CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-995

PHARMACOLOGY REVIEW

MEMORANDUM
Oct. 15, 2006

TO: File
FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-995

I concur with Drs. Todd Bourcier and Karen Davis-Bruno that the marketing application for Januvia (Sitagliptin) may be approved based on review of nonclinical data submitted by the sponsor.

Kenneth L. Hastings, Dr.P.H., D.A.B.T.
Associate Director
Office of New Drugs

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/s/

Kenneth Hastings
10/16/2006 11:05:05 AM
PHARMACOLOGIST

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-995**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**12/16/05**
PRODUCT:	**Januvia (Sitagliptin)**
INTENDED CLINICAL POPULATION:	**Type 2 Diabetics**
SPONSOR:	**Merck**
DOCUMENTS REVIEWED:	**eCTD**
REVIEW DIVISION:	**Division of Metabolic and Endocrine Products**
PHARM/TOX REVIEWER:	**Todd Bourcier, Ph.D.**
PHARM/TOX SUPERVISOR:	**Karen Davis-Bruno, Ph.D.**
DIVISION DIRECTOR:	**Mary Parks, M.D.**
PROJECT MANAGER:	**Lina Aljuburi, Pharm. D., M.S.**

Date of review submission to Division File System (DFS): 31 August 2006

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability
AP (Approval)
Pharmacology/Toxicology recommends approval of NDA 21,995 (Januvia®)

B. Recommendation for nonclinical studies
No additional nonclinical studies are required.

C. Recommendations on labeling

8. Use in Specific Populations
8.1 Pregnancy
Pregnancy Category B.
There are no adequate and well-controlled studies in pregnant women; [redacted] recommended for use in pregnancy unless clearly needed. Merck & Co., Inc. maintains a registry to monitor the pregnancy outcomes of women exposed to Januvia while pregnant. Health care providers are encouraged to report any prenatal exposure to Januvia by calling the Pregnancy Registry at (800) 986-8999.

Sitagliptin administered to pregnant female rats and rabbits [redacted] was not teratogenic at oral doses up to 250 mg/kg (rats) and 125 mg/kg (rabbits), or approximately 30- and 20-times human exposure at the maximum recommended human dose (MRHD) of 100mg/day based on AUC comparisons. Higher doses [redacted] increased the incidence of [redacted] rib malformations in offspring at 1000 mg/kg, [redacted]

Sitagliptin administered to female rats [redacted] decreased the average body weight in male and female offspring at 1000 mg/kg [redacted]. No functional or behavioral toxicity was observed in offspring of rats.

[redacted] placental transfer was approximately 45% at 2 hours and 80% at 24 hours postdose. [redacted] placental transfer was approximately 66% at 2 hours and 30% at 24 hours.

8.3. Nursing Mothers

Sitagliptin is excreted in the milk of lactating rats at a milk to plasma ratio of 4:1. It is not known whether sitagliptin is excreted in human milk. Because many drugs are excreted in human milk [redacted] a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

13. Nonclinical Toxicology

13.1 Carcinogenesis, mutagenesis, impairment of fertility

A two year carcinogenicity study was conducted in male and female rats given oral doses of sitagliptin of 50, 150, and 500 mg/kg. There was an increased incidence of combined liver adenoma/carcinoma in males and females and of liver carcinoma in females at 500 mg/kg. This dose results in approximately 60 times the human exposure at the maximum recommended daily adult human dose (MRHD) of 100 mg/day based on AUC comparisons. Liver tumors were not observed at 150 mg/kg, approximately 20 times human exposure at the MRHD.

A two year carcinogenicity study was conducted in male and female mice given oral doses of sitagliptin of 50, 125, 250, and 500 mg/kg. There was no increase in the incidence of tumors in any organ up to 500 mg/kg, approximately 70 times human exposure at the MRHD.

Sitagliptin was not mutagenic or clastogenic with or without metabolic activation in the Ames bacterial mutagenicity assay, a Chinese hamster ovary (CHO) chromosome aberration assay, an *in vitro* cytogenetics assay in CHO, an *in vitro* rat hepatocyte DNA alkaline elution assay, and an *in vivo* mouse micronucleus assay.

In rat fertility studies with oral gavage doses of 125, 250, and 1000 mg/kg, males were treated for – weeks prior to mating and females were treated – weeks prior to mating through gestation day 7. No adverse effect on fertility was observed at 125 mg/kg (approximately 12 times human exposure at the MRHD of 100 mg/day based on AUC comparisons). Higher doses increased resorptions [redacted] in females at approximately 25 times human exposure at the MRHD based on AUC comparisons.

[redacted]

II. Summary of non-clinical findings

A. Brief overview of non-clinical findings

Pharmacology

MK-0431 (sitagliptin phosphate) is a competitive inhibitor of dipeptidyl peptidase 4 (DPP4), an enzyme principally responsible for degrading incretin peptides glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). MK-0431 prolongs incretin half-life and biological activity and thus potentiates glucose-dependent insulin release and delays gastric emptying. In non-clinical models of diabetes, MK-0431 moderates glucose excursion and improves insulin release and islet cell function/mass without provoking hypoglycemia. MK-0431 is body weight-neutral, unlike marketed glitazones (weight gain) and GLP-1 analogues (weight loss).

Immunomodulatory effects of DPP4 (aka CD26) are reportedly not altered by MK-0431, based on normal responses of murine T- and B-cells to antigens and mitogens. However, rodent DPP4/CD26 differs in some aspects from human DPP4/CD26 (e.g., binding of adenosine deaminase) and Merck's experiments did not directly test the T-helper memory function ascribed to CD26. Therefore, the non-clinical data do not adequately predict potential effects of MK-0431 on DPP4/CD26's role in human immunity.

Safety pharmacology assessment of neurological, renal, pulmonary, and gastrointestinal effects of MK-0431 did not identify any significant liabilities.

Absorption, Distribution, Metabolism, and Excretion

An oral dose of MK-0431 is rapidly absorbed and is 60-90% bioavailable in rats and dogs. MK-0431 distributes to most rat tissues with low amounts distributing to the brain, eyes, and bone. Plasma protein binding is moderate (30%). Metabolism of MK-0431 is minimal with 80% of unchanged parent compound being eliminated in the urine of rats, dogs, and humans. Oxidative metabolism by CYP3A4 and 2C8 is a minor metabolic pathway. MK-0431 has a longer plasma half-life in humans (13hrs) than in rats and dogs (2-5hrs) probably due to different rates of renal elimination. MK-0431 slightly accumulates in humans but not in dogs or rats after multiple dosing.

MK-0431 is a P-glycoprotein and hOAT3 substrate, but does not interfere in the shuttling of other substrates via these transporters *in vitro*. MK-0431 does not inhibit CYP450 enzymes or induce CYP3A4. The results predict a low probability for pharmacokinetic drug interactions via these pathways.

General Toxicology (MRHD, Maximum Recommended Human Dose, or 100mg)

Single dose studies identified minimum lethal doses of 2000mg/kg (200-400x MRHD) in mice and 3000mg/kg (150-300x MRHD) in rats. Little other toxicological information was obtained in these studies.

Repeat dose studies were conducted in Sprague-Dawley rats and Beagle dogs up to 6 months and 12 months duration, respectively.

A high-dose 3-month study in rats identified kidney and liver necrosis, myocardial degeneration, bone marrow necrosis, and death at 1500 and 2000mg/kg (150-200x MRHD). Kidney toxicity was also observed in mice at 500mg/kg. Note that exposure at these high doses is theoretically sufficient to inhibit off-target enzymes DPP8/9, proteases that are associated with these toxicities.

Administration of doses up to 20x the MRHD for 6 months in rats did not elicit significant toxicity.

Studies in dogs identified NOAEL doses based on clinical signs that consisted of reduced activity, hunched posture, ataxia, tremor, and sporadic emesis observed at 50mg/kg (20x MRHD). Respiratory distress, described as audible and labored breathing and open-mouthed breathing, was also reported. No consistent target organs were identified in these studies.

Administration of doses up to 5x the MRHD for up to 12 months in dogs did not elicit significant toxicity.

Special Toxicology

MK-0431 did not produce vascular/skin lesions in rhesus monkeys, as seen with some DPP4 inhibitors, after three months administration of doses up to 25x the MRHD. Mechanistic data provided by Merck suggests that inhibiting DPP4 activity alone is not sufficient to produce this toxicity.

The combination of MK-0431 and high-dose (50 mg/kg) but not low-dose (20 mg/kg) metformin in dogs may have resulted in more numerous and earlier deaths than observed with metformin alone. The lower dose of metformin (20 mg/kg) better approximates maximum human exposure to metformin (2500mg/day). Convincing evidence is provided by Merck that high-dose metformin is responsible for the deaths observed in combination with MK-0431. Nevertheless, there is a slight possibility of exacerbated toxicity in the setting of high metformin exposure and clinical exposure to MK-0431.

Reproductive Toxicology

Exposure to MK-0431 in the definitive studies ranged from 12x to 90x MRHD in the rat and 6x to 50x in the rabbit. Resorptions and post-implantation losses increased in females in a fertility study at ~25x MRHD; male fertility was not effected. MK-0431 was not teratogenic but increased the incidence of skeletal malformations in rat pups at maternally toxic doses. At maternally non-toxic doses, a single rat pup had multiple skeletal abnormalities (incidence within historical range), and a single rabbit pup had multiple cardiovascular abnormalities, but a relationship to drug treatment is not conclusive. MK-0431 crosses the placenta in rats and rabbits and is excreted in maternal milk at a 4:1 ratio to plasma. As with other oral hypoglycemic agents, MK-0431 should not be given to pregnant or nursing mothers and Merck will maintain a pregnancy register. Pregnancy Category 'B' is recommended.

There were no conclusive drug-related effects on embryonic/post-natal development in rats at 125mg/kg (12x MRHD) or in rabbits at 125mg/kg (20x MRHD).

Genetic Toxicology

MK-0431 was not mutagenic or clastogenic in three *in vitro* assays (Ames, hepatocyte alkaline elution, and chromosome aberration) and one *in vivo* assay (murine micronucleus induction).

Carcinogenicity

Carcinogenic potential of MK-0431 was evaluated in 2 year studies in mice and rats. Both studies adequately assessed carcinogenesis. MK-0431 significantly increased the incidence of combined liver adenoma/carcinoma in male and female rats, and increased liver carcinomas in female rats at 500mg/kg (62x MRHD). Non-genotoxic, chronic hepatotoxicity is the suggested etiological event but this is based on weak correlative evidence of liver toxicity. MK-0431 did not produce any drug-related tumors in CD-1 mice up to 500mg/kg (72x MRHD). MK-0431 poses a minimal carcinogenic risk to humans.

B. Non-clinical safety issues relevant to clinical use

1. DPP4 cleaves several substrates in addition to GLP-1. Therefore, MK-0431 may have undesirable effects related to inhibiting cleavage of non-incretin substrates. Effects on human immunity, specifically recall responses to antigens and immune cell trafficking, may be adversely effected by DPP4 inhibition. This risk is an unavoidable characteristic of MK-0431 and the drug class. There is currently no clinical evidence of such effects with Januvia.

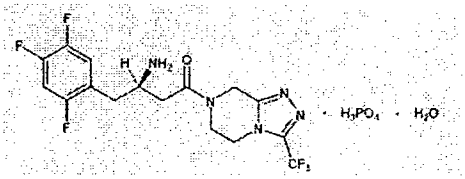
2. MK-0431 presents a marginal clinical risk of producing skin lesions with prolonged administration. This conclusion is based on the absence of skin findings in the 3-month monkey study, on mechanistic data suggesting that inhibiting DPP4 activity alone is not sufficient to produce this toxicity, and on the high DPP4 selectivity of MK-0431 at clinical exposure. Risk assessment for skin lesions must be done on a case-by-case basis and is not evidence of similar safety with other DPP4 inhibitors currently in clinical development.

3. The combination of MK-0431 and high-dose metformin (50 mg/kg) in dogs may have resulted in more numerous and earlier deaths than observed with metformin alone. The combination of MK-0431 and a lower dose of metformin (20 mg/kg) that better approximates human exposure at 2500mg/day resulted in no deaths and yielded no evidence of exacerbated toxicity. Convincing evidence is provided that the deaths at 50 mg/kg is due to metformin toxicity and not to the combination. Nevertheless, there is a slight possibility of exacerbated toxicity in the setting of high metformin exposure ($\geq$ 400μM*h AUC) and clinical exposure to MK-0431 (~10μM*h AUC).

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-995
Review number: 1
Sequence number/date/type of submission:
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Merck Research Laboratories
Manufacturer for drug substance:
Merck in Barceloneta, Puerto Rico, Vincenza, Italy, and Visp, Switzerland
Reviewer name: Todd Bourcier
Division name: Metabolic and Endocrine Products
Review completion date: 31 August 2006
Drug:
Trade name: Januvia
Generic name: Sitagliptin phosphate
Code name: MK-0431; L-000224715-010X
Chemical name:
7-[(3*R*)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-[3-(trifluoromethyl)-1,2,4-triazolo[4,3-*a*]pyrazine phosphate (1:1) monohydrate
CAS registry number: 654671-77-9
Molecular formula/ weight: $C_{16}H_{15}F_6N_5O \cdot H_3PO_4 \cdot H_2O$ /523.32 MW
Structure:



Relevant INDs/NDAs/DMFs: 62,278 (Novartis); [redacted]; 63,634 (BMS); 67,369 (GSK); 65,495 (Merck); [redacted]; [redacted] 69,707 (PPD)

Drug class: dipeptidyl-peptidase IV (DPP-IV) inhibitor

Intended clinical population: Type 2 Diabetics

Clinical formulation: MK-0431 monohydrate phosphate salt (25, 50, 100 mg tablets)
Tablets contain microcrystalline cellulose, calcium phosphate dibasic, croscarmellose sodium, magnesium stearate, sodium stearyl fumarate. Tablets are pink, light beige, or beige depending on dosage strength.

Route of administration: Oral

Maximum Recommended Human Dose: Merck seeks approval of 25, 50, and 100mg. The 100mg qd strength provides an average AUC of 10 μM*h and a Cmax of 1 μM.

Disclaimer: Some Tables and Figures from the electronic NDA submission have been copied for use in this review

Studies reviewed within this submission:

Primary Pharmacodynamics
Affinity for human and animal DPP-IV Human, mouse, rat, dog In vitro
Activity in T cell activation assays Mouse In vitro
Acute efficacy in oral glucose tolerance test Mouse P.O.
Pharmacodynamics in oral glucose tolerance test Mouse P.O.
Acute efficacy in model of diet-induced obesity Mouse P.O.
Acute efficacy in db/db mice Mouse P.O.
Selectivity of MK-0431 for DPP-IV Human, cow, pig, rabbit, rat In vitro
Selectivity of comparator compounds for DPP-IV Human, pig In vitro

Safety Pharmacology
Respiratory assay Rat P.O. MK-0431 Tablets
Cardiovascular telemetry assay Dogs P.O.
Oral functional observational battery assay Rats P.O.
Cellular electrophysiological evaluation of MK-0431 on HERG CHO In vitro
Cardiovascular effects: rising dose study Dog IV
Renal function and electrolyte excretion Dog P.O.
Respiratory function, hemostasis, and platelet function Dog IV
Gastric acid secretion Dog P.O.
Gastrointestinal motility Mouse P.O.
Behavioral and Other CNS Effects

Pharmacokinetics
Absorption
Pharmacokinetics in rat and dog
Oral bioavailability and dose dependence in rat and dog

Distribution
Single-dose tissue distribution in rat
Placental transfer in rat and rabbit
Reversible plasma protein binding
Serum albumin and α1-acid glycoprotein binding
Blood-to-plasma partitioning
P-glycoprotein mediated transport, mouse and human
Uptake by renal transporters, human

Metabolism
Metabolites in plasma, mouse and rabbit
Metabolites in plasma, liver, kidney, urine, and bile in rat
Metabolites in plasma, urine, and bile in dog
Metabolites in plasma, urine, and feces in human
Identification of metabolites M2 and M5 in dog
Metabolism in liver microsomes, mouse, rat, rabbit, dog, monkey, human
Metabolism in hepatocytes, rat, dog, human
Metabolism in recombinant cytochromes P450 in human
Inhibition of cytochromes P450
Induction of cytochrome P450 3A4
Effect on MDR1 P-glycoprotein-mediated transport

Excretion
Mass balance in rats and dogs
Urinary and biliary excretion in rats and dogs
Excretion into milk in rat

General Toxicology
Single dose toxicity in mouse and rat (anhydrous and monophosphate salt formulations)
Repeat dose toxicity studies and their duration:

CD-1 Mouse:	1 and 3 months
Sprague Dawley Rat:	2 weeks, 3 months, 3 months high-dose, 6 months
Beagle Dogs:	2 weeks, 3, 6, and 12 months

Genetic Toxicology
Ames Assay (in vitro)
Primary rat hepatocytes (in vitro)
Chinese hamster ovary cells (in vitro)
Micronucleus induction in mice after single oral dose (in vivo)

Carcinogenicity
106 week oral gavage in CD-1 mice and toxicokinetic analysis
106 week oral gavage in SD rats and toxicokinetic analysis

Reproductive/Developmental Toxicology
Male and female fertility in rat
Rat Embryonic Development (dose-ranging and definitive studies)
Rabbit Embryonic Development (dose-ranging and definitive studies)
Rat Post-natal Development

Special Toxicology Studies
Dermal sensitization in mice, rabbits, and humans [redacted]
Ocular toxicity in bovine cornea (in vitro) and in rabbits (in vivo)
Intravenous administration of MK-0431 for 16 consecutive days in rats and dogs
Skin lesion assessment of sitagliptin in a 14-week oral toxicity study in monkeys
Skin lesion assessment of L-000000826 in a 12-week oral toxicity study in monkeys
Interim Report: Skin lesion assessment of L-000233357 in a 14-week oral toxicity study in monkeys
MK-0431 + Metformin: Combination Toxicity Studies in Dogs: Summary
MK-0431 + Metformin: 14 week oral toxicity study in dogs
Exploratory 5-week oral tolerability study with Metformin in female dogs
MK-0431 + Metformin: 16 week oral toxicity in female dogs

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

MK-0431 is a triazolopiperazine-based competitive inhibitor of dipeptidyl peptidase 4 (DPP4). MK-0431 selectively inhibits DPP4 activity in serum from humans, rodents, and dogs with high potency (IC_{50}, 18-69nM; Ki, 9nM). Inhibitory activity against closely related proteases, including DPP8/9, and a panel of unrelated enzymes and ion channels is minimal (IC_{50}, 48μM to >100 μM) and not relevant at clinical drug concentrations (~1.0 μM at a 100mg dose). The DPP4 selectivity of MK-0431 is superior to vildagliptin, a DPP4 inhibitor being developed by Novartis. The selectivity of MK-0431 for DPP4 minimizes the potential for toxicities associated with inhibition of DPP8/9.

MK-0431 bound to serotonin receptors with a Ki of 2-5 μM, but was devoid of agonist activity; it is not known if MK-0431 interferes with endogenous serotonergic activity. Merck states that MK-0431 distributes poorly to the brain ($1/10^{th}$ plasma) and that 5HT2A antagonists are used clinically.

DPP4, also known as CD26, contributes to the co-activation of memory/helper T-cells to recall antigens. MK-0431 did not suppress murine T- and B-cell activation in a series of *in vitro* activation assays. Other selective DPP4 inhibitors did not suppress reactivity of human peripheral lymphocytes, but MK-0431 was not specifically tested. These experiments did not address the memory T-cell function of CD26 and are of uncertain value in predicting the effect of MK-0431 on human immunity.

MK-0431 showed efficacy in lean mice, diet-induced obese mice, and in db/db mice. MK-0431 inhibited plasma DPP4 activity, increased plasma GLP-1, and reduced blood glucose excursion in a dose-dependent manner. Efficacious plasma drug concentrations were 200-700nM, sufficient to inhibit plasma DPP4 activity more than 90%. For comparison, the Cmax at the 100mg clinical dose is 1000nM.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

MK-0431 inhibits DPP4 in vitro: MK-0431 inhibits activity of human recombinant DPP4 by 50% at 17.9 nM (IC_{50}, **Figure 1**). The range for inhibitory activity is ~5nM to 1000nM, representing ~20% to 99% inhibition of DPP4 activity against a fluorogenic dipeptide substrate (Gly-Pro-AMC). Inhibitory activity of MK-0431 was competitive and reversible.
MK-0431 inhibits activity of native DPP4 from humans and from species used for toxicology testing with similar potency (16-69nM, **Table 1**). MK-0431 inhibits free DPP4 in serum as well as membrane-bound enzyme (CACO-2 extracts).

Figure 1: *In vitro* inhibition of human recombinant DPP4 by MK-0431

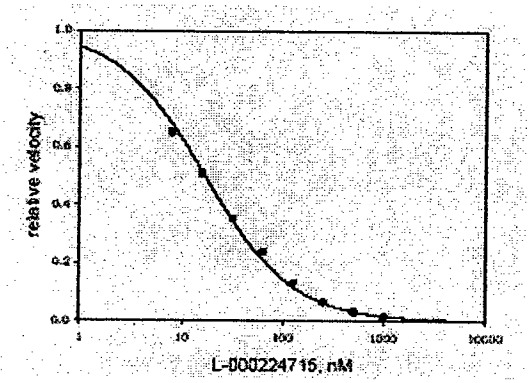


Table 1: Inhibition of DPP4 from various sources by MK-0431

DPP-IV Source	IC_{50}, nM	SD (n)
Human recombinant	17.9	7.4 (3)
Human serum	12.9	0.6 (3)
CACO-2 extract	20.3	1.2 (3)
Rat serum	52.4	6.2 (3)
Mouse serum	69.3	7.0 (3)
Dog serum	16.3	4.2 (5)

MK-0431 is selective for DPP4 in vitro: MK-0431 selectively inhibits activity of DPP4 relative to closely-related proline specific serine proteases (**Table 2**), although FAPα was not assayed. MK-0431 inhibits DPP8 activity with ~2,500 fold less potency compared to DPP4, based on IC_{50} values.

MK-0431 was also screened for activity against a panel of unrelated proteases and ion channels (**Tables 3 & 4**). Granzyme B and gamma-secretase were inhibited with an IC_{50} $\geq$ 10μM, and L-type calcium channels with an IC_{50} of 22μM. These concentrations are approximately 500-fold and 1000-fold higher than the IC_{50} for DPP4 activity.

MK-0431 bound to rat serotonin receptors 5HT2 (Ki, 5.8μM) and 5HT2A (Ki, 2.1μM), but no agonist activity was observed up to 10μM concentration.

The potential for MK-0431 to exhibit off-target inhibitory activity at clinically relevant concentrations is minimal. The low inhibitory activity against related DASH members (**D**PP4 Activity & **S**tructural **H**omologs) minimizes the toxicities associated with DPP8/9 inhibition in rats and dogs (e.g., thrombocytopenia, mortality in rats, gastrointestinal toxicity in dogs). Despite the minimal off-target potential of MK-0431, substrate promiscuity of DPP4 activity and its possible sequalae is an unavoidable characteristic of MK-0431 and the drug class.

Table 2

Activities of L-000224715 in Assays for Proline Specific Enzymes

Screening Target	IC_{50}, µM (n)
DPP8	48 ± 20 (4)
DPP9	>100 (3)
QPP	>100 (3)
APP	>100 (2)
PEP	>100 (2)
Prolidase	>100 (3)

Table 3:
MK-0431 inhibition of selected proteases

Screening Target	IC_{50}, µM
Cathepsin B	>100
Cathepsin H	>100
Caspases 1-10, 13	>100
Granzyme B	>10
Gamma-secretase	>10
Beta-secretase	>50
Thrombin	>100
Trypsin	>100
Factor Xa	>100
TAFI	>100

Table 4:
MK-0431 inhibition of selected ion channels

Screening Target	IC_{50}, µM (n)
IKr (MK-0499)	67 ± 39 (4)
L-type Ca Channel	22 ± 5 (2)
Na Channel Site II	52 ± 7 (2)

MK-0431 selectivity vs. comparator compounds: MK-0431 (**L-000224715 in Table 5**) exhibits a superior selectivity profile compared to a panel of other DPP4 inhibitors, including the Novartis compound LAF237 (vildagliptin) currently in Phase 3 clinical trials. The threo-, allo- and DPP8/9 selective compounds produced toxicity in rats and dogs, including thrombocytopenia, anemia, multiple organ histopathology, and mortality ([1]Lankas 2005). The threo- and allo-Ile non-selective compounds also produced similar toxicity in DPP4 deficient mice. MK-0431 did not produce these toxicities in this study, indicating that several toxicities are associated with inhibition of DPP8/9 but not DPP4. A highly selective inhibitor of DPP4 would therefore avoid such DPP8/9-related toxicities.

[1]Lankas GR, et al. (2005) Diabetes (10):2988-94.
This study was conducted by the Dept. of Safety Assessment, Merck Research Laboratories

Table 5: *In vitro* selectivity of comparator DPP4 inhibitors (IC_{50}, μM)

Compound	DPP-IV	DPP8	DPP9	QPP	PEP	APP	Prolidase
threo-Ile thia	0.42	2.2	1.6	14	100	>100	>100
allo-Ile thia	0.46	0.22	0.32	18	>100	>100	>100
QPP selective	1.9	22	31	0.019	100	>100	>100
DPP8/9 selective	30	0.038	0.055	14	>100	>100	>100
L-000224715	0.018	48	>100	>100	>100	>100	>100
DPP-728	0.01	1.9	0.067	3	>100	>100	>100
LAF237	0.038	5.9	0.28	>100	>100	>100	>100

MK-0431 activity in murine T cell activation assays in vitro: DPP4, also known as CD26, is thought to contribute to co-activation of memory/helper T-cells. MK-0431 was therefore evaluated over a concentration range of 12nM to 50μM in several *in vitro* activation assays with murine T- and B-cells. MK-0431 did not inhibit T-cell proliferation in the mixed splenic lymphocyte reaction (MLR) or in response to antigen, and did not alter lipopolysaccharide-induced proliferation of B cells. The Lankas article[1] reported that DPP4-selective compounds do not suppress *in vitro* proliferation of human peripheral blood lymphocytes in response to phytohemagglutinin or staph enterotoxins but less selective compounds do have inhibitory activity. MK-0431 was not evaluated in that experiment.

DPP4/CD26 in mice and rats differs in some aspects from the human form[1,2], despite ~85% homology across species (e.g., ADA binding). In addition, the *in vitro* assays done by Merck do not clearly test the helper functions ascribed to CD26 on memory T-cells (e.g., human T-cell response to tetanus toxoid-loaded antigen presenting cells). At least in mice, MK-0431 does not suppress T- and B-cell activation, but the possible effect on human immunity is unknown.

[1]Lankas GR, et al. (2005) Diabetes (10):2988-94.
This study was conducted by the Dept. of Safety Assessment, Merck Research Laboratories

[2]Iwaki-Egawa S, et al. (1997) Cellular Immunology (178):180-186

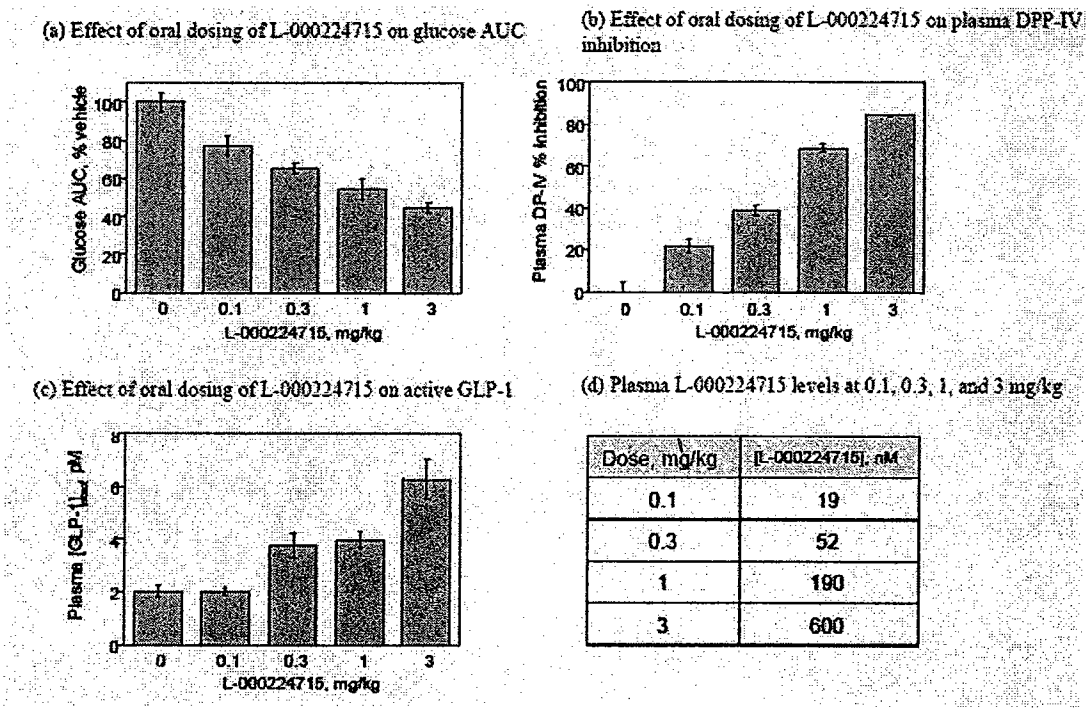
Drug activity related to proposed indication:

Non-clinical efficacy of MK-0431 was assessed in lean mice, diet-induced obese mice, and in db/db mice. MK-0431 inhibited plasma DPP4 activity, increased plasma GLP-1, and reduced blood glucose excursion in a dose-dependent manner. Efficacious plasma drug concentrations were 200-700nM, sufficient to inhibit plasma DPP4 activity more than 90%. Merck suggests that clinical efficacy will be achieved by maintaining 80% DPP4 inhibition and 2-fold GLP-1 elevation at trough plasma drug levels.

Lean mice were treated orally with MK-0431 (0.1, 0.3, 1, 3 mg/kg) and then challenged with dextrose (5g/kg, 10ml/kg) 1 hour post-dose **(Figure 3)**. The blood glucose excursion profile from 0 to 120 minutes was used to integrate an area under the curve (AUC) for each treatment. MK-0431 inhibited blood glucose excursion in a dosage-dependent manner achieving maximum efficacy at 1 mg/kg (46% inhibition).

In a separate experiment, plasma was collected at 20 minutes post-dose for measurement of plasma DPP4 inhibition, active GLP-1, and compound. Maximal efficacy, corresponding to plasma DPP4 inhibition of 70% and plasma concentrations ≥190 nM, resulted in a 2- to 3-fold increase in active GLP-1, analogous to what is observed upon glucose challenge in DPP4 deficient mice.

Figure 3: Pharmacodynamics of MK-0431 in lean mice



Dose, mg/kg	[L-000224715], nM
0.1	19
0.3	52
1	190
3	600

High fat diet-induced obese mice (DIO) mice (**Figure 4**) develop obesity, hyperglycemia, and hyperinsulinemia and have impaired blood glucose tolerance in response to a dextrose challenge. Following oral administration of 0.3, 3, and 30mg/kg MK-0431, dextrose-induced blood glucose excursion was significantly inhibited by 68, 90, and 82% (normalized to the dextrose-challenged lean controls), respectively. Maximum efficacy was seen at the 3 mg/kg dose in this study, corresponding to a plasma concentration of approximately 700nM based on a parallel PK study in DIO mice.

The db/db mouse is a murine model of type 2 diabetes (**Figure 5**) characterized by severe insulin resistance and marked hyperglycemia. Oral administration of MK-0431 (3, 10, and 30 mg/kg) to diabetic *db/db* mice (9 to 10 weeks of age) resulted in near normalization of blood glucose to lean controls. Maximal efficacy was observed at 3 mg/kg (76% correction of hyperglycemia at 4 hours postdose), corresponding to a maximum plasma concentration of approximately 400 nM based on a parallel PK study in *db/db* mice.

Figure 4:
Glucose AUC in DIO mice with MK-0431

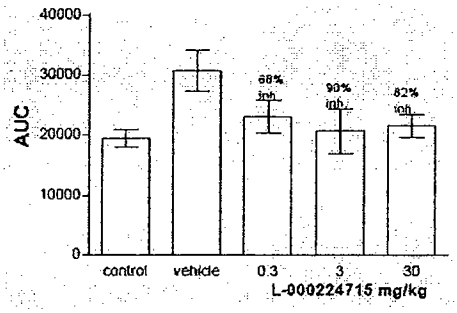
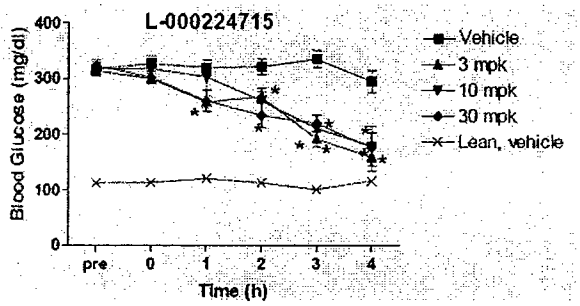


Figure 5:
Blood glucose in db/db mice with MK-0431



2.6.2.3 Secondary pharmacodynamics

2.6.2.4 Safety pharmacology

Brief Summary:
Safety assessment of neurological, renal, pulmonary, and gastrointestinal effects of MK-0431 did not identify any significant liability. MK-0431 clearly inhibits hERG potassium current *in vitro* at concentrations that markedly exceed human exposure, but nevertheless represents a potential cardiac conduction liability. Further cardiac telemetry and dose-rising studies did not identify a treatment-related change in QT or other ECG interval in dogs up to 50 mg/kg, reducing the importance of the hERG results. Other cardiovascular findings include a 56mmHg decrease in blood pressure and slight increase in heart rate in vagotomized dogs at 30mg/kg i.v., and a slight rise in heart rate in conscious dogs at 50mg/kg oral dose.

Neurological effects:
NOEL > 180 mg/kg (rats), >100 mg/kg (mice)

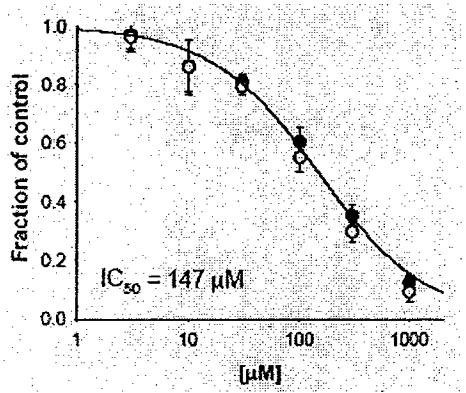
For CNS activity measurements, Sprague-Dawley rats were subjected to a functional observational battery assay (home cage, hand-held, and open-field observations, stimulus activity responses, and grip strength, foot splay, and body temperature measurements). There were no treatment-related effects after a single dose at 20, 60 or 180 mg/kg. CNS

function, behavior, motor activity, and thermoregulatory effects of MK-0431 (100mg/kg P.O) were also evaluated in 10 conscious mice. MK-0431 had no meaningful effect on these parameters when compared to vehicle-dosed animals.

Cardiovascular effects:
NOEL in vivo, 10mg/kg (dogs)

hERG activity: MK-0431 inhibits hERG potassium current with an IC_{50} of 147μM and an IC_{20} of ~50μM (**Figure 6**). Complete inhibition is achieved at 1000μM. Inhibitory activity is 80% reversible upon removal of MK-0431.

Figure 6: MK-0431 inhibition of hERG Current



Cardiovascular Rising Dose Study: Anesthetized and vagotomized dogs (n=3) were given an *intravenous* infusion of MK-0431 yielding cumulative doses of 1, 3, 10, and 30mg/kg over a 10 minute period (**Table 6**). No important changes in mean arterial pressure or heart rate were observed up to 10mg/kg, but at 30mg/kg blood pressure decreased 56 mmHg and heart rate decreased 40 bpm near the end of the 10 minute infusion.

Heart rate-corrected (Bazzet's) QT interval did not change at any dose. PR interval increased 7.4% at 30mg/kg without a change in QRS width or R-wave amplitude.

Plasma concentration was 202μM at 30mg/kg, and ≤ 59μM at 10mg/kg and lower. For comparison, the clinical Cmax is 1μM at 100mg.