | Rat | | Dog | | Dog | | Monkey ^a | |
|--|-------------------|---------------------|------------------|------------------|------------------|------------------|---------------------|--------|
| Study Duration | 26 Weeks | | 26 Weeks | | 52 Weeks | | 4 Weeks | |
| Sex | Male | Female | Male | Female | Male | Female | Male | Female |
| NOAEL Dose (mg/kg) | 50 | 100 | 40 | 40 | 40 | 40 | 2 | 2 |
| AUC_{0-24h} (ng·h/mL)^b | | | | | | | | |
| Aripiprazole | 2052.3 (598.6) | 14867.0 (4336.2) | 4902.9 (1430) | 5211.4 (1520) | 8434.3 (2460) | 6240.0 (1820) | 2263 | 2189 |
| OPC-14857 | 19.54 (5.7) | 1185.9 (345.9) | 1803.4 (526) | 1700.6 (496) | 3226.3 (941) | 2478.9 (723) | 554 | 688 |
| DM-1451 | 161.8 (47.2) | 174.5 (50.9) | 26.2 (7.64) | 23.8 (6.95) | 51.4 (15.0) | 29.3 (8.53) | ND | ND |
| DM-1452 | NC | 97.0 (28.3) | 668.6 (195) | 589.7 (172) | 1196.6 (349) | 994.3 (290) | ND | ND |
| OPC-3373 | NC | 165.9 (48.4) | 127.2 (37.1) | 170.7 (49.8) | 242.4 (70.7) | 206.1 (60.1) | ND | ND |
| DCPP | NC | 63.1 (18.4) | 72.7 (21.2) | 78.2 (22.8) | 161.1 (47.0) | 89.8 (26.2) | ND | ND |
| C_{max} (ng/mL) | | | | | | | | |
| Aripiprazole | 98.1 | 1135.3 | 263 | 295 | 438 | 306 | 697 | 767 |
| OPC-14857 | 1.8 | 101.1 | 89.7 | 87.5 | 163 | 122 | 43 | 49 |
| DM-1451 | 8.2 | 8.2 | 1.79 | 1.63 | 2.86 | 1.84 | ND | ND |
| DM-1452 | <LLOQ | 7.2 | 33.7 | 30.7 | 64.4 | 47.6 | ND | ND |
| OPC-3373 | <LLOQ | 14.8 | 6.73 | 10.4 | 13.8 | 11.4 | ND | ND |
| DCPP | <LLOQ | 5.9 | 4.06 | 3.99 | 8.71 | 5.33 | ND | ND |

AUC_{0-t} = area under the concentration-time curve; C_{max} = maximum concentration; LLOQ = lower limit of quantification (2 ng/mL); NC = not calculated; ND = not determined; NOAEL = no observed adverse effect level.

^aFor the monkey, a NOAEL was not established. The toxicokinetic parameters are shown for the lowest dose tested. For the AUC, the values are in units of ng h/mL.

^bThe AUC is shown as the AUC_{0-24h} (ng h/mL) and was calculated from the AUC_{7d} (ng d/mL) that is given in parenthesis
Source: Otsuka Report Nos. 019345 (26-week rat), 019346 (26-week dog), 022722 (52-week dog), 017831 (4-week monkey)

Safety margins

| Toxicity | Species | NOAEL (mg/kg) M/F | NOAEL (mg/m ²) M/F | Safety Margin* Based on body surface area (m ²) | AUC** at NOAEL (ng.h/mL) |
|------------------------|---------|---|--|---|---|
| General Repeat-dose | Rat | 50 mg/kg/week (M) 100 mg/kg/week (F) | 300 mg/m ² /week (M) 600 mg/m ² /week (F) | 4.9 9.7 | AUC _{7d} 599 (M) 4336 (F) |
| | Dog | 40 mg/kg/week(M/F) | 800 mg/m ² /week | 13 | AUC _{7d} 2460 (M) 1820 (F) |
| | Monkey | < 2 mg/kg/day (M/F) (NOAEL not reached: less than the lowest tested dose of 2 mg/kg/day) | < 24 mg/m ² /day | NA | AUC _{0-24 h} < 2263 (M) < 2189 (F) |

* MRHD in human: 400 mg/4 wks (100 mg/week = 1.7 mg/kg or 61.7 mg/m² for a 60 kg person)

**AUC_{0-28d} in human: 163 mcg h/mL at MRHD

Conclusion and recommendation: Based on the results from the nonclinical general toxicity and local tolerance studies and safety margins between animal exposures at NOAEL and human exposure at MRHD of 400 mg per 4 weeks (per m² of body surface area), aripiprazole IM Depot formulation is safe to administer to humans.

12 Appendix/Attachments

None

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

/s/

SONIA A TABACOVA
07/02/2012

AISAR H ATRAKCHI
07/02/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 202971 Applicant: Otsuka

Stamp Date: 9/26/2011

Drug Name: Aripiprazole NDA/BLA Type:
Extended Release Suspension
for injection

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? | √ | | |
| 2 | Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin? | √ | | |
| 3 | Is the pharmacology/toxicology section legible so that substantive review can begin? | √ | | |
| 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)? | √ | | The pharmacology, safety pharmacology, genetic toxicity, carcinogenicity and reproductive/developmental toxicity studies are cross-referenced from the original NDA 21-436 (oral tablet) and from supplemental NDAs 21-713 (oral solution), 21-729 (oral disintegrating tablet) and 21-866 (injectable IM rapid release formulation). A carcinogenicity study of the intramuscular depot formulation was not conducted in accordance with the Executive CAC conclusion (Executive CAC Minutes, 9/26, 2008). A waiver for undertaking developmental and reproductive toxicity studies was granted by the FDA (e-mail from K. Kiedrow 6/23, 2010). |
| 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). | | | N.A. (the formulation to be marketed is not different from the formulation used in the toxicology studies) |
| 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 <u>submitted</u> a rationale to justify the alternative route? | √ | | |
| 7 | Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations? | √ | | |

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

**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? | √ | | |
| 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? | √ | | |
| 10 | Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.) | √ | | see CMC review |
| 11 | Has the applicant addressed any abuse potential issues in the submission? | √ | | Cross-referenced from the original NDA 21-436 |
| 12 | If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted? | | | 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.

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

Sonia Tabacova 11/09/2011

 Reviewing Pharmacologist Date

Aisar Atrakchi 11/14/2011

 Team Leader/Supervisor Date

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

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

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

Reference ID: 3042662

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

/s/

SONIA A TABACOVA
11/10/2011

AISAR H ATRAKCHI
11/14/2011