CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

022426Orig1s000

PHARMACOLOGY REVIEW(S)

Memorandum

PHARMACOLOGY/TOXICOLOGY MEMO TO FILE

Date:	24 August, 2012
NDA #	22-271 – Complete Response, 7/26/12 (SDN-88) 22-426 – Complete Response, 7/27/12 (SDN-71)
Sponsor:	Takeda
Drug:	Alogliptin (Proposed Nesina®) Alogliptin/pioglitazone FDC (Proposed Oseni™)
Reviewer:	David B. Carlson, Ph.D.

Summary: Takeda submitted data to respond to a 'Complete Response'[1] action letter for alogliptin tablets and alogliptin plus pioglitazone FDC tablets under NDA 22-271 and NDA 22-426, respectively. No pharmacology or toxicology issues were identified in the current Complete Response letter and no nonclinical data were included in the new submissions.

Comprehensive Pharmacology/Toxicology Reviews have been completed for alogliptin monotherapy and alogliptin fixed dose combinations with pioglitazone or metformin. Alogliptin was well tolerated in animals and this pharmacology/toxicology reviewer recommended approval for each of the three alogliptin drug products indicated for treatment of type 2 diabetes.[2]

No comprehensive nonclinical review of the new clinical data is anticipated. Data from the nonclinical pharmacology and toxicology studies previously reviewed did not predict clinical hepatotoxicity and have not proved informative about potential risks of clinical hepatotoxicity.

General labeling recommendations were described in prior nonclinical reviews. Any further specific nonclinical labeling changes will be completed as necessary during the current review cycle.

Conclusions: Nonclinical approval recommendations remain unchanged from prior reviews. Specific nonclinical labeling recommendations will be completed as necessary during the current review cycle.

Internal comments: No additional nonclinical review is anticipated with the exception of any consultations requested by the clinical team or other review disciplines.

[1] M. Parks, Complete Response, 4/25/12

[2] D. Carlson, Pharmacology/Toxicology NDA Review and Evaluation for NDA 22-271 (8/27/08 and 1/18/12), NDA 22-426 (6/8/09 and 1/18/12) and NDA 203-414 (7/23/12)

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/s/

DAVID B CARLSON
08/27/2012
Pharmtox approval recommendations unchanged (no nonclinical data in CR #2 submissions)

TODD M BOURCIER
08/27/2012
I concur

Tertiary Pharmacology/Toxicology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

NDA: 22-426
Submission date: 25 July, 2011 (resubmission)

Drug: alogliptin/pioglitazone
Sponsor: Takeda Pharmaceuticals
Indication: treatment of type 2 diabetes mellitus

Reviewing Division: Division of Metabolism and Endocrinology Products

Comments:
In this resubmission, the pharm/tox reviewer and supervisor both found the nonclinical information adequate to support approval of the alogliptin/pioglitazone combination product for the intended indication. This does not differ from the previous recommendation from pharm/tox for this NDA.

Conclusions:
I read the pharm/tox review and supervisory memorandum and I agree that the information is adequate from a pharm/tox perspective to support approval of this NDA. I have discussed some aspects of labeling with the reviewer and supervisor.

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/s/

PAUL C BROWN
04/24/2012

SUPERVISOR'S MEMO

Date:	17 Jan 2012
RE:	NDAs 22426 and 22271
Sponsor:	Takeda Pharmaceuticals
Drug/Indication	Alogliptin monotherapy (22271) Alogliptin + Pioglitzaone FDC (22426)

In 2009, the FDA issued a Complete Response to Takeda for NDA 22271 which sought marketing of alogliptin, a DPP4 inhibitor, as monotherapy for type 2 diabetes. The same regulatory action was taken for the related NDA 22426, a fixed dose combination of alogliptin + pioglitazone.

Clinical and nonclinical issues were cited as issues in the CR letter from 2009. The nonclinical issue concerned a potential teratogenicity signal that emerged at that time in studies with another DPP4 inhibitor when used in conjunction with metformin. Because alogliptin would be frequently used in combination with metformin, Takeda was required to submit an embyrofetal development study in rats administered alogliptin with and without metformin.

The requested embyrofetal study was submitted by Takeda in their complete response submission for both NDAs (monotherapy and pioglitazone FDC). No drug-related fetal abnormalities considered relevant to human subjects was identified in a combination embyrofetal toxicology study conducted in rats. The study included separate arms for alogliptin and metformin in addition to the drugs in combination, as requested by FDA. Two dams from the high dose combination group of 100/500 mg/kg alogliptin/metformin produced 4 fetuses with abnormalities, but this was associated with evidence of toxicity in the dams. Exposure margins at these doses are approximately 23x for alogliptin and 6x metformin relative to the recommended clinical doses. Of note, the teratogenicity issue (craniofacial abnormalities) that emerged in 2009 with another DPP4 inhibitor was subsequently resolved with additional studies, and no further evidence of augmented teratogenicity has been observed with the combination of DPP4 inhibitors and metformin. The nonclinical CR issue has therefore been adequately addressed by the sponsor and the data supports an approval recommendation for both NDAs.

Other nonclinical studies submitted in the CR submission, notably a juvenile animal study, do not change our recommendations for either NDA.

Labeling recommendations appropriate to start negotiations with Takeda are recommended in Dr Carlson's review.

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/s/

TODD M BOURCIER
01/18/2012
pharm/tox supports AP

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	22-271 22-426
Supporting document/s:	SDN-49(N-48) – NDA 22-271 Complete Response SDN-31(N-30) – NDA 22-426 Complete Response
Applicant's letter date (CDER Stamp Date):	25 July, 2011
Product:	Alogliptin (Nesina™ - proposed) Alogliptin + pioglitazone FDC (Oseni™ - proposed)
Indication:	Type 2 diabetes mellitus (T2DM)
Applicant:	Takeda Pharmaceutical Co. Ltd.
Review Division:	Metabolism and Endocrinology Products
Reviewer:	David B. Carlson, Ph.D.
Supervisor/Team Leader:	Todd Bourcier, Ph.D.
Division Director / Deputy Director:	Mary Parks, M.D. Eric Colman, M.D.
Project Manager:	Mehreen Hai, Ph.D.
Review Completion Date:	17 January, 2012

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 22-271 and NDA 22-426 are owned by Takeda or are data for which Takeda has obtained a written right of reference. Any information or data necessary for approval of NDA 22-271 and NDA 22-426 that Takeda 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 described 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 22-271 or NDA 22-426.

Review Notes and Abbreviations/Key

Some of the sponsor's tables and figures from the electronic NDA submission have been included and cited in this review. All drug-related trends are discussed in relation to concurrent vehicle control groups in each study unless otherwise noted. Vehicle for oral gavage administration was 0.5% methylcellulose for alogliptin and water for injection (sterile water) for metformin unless otherwise noted. Common animal strains were used and abbreviated by common animal name, unless noted, as follows: Sprague-Dawley rat, CD-1 mouse, Beagle dog, Cynomolgus monkey, New Zealand White rabbit. Alogliptin benzoate salt (SYR-322) and metformin HCl drug substances were used for dosing solutions, with all nominal doses expressed as free base SYR-322Z (SYR-322/SYR-322Z = 1.360) or metformin (metformin HCL/metformin = 1.282).

Key: Alogliptin benzoate (alo; alogliptin; SYR-322; SYR-322Z; SYR322S); pioglitazone HCl (pio; pioglitazone), SYR-322Z (free base of SYR-322); alogliptin + pioglitazone FDC (alo + pio; SYR-322-4833); metformin HCl (met; metformin); fixed-dose combination (FDC), once daily dosing (QD), dosing groups – LD (low dose), MD (mid dose), LMD (low mid dose), HMD (high mid dose), HD (high dose); mg/kg (mg/kg/day); MRHD (maximum recommended human dose); NOAEL (no observed adverse effect level); LOAEL (lowest observed adverse effect level); MTD (maximum tolerated dose); statistically significant (ss); not statistically significant (nss); PD (pharmacodynamic), PK (pharmacokinetic), TK (toxicokinetic); BW (body weight); GD (gestation day); CNS (central nervous system); GI (gastrointestinal tract); CV (cardiovascular); 5-methyltetrahydrofolic acid (5-MT), S-(5'-adenosyl)-L-methionine (SAM), S-(5'-adenosyl)-L-homocysteine (SAM); CR (Complete Response, pertaining to regulatory action for NDA review); T2DM (type 2 diabetes mellitus).

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1 Executive Summary

1.1 Introduction

NDAs for alogliptin tablets and alogliptin plus pioglitazone FDC tablets were previously submitted and reviewed by this pharmacology/toxicology reviewer. The pharmacology/toxicology reviews recommended approval for both alogliptin monotherapy (NDA 22-271) and FDC (NDA 22-426) applications indicated for treatment of type 2 diabetes mellitus.

A Complete Response (CR) letter was issued requiring additional clinical data to support approval of the monotherapy and FDC drugs. A pharmacology/toxicology comment was included in the CR letter requiring an embryofetal rat study with combination alogliptin plus metformin treatment because of expected alogliptin treatment on background metformin therapy. Class-related reproductive toxicity had been suspected with DPP4 inhibitor drugs and metformin when given in combination. Takeda submitted their proposed embryofetal rat protocol for discussion and a general design was agreed upon by DMEP pharm/tox and Takeda. There were no other nonclinical requirements or requests communicated in the CR letter.

Takeda submitted “Complete Response” data for both alogliptin and alogliptin plus pioglitazone FDC tablets at the same time, with cross-references between the NDAs where appropriate. All of the information in the submissions were reviewed together and will be discussed in this single review for both NDAs.

1.2 Brief Discussion of Nonclinical Findings

Prior Pharmacology/Toxicology reviews and approval recommendations for alogliptin and alogliptin plus pioglitazone FDC tablets were based on maximum recommended human dose (MRHD) exposure estimates for 25 mg QD alogliptin ($AUC_{0\text{-}24\ h}$ = 1.5 µg*h/ml; C_{max} = 140 ng/ml) and 45 mg QD pioglitazone ($AUC_{0\text{-}24\ h}$ = 10 µg*h/ml). The same clinical dose and MRHD exposure estimates were used in this review for new toxicology studies.

The only pivotal nonclinical studies submitted with the Complete Response was the combination alogliptin and metformin embryofetal rat study, which could have important implications for alogliptin monotherapy on top of background metformin therapy for T2DM. Early reproductive toxicity data suggested a potential interaction between DPP4 inhibitor drugs and metformin leading to craniofacial malformations in rat fetuses exposed during development. This review focused on the combination reproductive toxicity studies and other new studies submitted in the CR without recapitulating the original NDA reviews for alogliptin. In addition to the combination embryofetal rat study, new studies in the CR included alogliptin plus metformin combination subchronic (three-month) rat toxicity, alogliptin juvenile rat, environmental toxicology, and pharmacology and PK studies.

No new nonclinical safety signals have emerged in the DPP4 inhibitor class or with pioglitazone (and the PPARα/TZD class) since the original alogliptin ± pioglitazone NDA reviews. It is important to note, though, that additional clinical evidence has emerged consistent with the nonclinical signals of cardiac toxicity and bladder cancer risk with long term pioglitazone use. Bladder cancer risk has been added as a “precaution” on the pioglitazone label.

Pancreatitis has been identified as a potential clinical risk with DPP4 inhibitor use. Previously reviewed alogliptin nonclinical data were reevaluated to identify any potential evidence or signals of alogliptin-induced pancreatitis. No apparent pancreas toxicity was evident upon reanalysis of toxicology studies in rodents and non-rodents.

Adverse event reports analyzed during the clinical review of alogliptin NDAs indicate a potential clinical signal for alogliptin-induced liver toxicity. Alogliptin animal data have not indicated a strong signal for liver toxicity. Signs of modest liver toxicity were seen in chronic/lifetime rat studies with alogliptin, which showed liver hepatocellular hypertrophy, periportal vacuolation, and basophilic ‘focus of cell alteration’ at greater than 200-times estimated clinical dose. The NOAEL for hepatotoxicity was at least 30-times MRHD in all animal species (mouse, rat, dog, monkey). Data from combination alogliptin toxicity studies (with pioglitazone or metformin coadministration) did not indicate any drug interactions leading to exacerbated liver toxicity.

New animal toxicity studies were designed to identify additive or synergistic effects of well tolerated alogliptin doses in combination with MTD metformin doses. There was no unexpected toxicity and no apparent synergistic increased toxicity in a three-month alogliptin plus metformin rat study. Toxicity was generally driven by metformin and included reduced BW gain, plasma lactic acid accumulation without concomitant increased bicarbonate, increased serum ALT and CK, slight alterations in plasma electrolytes, and increased organ weights with correlative histological lesions. Heart cardiomyopathy and myocardial hypertrophy, liver hepatocyte hypertrophy, kidney tubule regeneration and hypertrophy, salivary gland hypertrophy, and adrenal vacuolation and hypertrophy were correlated with organ/tissue weight increases. GI tract was also a target based on stomach gross foci and erosion, duodenum erosion, and cecum hyperplasia.

As noted above, DMEP required a combination alogliptin plus metformin rat embryofetal development study with the CR submission because of potential class-related craniofacial malformations in rats treated with DPP4 inhibitors and metformin. There were no treatment related fetal findings in alogliptin or metformin controls or in the low dose combination treatment (34X alogliptin / 3X metformin MRHD at the NOAEL). In the high dose combination treatment group there was evidence of maternal toxicity based on reduced body weight gain. Eye and vertebral malformations were seen in 4 fetuses from 2 (of 20) dams in the 100/500 mg/kg (alogliptin/metformin) combination (23X alogliptin / 6X metformin MRHD). A relationship to treatment could not be ruled out for malformed fetuses, but findings were limited to microphthalmia in three fetuses from one dam with markedly reduced body weight gain (suggesting significant maternal

toxicity) and a single fetus with multiple abnormalities in a separate dam (microphthalmia, cleft palate, microglossia, and mandibular micrognathia). There were no other treatment-related malformations and no apparent treatment-related external, visceral, or skeletal variations. There were no apparent treatment-related effects on pregnancy success or other pregnancy-related or fetal outcomes in any group. The fetal malformations were not consistent with prior craniofacial malformation signals in the class. It is also important to note that no further evidence of craniofacial abnormalities has emerged in embryofetal studies with DPP4 inhibitor and metformin coadministration. With the exception of fetal malformations from two HD combination dams but none in dams treated only with alogliptin or metformin, there was no clear evidence of unexpected or synergistic increase in maternal or embryofetal toxicity.

Alogliptin plasma exposure (C_{max} and AUC_{0-24h}) consistently decreased slightly, approximately 25-40%, with metformin coadministration. Metformin co-treatment did not affect alogliptin metabolism. Conversely, alogliptin treatment did not alter metformin plasma exposures.

Pediatric trials have not been allowed with pioglitazone. Nonclinical data showing bladder tumors in rats at estimated human exposures caused sufficient clinical concern to prohibit clinical trials in children and adolescents. Recent clinical data from post-market monitoring confirmed potential for bladder tumors in humans and support a continued hold on pediatric clinical trials with pioglitazone. Potential pioglitazone effects on bone are also a concern for a pediatric patient population expected to have ongoing bone development.

Juvenile animal studies have not been required for other DPP4 inhibitors and they were not considered necessary prior to pediatric clinical trials for alogliptin. However, because testes-related toxicity was seen at high doses in monkeys and rats at high exposure multiples (>30- to >200-times MRHD at NOAELs), there is a low probability of risk to the developing reproductive system in male children. Takeda investigated alogliptin effects on juvenile development in male and female rats in two studies: a relatively short, 4-week juvenile animal study with limited endpoints; and, a separate study dedicated to juvenile male reproductive system development. There were no apparent alogliptin effects up to 77- or 88-times (male reproductive study) expected clinical exposures. The acute toxicology profile of alogliptin appears to be similar in juveniles and adults; however, the juvenile animal studies did not evaluate typical endpoints of pubertal onset, behavioral maturation, or fertility. It is important to note that DMEP did not require these studies and DMEP pharmacology/toxicology was not consulted on study designs or informed of any specific rationale for Takeda conducting the studies. Since the therapeutic index is high for alogliptin and the target population for future alogliptin pediatric trials is children between 10 and 17 years old with immune system and brain development beyond critical early life stages, nonclinical data support safety of alogliptin in the target pediatric population.

No new issues were identified with alogliptin alone or in combination with pioglitazone (or metformin) to change prior pharmacology/toxicology conclusions. Alogliptin is well

tolerated in animals with large exposure margins to toxic animal doses. There is no unique toxicity concern for alogliptin compared to other DPP4 inhibitor drugs and TZD class-related toxicity is well established.

Safety issues relevant to clinical use of alogliptin alone or in combination with pioglitazone were summarized in prior pharmacology/toxicology reviews and they are reiterated and updated here where appropriate.

1. Clinical risks of pioglitazone (included on current labels) are well known from years of clinical use and previous nonclinical reviews. The major risks identified in pioglitazone nonclinical studies are cardiac toxicity and bladder tumors.
    a. Cardiac hypertrophy and increased heart weights with concomitant physiologic sequelae were seen in animals at low multiples of human exposure. Nonclinical cardiac changes may be adaptive in nature due to PPARγ-mediated (or other unknown mechanism) plasma volume expansion and changes in hemodynamic parameters and water distribution. Nonclinical data show no apparent risk of alogliptin on cardiac toxicity and, importantly, establish there is no apparent additive or synergistic effect of alogliptin co-treatment on pioglitazone-mediated cardiac or other toxicity.
    b. Bladder tumors were seen at low clinical exposures in male rats and bladder cancer risk has been added as a “precaution” on the pioglitazone label due to post-marketing clinical evidence. Simple transitional cell hyperplasia and gross calculus/calculi were seen in bladders of rats exposed chronically to very high levels of alogliptin. No chronic or lifetime combination alogliptin and pioglitazone studies have been conducted so potential interactions during chronic use are not known.

2. Combination treatment in rats did not cause an increase in hypoglycemia compared to alogliptin or pioglitazone monotherapy. Hypoglycemia risk is expected to be low due to glucose-dependent and insulin-dependent mechanisms of the two drugs. Nevertheless, because of the independent, complementary mechanisms for glucose lowering, clinical hypoglycemia risks may be higher compared to either monotherapy.

3. Hypersensitivity and/or pseudoallergy are predicted in sensitive individuals in the clinical population based on findings in dogs administered alogliptin. The reactions in dogs include facial swelling/edema at high multiples of human exposure (≥ 32X MRHD), which is also notable since edema is listed in the ‘precautions’ section of the ACTOS label. The reaction in dogs seemed to be separate from DPP4-inibitor induction of necrotic skin lesions in monkeys. The risk of skin lesions from prolonged alogliptin treatment cannot be ruled out, but there was no evidence of skin lesions in any species in the non-clinical program.

4. Since DPP4 cleaves substrates other than the targeted incretin hormones, inhibition of DPP4 may have unintended consequences with prolonged dosing. In

particular, antigen-mediated responses and immune cell trafficking may be affected by DPP4 inhibition. Immune-related effects remain an unresolved, unavoidable risk with DPP4 inhibitor drugs.

5. Both alogliptin and pioglitazone are secreted in milk of nursing rats and combination treatment in rats showed alogliptin potentiated some pioglitazone-mediated fetal effects including low fetal weights and increased non-teratogenic visceral variations. The ACTOS label and proposed OSENI label accurately note fetal toxicity in animals and recommend against drug use in pregnant women or nursing mothers unless medically necessary.

1.3 Recommendations

1.3.1 Approvability

The Pharmacology/Toxicology approval recommendation for alogliptin tablets and alogliptin plus metformin FDC tablets remains unchanged after review of the Complete Response submission. Pioglitazone's clinical cardiac and bladder tumor risks, previously identified in nonclinical reviews, are addressed in current labels.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

The only new nonclinical data submitted with potential labeling implications comes from the alogliptin plus metformin combination embryofetal rat study. While alogliptin alone did not cause any reproductive or fetal effects, the observed malformations with combination metformin treatment cannot be ruled out as unrelated to treatment. The data should be included in the label for the alogliptin plus metformin FDC (under review in a separate application, NDA 203-414) but the data is not relevant to alogliptin monotherapy use without background metformin therapy. Therefore, the pharmacology/toxicology recommendation is to include only data from reproductive toxicity studies with alogliptin alone in the alogliptin monotherapy label.

Takeda used slightly different human exposure estimates (1.40 and 1.73 µg*h/ml in males and females, respectively) than those used in this review (1.5 µg*h/ml sexes combined). Exposure estimates were within ± 13% and differences were not considered biologically significant. However, slight differences in human exposure estimates lead to slightly different exposure multiple calculations in the Sponsor's label compared to multiples in this review.

Specific labeling revisions and suggestions were made directly on the proposed labels in the Division files (eRoom).

2 Drug Information

2.1 Drug

Nesina™ (alogliptin) / Oseni™ (alogliptin + pioglitazone)

2.1.1 CAS Registry Number

Alogliptin – 850649-62-6
Pioglitazone – 112529-15-4

2.1.2 Generic Name

Alogliptin (alogliptin benzoate); Pioglitazone HCl

2.1.3 Code Name

Alogliptin – SYR-322 (SYR-322S; SYR110322; SYR110322S; SYR110322 benzoate)
Pioglitazone – AD-4833 (HCl); U-72,107A

2.1.4 Chemical Name

Alogliptin – 2-({6-[(3*R*)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl}methyl)benzonitrile monobenzoate; 2-[[6-[(3*R*)-3-Amino-1-piperidinyl]-3,4-dihydro-3-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl]methyl]benzonitrile monobenzoate

Pioglitazone – (±)-5[[4-[2-(5-ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-2,4-thiazolidinedione monohydrochloride

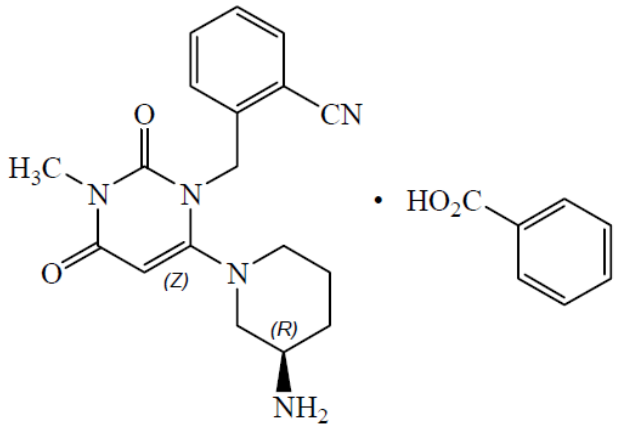
2.1.5 Molecular Formula/Molecular Weight

Alogliptin – $C_{18}H_{21}N_5O_2 \cdot C_7H_6O_2$ / 461.51 g/mol (benzoate salt); 339.30 g/mol (free base)

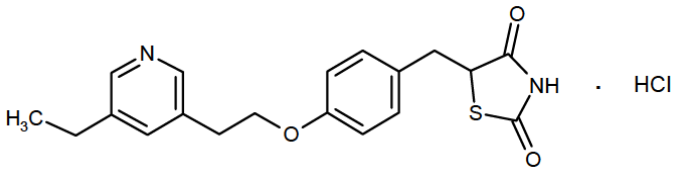
Pioglitazone – $C_{19}H_{20}N_2O_3S$ • HCl / 392.90 g/mol (HCl salt); 356.43 g/mol (free base)

2.1.6 Structure (or Biochemical Description)

Alogliptin



Pioglitazone



2.1.7 Pharmacologic class

Dipeptidyl peptidase IV (DPP4) inhibitor (alogliptin) plus thiazolidinedione (TZD) (pioglitazone).

Pioglitazone is also listed as a peroxisome proliferator-activated receptor (PPAR) alpha- and gamma- agonist.

2.2 Relevant IND/s, NDA/s, and DMF/s

Alogliptin – NDA 22-271; IND 69,707 (alogliptin), IND 73,193 (alogliptin + pioglitazone), IND 101,628 (alogliptin + metformin); NDA 203-414 (alogliptin + metformin)

Pioglitazone – NDA 21-073, IND 33,729

DPP4 Inhibitor NDAs – NDA 21-995 (sitagliptin, Januvia®) and NDA 22-044 (sitagliptin + metformin, Janumet®); NDA (b) (4) (vildagliptin); NDA 22-350 (saxagliptin, Onglyza®) and NDA 200-678 (saxagliptin + metformin, Kombiglyze XR ®); NDA 201-280 (linagliptin), NDA 201-281 (linagliptin + metformin)

2.3 Drug Formulation

No new drug formulation data were submitted with the CR submission. No safety concerns from drug substance impurities/degradants or drug product excipients were identified in the original Pharmacology/Toxicology Reviews for NDA 22-271 (alogliptin monotherapy) and NDA 22-426 (alogliptin + pioglitazone FDC)[1] Comprehensive drug formulation information can be found in the CMC reviews for monotherapy and FDC tablets.

2.6 Proposed Clinical Population and Dosing Regimen

Alogliptin and alogliptin plus metformin FDC tablets are indicated as adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus.

The recommended daily dose of alogliptin is 25 mg once daily, as monotherapy or in combination with anti-diabetic drug(s), taken with or without food. Dosing adjustment is recommended for patients with moderate or severe renal impairment or end stage renal disease.

The maximum recommended daily dose of the FDC is 25 mg alogliptin/45 mg pioglitazone. Dosing adjustments are recommended for patients with moderate renal

[1] D. Carlson, Pharmacology/Toxicology Review NDA 22-271 (8/27/08) and NDA 22-426 (6/8/09)

impairment (not recommended for severe renal impairment or end stage renal disease) or taking strong CYP2C8 inhibitors.

The Sponsor requested a deferral for alogliptin clinical trials for pediatric patients aged 10-17 and a waiver for ages under 10 (based on a limited T2DM population in young children. The major potential risks to children in pediatric trials are toxicity to tissues and organ systems that continue to develop until adulthood, such as brain, bone, muscle, and reproductive and immune systems. DPP4 is synonymous with CD26, which is present on various T-cells and is an active immune system component as a receptor/co-stimulatory molecule and adhesion molecule, and it has enzymatic activity on various chemokine substrates. DPP4 is also present in developing brain and skeletal muscle in low levels but evidence suggests it may lack enzymatic activity developmentally. It is not clear whether alogliptin or other DPP4 inhibitors alter the immune system or brain very early in development. In animals alogliptin readily crosses the placenta and it is excreted in breast milk, which suggests it may cross the blood:brain barrier resulting in brain and nervous system exposure although rat distribution studies showed limited brain accumulation. Whatever functions DPP4/CD26 may have in the developing nervous and immune systems, data from $DPP4^{-}$ knockout mice lacking DPP4 show it is not essential for normal development and there is apparently redundancy for DPP4-mediated developmental activity. Importantly, the target clinical trial population is limited to children older than 10, thus immune system and brain development should be advanced past critical early life stages.

Juvenile animal studies have not been required for other DPP4 inhibitors and they were not considered necessary prior to pediatric clinical trials for alogliptin. However, because testes-related toxicity was seen at high doses in monkeys and rats at high exposure multiples (>30- to >200-times MRHD at NOAELs), there is a low probability of risk to the developing reproductive system in male children. Takeda investigated alogliptin effects on juvenile development in male and female rats in two studies: a relatively short, 4-week juvenile animal study with limited endpoints; and, a separate study dedicated to juvenile male reproductive system development. There were no apparent alogliptin effects up to 77- or 88-times (male reproductive study) expected clinical exposures. The acute toxicology profile of alogliptin appears to be similar in juveniles and adults; however, the juvenile animal studies did not evaluate typical endpoints of pubertal onset, behavioral maturation, or fertility. Since the therapeutic index is high for alogliptin and the target population for future alogliptin pediatric trials is children between 10 and 17 years old with immune system and brain development beyond critical early life stages, nonclinical data support safety of alogliptin in the target pediatric population.

Pediatric trials have not been allowed with pioglitazone. Nonclinical data showing bladder tumors in rats at estimated human exposures caused sufficient clinical concern to prohibit clinical trials in children and adolescents. Recent clinical data from post-market monitoring confirmed potential for bladder tumors in humans and support a continued hold on pediatric clinical trials with pioglitazone. It is worth noting potential

pioglitazone effects on bone are also a concern for a pediatric patient population expected to have ongoing bone development.

2.7 Regulatory Background

Alogliptin (NDA 22-271) and alogliptin plus metformin FDC (NDA 22-426) NDAs were previously submitted and reviewed by this pharmacology/toxicology reviewer. A Complete Response (CR) letter was issued requiring additional clinical data to support approval of the monotherapy and FDC drugs. A pharmacology/toxicology comment was included requiring a combination alogliptin plus metformin embryofetal rat study based on concerns about potential interactions with DPP4 inhibitors and metformin on fetal development. Metformin is the standard of care for T2DM and alogliptin is expected to be used on a background of metformin treatment (among other potential background treatments). In addition, during the course of this review the Sponsor submitted a separate NDA (NDA 203-414) for alogliptin plus metformin FDC tablets.

Pioglitazone is currently marketed by the Sponsor as monotherapy and in FDC products.

3 Studies Submitted

3.1 Studies Reviewed

See Table of Contents, above (all studies were reviewed).

3.2 Studies Not Reviewed

None.

3.3 Previous Reviews Referenced

Pharmacology/Toxicology Reviews from the original NDA submissions are referenced where appropriate[2].

[2] *IBID*

4 Pharmacology

4.1 Primary Pharmacology

DPP4 and serine peptidases inhibition by alogliptin and other DPP4 inhibitors (Report No. TSD0322-112)
Non-GLP (research study), signed 3/11/08

Inhibition of DPP4 and related serine peptidases by alogliptin and other drugs was determined in 96- and 384-well microtiter plate assays. Results confirmed highly selective DPP4 inhibition by alogliptin, with no detected inhibition of DPP8 or DPP9.

Table 1 – DPP4 inhibition comparison

Summary of mean IC_{50} values of alogliptin, vildagliptin and sitagliptin for DPP4 and related serine peptidases

Enzyme	IC_{50} (nM) [a]		
	Alogliptin	Vildagliptin	Sitagliptin
DPP4	6.9 ± 1.5	23.8 ± 1.6	12.1 ± 0.8
DPP2	>100,000	>100,000	>50,000
DPP8	>100,000	1,400 ± 200	19,000 ± 2,000
DPP9	>100,000	81.5 ± 8.1	62,000 ± 4,000
PREP	>100,000	>50,000	>100,000
FAP/seprase	>100,000	73,000 ± 8,000	>100,000
Tryptase	>390,000	>200,000	>400,000

DPP=dipeptidyl peptidase; PREP=prolyl endopeptidase; FAP=fibroblast activation protein.
[a] Values are expressed as mean ± SE and represent the mean of 2 to 17 individual evaluations for each compound against each enzyme.

DPP4 inhibition by alogliptin metabolites M-I and M-II (Study TCAD2007-TA-09)
Non-GLP (research study), signed 2/18/08

Inhibitory potential of alogliptin metabolites M-I and M-II was determined on DPP4 isolated from Caco-2 cells and plasma from various species. DPP4 activity was inhibited by M-I but not by M-II. M-I potently inhibited DPP4 activity at nanomolar concentrations, with calculated IC_{50}s of 21 nM against Caco-2 DPP4 and 14, 17, and 19 nM against human, dog, and rat plasma DPP4, respectively. M-II showed “very weak inhibitory activity” with no calculable IC_{50} up to 30 µM M-II for any isolated DPP4.

4.2 Secondary Pharmacology

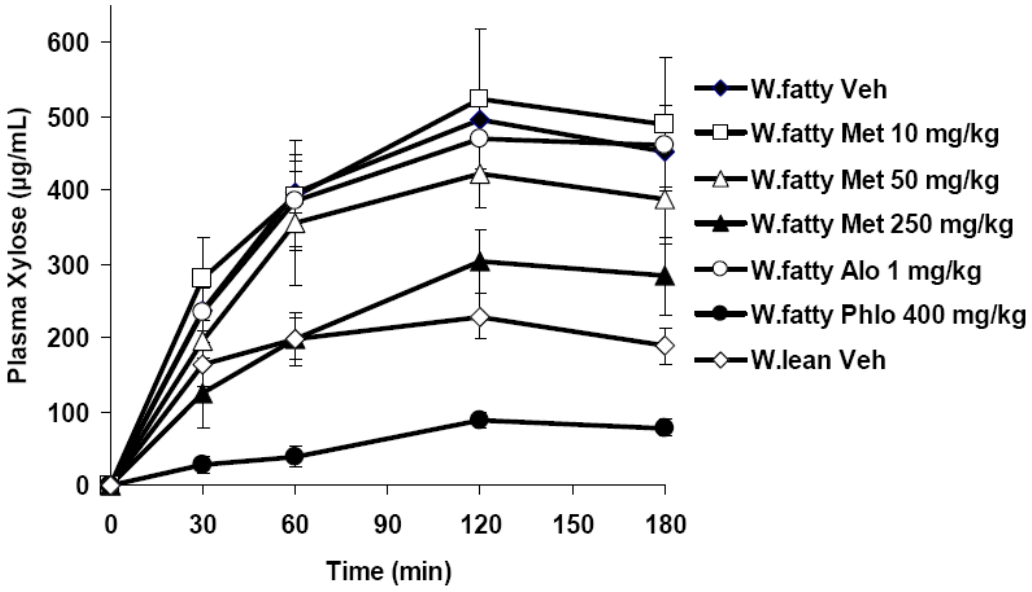
Effect of alogliptin and metformin on intestinal xylose absorption (Study SD1AD2007-KT-184)

Non-GLP (research study), signed 4/3/08

Summary: Intestinal xylose absorption was investigated in Wistar fatty (WF) rats to determine if the plasma glucose lowering mechanism of alogliptin or metformin may involve decreased glucose absorption. A single 1 mg/kg oral alogliptin dose had no effect on xylose absorption, whereas ≥ 50 mg/kg metformin decreased xylose absorption by ≥ 14% (ss) compared to vehicle (0.5% methylcellulose) controls. Intestinal sugar absorption occurs primarily through sodium glucose cotransporter 1 (SGLT1) and is not insulin-dependent (confirmed by the SGLT inhibitor positive control, phlorizin), Results suggest improved glucose tolerance after combination alogliptin plus metformin in WF rats may involve independent and/or complementary mechanisms.

Figure 1 – Alogliptin and metformin effects on intestinal sugar absorption

(A)



(B)

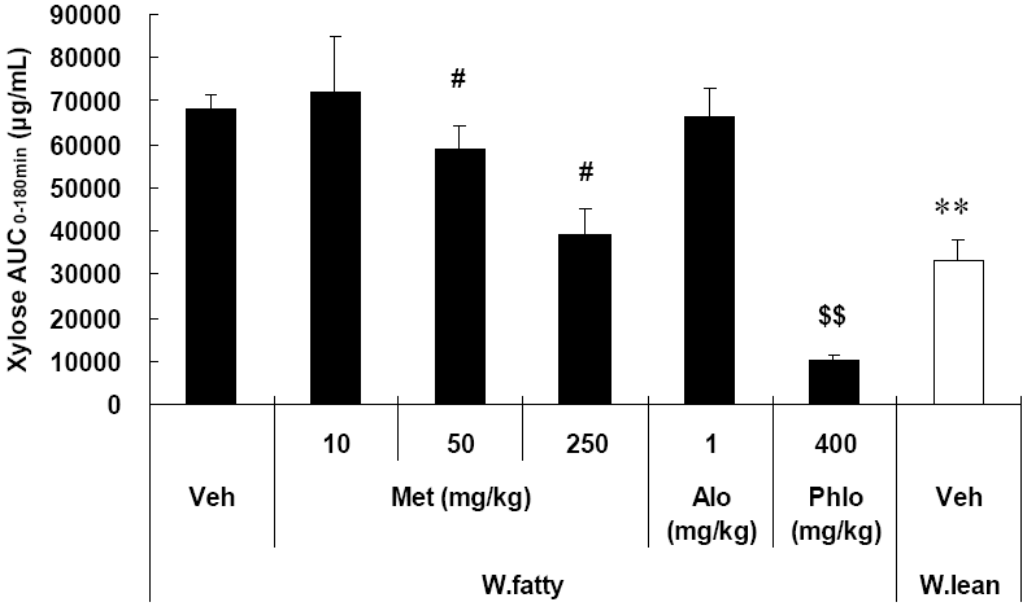


Figure Legend – Rats were fasted for 18 h, and administered 10, 50, and 250 mg of metformin (Met), or 1 mg of alogliptin (Alo) 1 hr before an oral xylose load (0.8 g/kg). Phlorizin (Phlo) (400mg/kg) was simultaneously administered with xylose. Plasma xylose levels (A) and $AUC_{0\text{-}180min}$ of plasma xylose (B) were measured. Values are mean ± S.D. (n=6 for Wistar fatty rats, n=4 for Wistar lean rats). #$p \leq 0.025$ vs vehicle-treated Wistar fatty rats by Shirley-Williams test. $$ $p \leq 0.01$ vs vehicle-treated Wistar fatty rats by Aspin-Welch test. **$p \leq 0.01$ vs vehicle-treated Wistar fatty rats by Student's t-test.

Pharmacodynamic Drug Interactions

Alogliptin is likely to be used in T2DM patients on background diabetic therapies, particularly if glucose control becomes inadequate with individual or multiple therapies. Alogliptin efficacy was investigated in several different animal diabetes models in combination with other glucose lowering drugs, including: a biguanide (metformin) ± a TZD (pioglitazone); a sulfonylurea (glibenclamide); and an α-glucosidase inhibitor (voglibose).

Effect of alogliptin and metformin on glucose tolerance and GLP-1 (Study SYAD2009-KT-024)

Non-GLP (research study), signed 11/9/09

Summary: Female Wistar fatty (WF) rats were treated orally with vehicle (0.5% methylcellulose), 3 mg/kg alogliptin, 50 mg/kg metformin, or alogliptin (3 mg/kg) plus metformin (50 mg/kg). Plasma glucose, plasma insulin, and plasma active GLP-1 were measured in fasted animals for 2 h after a glucose load (i.e., oral glucose tolerance test (OGTT) given 60 min post-dose). Results were also compared to non-diabetic Wistar lean (WL) rats treated with vehicle. The alogliptin plus metformin combination improved the glucose response to OGTT in an additive manner (ss 27% decreased AUC vs. 12-13% for monotherapies) and improved GLP-1 activity in a synergistic manner (ss 20-fold increased activity vs. 7-fold for alogliptin alone). Combination treatment did not increase plasma insulin levels, rather, alogliptin alone had the largest effect on plasma insulin. Data are summarized in the Sponsor's figure below (Figure 2).

Figure 2 – Glucose, insulin, and DPP-4 response to alogliptin + biguanide

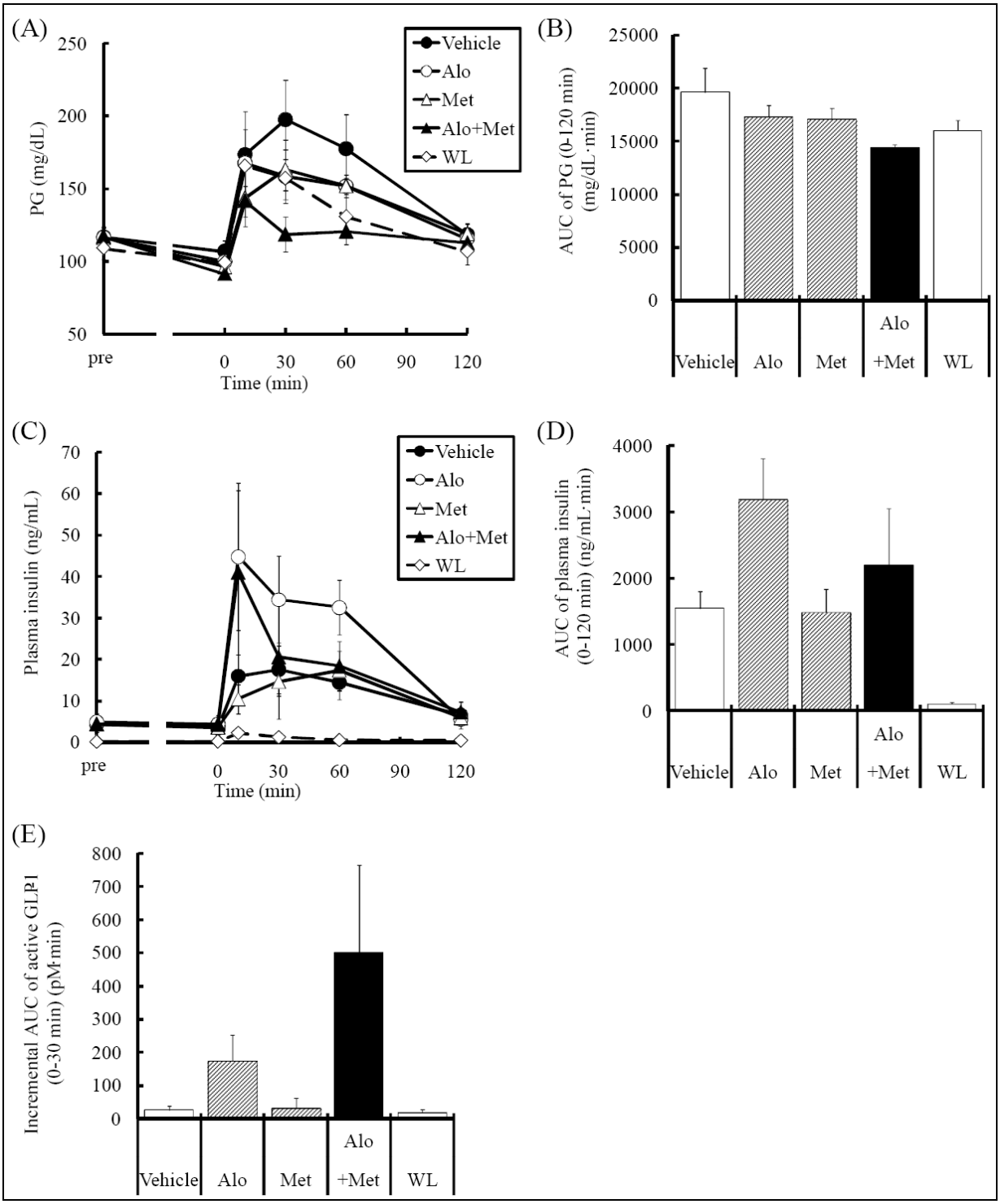


Figure Legend – Combined effects of alogliptin and metformin on plasma glucose, insulin levels and active GLP-1 levels in female WF rats (A) and (C) show time-dependent changes of PG and plasma insulin after 1 g/kg glucose challenge, respectively. Data in (B) and (D) represent AUC0-120min of PG shown in (A) and AUC0-120min of plasma insulin shown in (C), respectively. Data in (E) represent

incremental AUC0-30min of plasma active GLP-1. Two-way ANOVA showed significant main effects of alogliptin ($p \leq 0.01$) and metformin ($p \leq 0.01$) with no interaction between alogliptin and metformin on AUC0-120min of PG, indicating that the combined effect is additive. Two-way ANOVA showed significant main effects of alogliptin ($p \leq 0.01$) and metformin ($p \leq 0.01$) with significant interaction between alogliptin and metformin ($p \leq 0.05$) on incremental AUC0-30min of plasma active GLP-1, indicating that the combined effect is synergistic. Values are mean ± SD (n=5-6). Alo, 0.3 mg/kg of alogliptin; Met, 50 mg/kg of metformin; Alo + Met, 0.3 mg/kg of alogliptin + 50 mg/kg of metformin.

Effect of alogliptin, metformin, and pioglitazone combination on glucose tolerance (Study SD1AD2007-KT-124)

Non-GLP (research study), signed 3/25/08

Summary: Male Wistar fatty (WF) rats were pre-treated (6 d) with 1 mg/kg pioglitazone (or vehicle) and response to oral glucose tolerance test (OGTT) was assessed after 1 mg/kg alogliptin and 50 mg/kg metformin alone or in combination. Triple combination of drugs improved glucose excursion as decreased $AUC_{0\text{-}120\ min}$ by 55% compared to 37-38% decrease by combination pio + alo, pio + met, or alo + met. As expected, alogliptin increased plasma active GLP-1, while metformin slightly increased active GLP-1 and a supra-additive increase in active GLP-1 was observed with alo + met combination (consistent with a separate study). Alogliptin treatment did not further improve insulin response from pioglitazone ± metformin treatment.

Figure 3 – Plasma active GLP-1 with alo ± met on background pio in WF rats

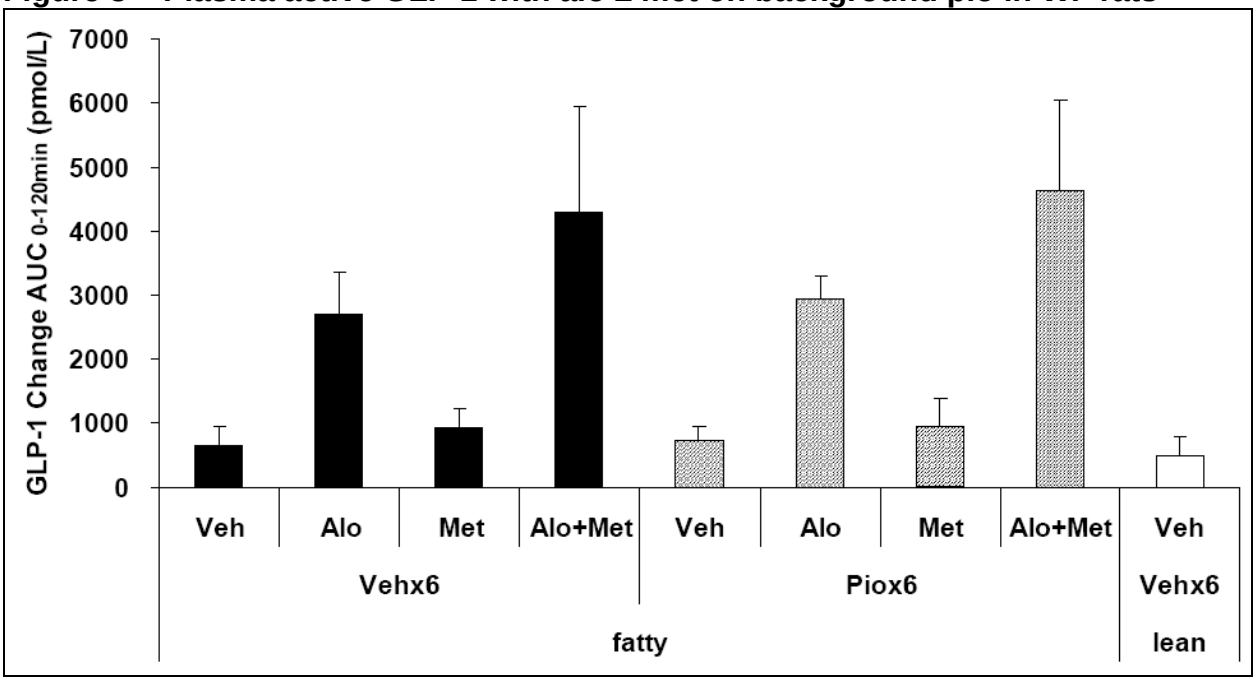


Figure legend – AUC of $AUC_{0\text{-}120min}$ of incremental active GLP-1. Values are mean ± SD (n=6 for Wistar fatty, n=5 for Wistar lean rats). Veh, vehicle; Alo, alogliptin; Met, metformin; Pio, pioglitazone.

Figure 4 – Glucose AUC with alo ± met on background pio in WF rats

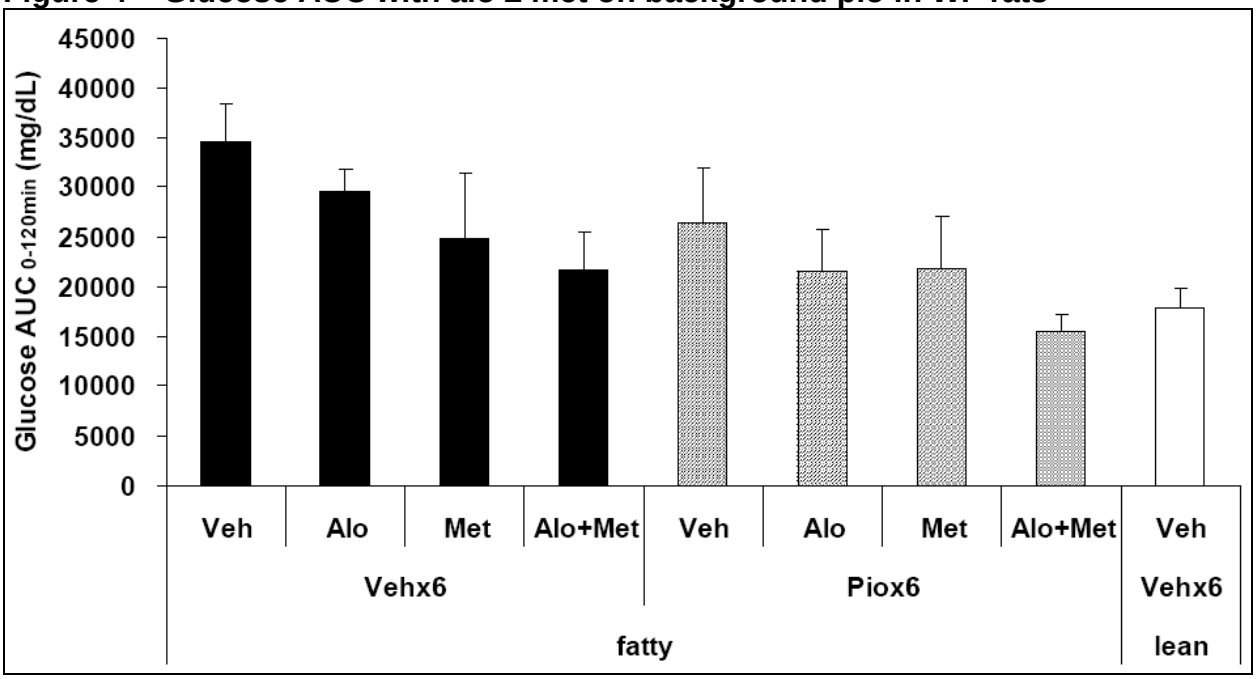


Figure legend – Effect of triple combination of alogliptin, pioglitazone, and metformin on plasma glucose $AUC_{0\text{-}120\ min}$ after OGTT in Wistar fatty rats. Values are mean ± SD (n=6 for Wistar fatty, n=5 for Wistar lean rats). Veh, vehicle; Alo, alogliptin; Met, metformin; Pio, pioglitazone.

Effect of alogliptin and glibenclamide combination on glucose tolerance and insulin (Study SYAD2009-KT-025)

Non-GLP (research study), signed 12/24/09

Summary: Male N-STZ-1.5 rats, a type 2 diabetes model (Wistar Kyoto (WKY) rats injected with STZ day 1.5 after birth), were treated orally with 0.3 mg/kg alogliptin alone or in combination with the sulfonylurea glibenclamide. Effects on plasma glucose and insulin responses after oral glucose tolerance test (OGTT; 60 min post-dose) were compared to vehicle treated (0.5% methylcellulose) and untreated non-diabetic WKY controls. Combination treatment resulted in additive improvement of plasma glucose AUC (ss 32% decrease compared to ss 13% alogliptin and 26% glibenclamide monotherapies) and insulin secretion (ss 60% increase compared to ss 30-40% increase with each monotherapy). Time of maximum insulin secretion shifted earlier in combination treatments (T_{max} = 10 min) compared to monotherapy, vehicle controls, and untreated non-diabetic controls (T_{max} = 30 min).

Figure 5 – Glucose and insulin response to alogliptin + sulfonylurea

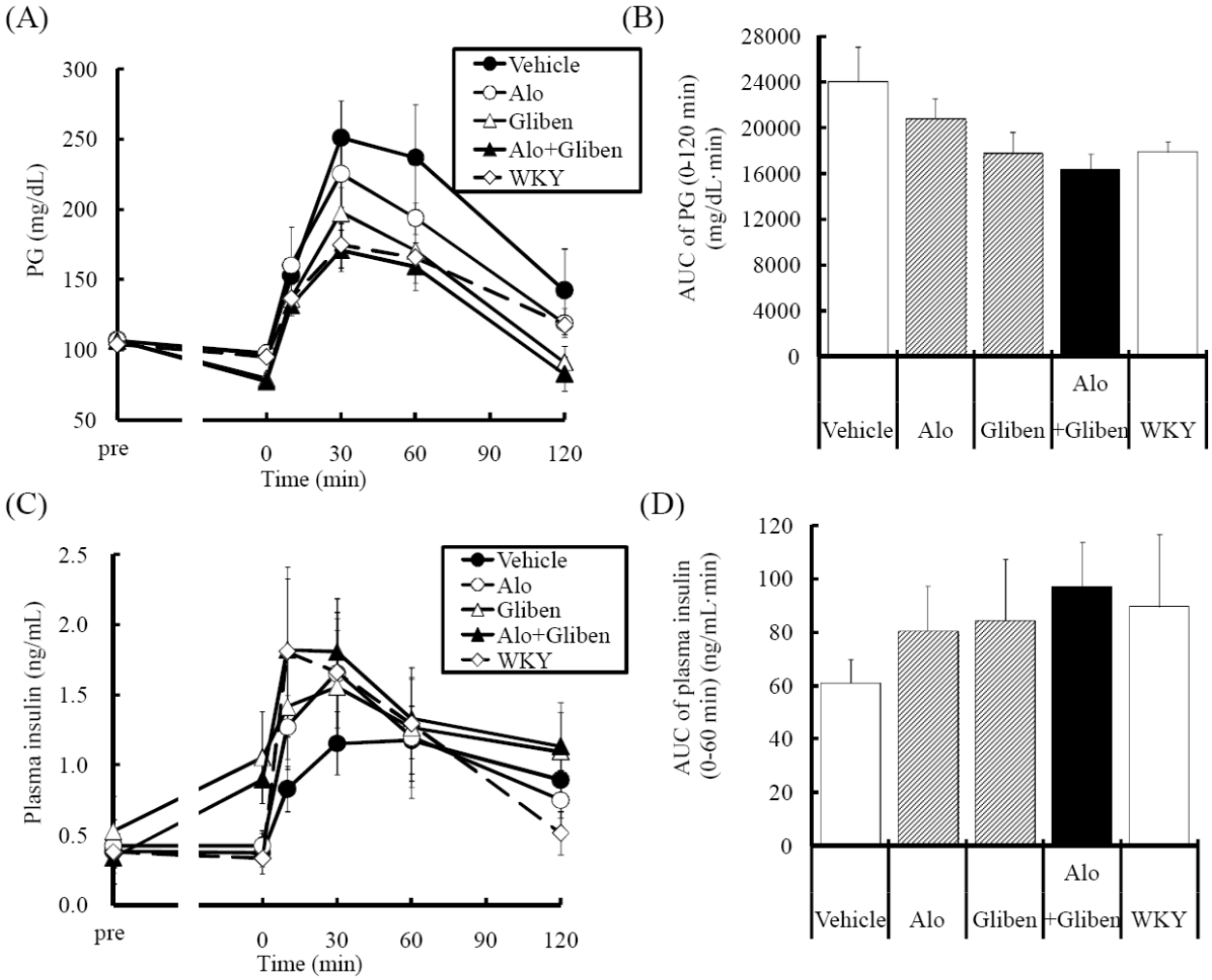


Figure Legend – Combined effects of alogliptin and glibenclamide on PG and insulin levels in male N-STZ-1.5 rats (A) and (C) show time-dependent changes of PG and plasma insulin after 1 g/kg glucose challenge, respectively. Data in (B) and (D) represent $AUC_{0\text{-}120min}$ of PG shown in (A) and $AUC_{0\text{-}60min}$ of plasma insulin shown in (C), respectively. Values are mean ± SD (n=5-6). Alo, 0.3 mg/kg of alogliptin; Gliben, 10 mg/kg of glibenclamide; Alo + Gliben, 0.3 mg/kg of alogliptin + 10 mg/kg of glibenclamide.

Effect of alogliptin and voglibose combination on pancreatic islet cells (Study SD1AD2006-KT-063)

Non-GLP (research study), signed 3/13/08

Summary: Male pre-diabetic *db/db* mice were treated orally in diet for 27 days with 0.03% alogliptin and 0.001% voglibose alone or in combination and compared to vehicle treated and untreated (non-diabetic *db/+* male mice) controls. Studies were conducted to further investigate a mechanism for observed improved glycemic control and β-cell preservation in mice treated with combination treatment compared to individual drug treatment. The study report concluded β-cell immunohistochemical staining showed

combination treatment caused “potent” insulin staining and improved glucagon-positive cell distribution, in contrast to monotherapy or vehicle controls with: reduced insulin staining; abnormal glucagon-positive cell distribution;, decreased pdx-1 nuclear expression; and, decreased glut2 membrane localization. Only a single summary figure of immunohistochemical staining was shown and no discussion of quantitative analyses was provided, preventing independent verification of the reported conclusions.

4.3 Safety Pharmacology

No safety pharmacology issues were identified in the original alogliptin review for CNS, CV, pulmonary, renal, GI, or abuse liability endpoints.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Analytical Methods and Validation

Method validation for metformin in rat plasma (Study P08-22601)
GLP statement, signed 9/26/08

Summary: A LC/MS/MS method was fully validated for detection of metformin in rat plasma from 50 to 20,000 ng/ml, with stability confirmed for 3 months storage at ≤ -15°C.

Absorption

Plasma PK of alogliptin, M-I and M-II after single oral and *iv* doses in rat (Study A883-322-038, Amendment 2)
Non-GLP (research study), signed 10/21/09

Summary: Amendment #2 to the final study report was amended with the proper data for metabolite M-II (which had been inadvertently transposed from M-I data). The corrected data table is shown below.

Concentrations of M-II in plasma after intravenous administration of SYR-322 at a dose of 1 mg/kg (as SYR-322Z) to rats

Time after dosage	Concentration (ng/mL)			
	No. 10	No.14	No.15	Mean ± S.D.
Pre	0	0	0	0 ± 0
5 min	2.08	2.98	2.88	2.65 ± 0.49
10	2.01	5.03	3.84	3.63 ± 1.52
15	3.13	2.64	3.61	3.13 ± 0.49
30	2.92	3.82	3.23	3.32 ± 0.46
1 h	4.23	2.51	3.17	3.30 ± 0.87
2	1.54	1.05	0.887	1.16 ± 0.34
3	0	0.512	0	0.171 ± 0.296
4	0	0	0	0 ± 0
6	0	0	0	0 ± 0
8	0	0	0	0 ± 0
24	0	0	0	0 ± 0
T_{max} (h)	1.0	0.167	0.167	0.4 ± 0.5
C_{max} (ng/mL)	4.23	5.03	3.84	4.37 ± 0.61
$AUC_{0\text{-}24h}$ (ng·h/mL)	7	6	6	6 ± 1

Pre; Prior to dosing.
Data were obtained from the Study No. P06-17303.

Metabolism

***In vitro* alogliptin metabolism by cytochrome P450s (Study A970-322-047)**
Non-GLP (research study), signed 12/7/07

Summary: Metabolism of alogliptin was investigated in microsome preparations from cells expressing specific human P450s from baculovirus infection. Alogliptin was metabolized "slightly" (2-3 pmol/h/pmol P450) by human CYP2D6 and CYP3A4 in microsomal incubations. M-I was formed primarily by CYP2D6 and other unidentified metabolites resulted from CYP3A4-mediated metabolism.

Alogliptin metabolism in primary hepatocytes (Study AE-5462-G)
Non-GLP (research study), signed 12/25/07

Summary: Alogliptin metabolism was investigated in primary cultures of cryopreserved rat, dog, and human hepatocytes. Metabolism was limited in all species, with M-I produced in rats (2-8%), dogs (1-3%) and humans (0.1-.02%) and M-II produced in rats (1-3%) and humans (0.2-1%). Low levels of additional, unidentified metabolites were seen in rats and humans.

Alogliptin metabolism in rat after single oral and *iv* treatment (Study SYR-322(15))
Signed GLP-like statement and QA statement, signed 11/15/06

Summary: Alogliptin was found largely unchanged in rat plasma after single oral and *iv* doses. Oral bioavailability was approximately 46%. Low levels of M-I and M-II were also detected.

5.2 Toxicokinetics

Single combination oral gavage alogliptin + metformin rat TK (Study 08-329/tk)
Non-GLP range-finding, signed 3/3/09

Summary: Alogliptin and metformin were coadministered by oral gavage to SD rats (n=2/sex/group) at doses of 100/0, 0/100, 0/300, 0/1000, 100/1000 mg/kg alogliptin/metformin. Maximum (C_{max}) and total (AUC_{0-24h}) metformin exposure increased with increasing dose and exposure was not affected by alogliptin coadministration. Both C_{max} and AUC_{0-24h} of alogliptin were decreased, generally 2-fold or more, with HD metformin coadministration compared to alogliptin alone. Alogliptin metabolite M-I and M-II exposures were also lower with metformin coadministration.

6 General Toxicology

Alogliptin benzoate salt (SYR-322) and metformin HCl drug substances were used for dosing solutions, with all nominal doses expressed as free base SYR-322Z (SYR-322/SYR-322Z = 1.360) or metformin (metformin HCL/metformin = 1.282). Metformin clinical exposure was estimated based on a maximum recommended dose of 2000 mg/day and the Sponsor's clinical trial results (SYR-322MET_102) showing mean metformin exposure of 26 µg*h/ml (estimated $AUC_{0\text{-}24\ h}$) in combination with alogliptin.

6.2 Repeat-Dose Toxicity

Four week dose-ranging alogliptin + metformin study in rats
Non-GLP, signed 5/25/09 (Study No. 08-332/su, Report No. SYR-322MET-10012)

0, 30/0, 100/0, 30/100, 100/100, 100/300, 100/1000 mg/kg alogliptin/metformin
*5/0, 25/0, 7/30, 30/32, 29/100, 25/265 µg*h/ml alogliptin/metformin*

NOAEL = 30/100 mg/kg alogliptin/metformin (5X alo / 1X met MRHD)

Summary: Isolated, low incidence and low severity findings were seen in kidney (hyaline droplets) and adrenal gland (increased weight) in 30/100 alo + met combination, which were not considered adverse in the absence of other signs of toxicity. Signs of modest toxicity in kidney (tubule hyaline droplets or vacuolation), heart (cardiomyopathy), and submandibular gland (decreased duct granules) were more evident at 100/100 and 100/300 combinations. The HD 100/1000 mg/kg combination dose showed clearly increased incidence and severity of toxicity in multiple target organs.

Key Study Findings:

- Kidney hyaline droplets and/or vacuolization in renal tubules in all groups, tubular basophilia in females ≥ 100/100 mg/kg group, with increased incidence and/or severity in 100/1000 mg/kg HD. Biological significance was unclear.[3]

- Submandibular gland minimal decreased granules (in granule ducts) in males ≥ 100/300 and male duct wall hypertrophy and female duct hypertrophy in 100/1000 HD combination.
    - Sublingual gland minimal duct hypertrophy in HD combo females

- More pronounced toxicity evident in HD combination 100/1000
    - Modest clinical signs– salivation, loose stool, crystalluria, soiled fur
    - ↓ Body weight gain (♂) and ↓ food consumption
    - ↑ Lactic acid

[3] *Reviewer's note – analyses in the 13-week study identified $\alpha 2\mu$-globulin in males, presumably in phagolysosomes, and lipid droplets in females at ≥ 100/100 mg/kg* alo/met

- ↑ Triglycerides
- Target organ toxicity (↑ liver (+30-40%), heart (+25-30%), ovary (+30%) and adrenal gland (+11-32%) weights; minimal centrilobular hepatocyte hypertrophy (♀); mild cardiomyopathy; and kidney tubule hyaline droplets, vacuolization, and/or basophilia.

- No clear treatment-related ophthalmoscopy, hematology, or gross pathology findings

Thirteen week combination alogliptin + metformin study in rats
GLP statement, signed 1/13/10

Doses (mg/kg/d) *0/0 (vehicle control)*
100/300, 100/1000 mg/kg (Alogliptin / Metformin)
0/300, 0/1000 mg/kg (metformin LD & HD controls)

*Exposures: 47/110, 34/242, 0/84, 0/230 µg*h/ml (alogliptin / metformin)*

***NOAEL < 100/300** alogliptin / metformin (31X alo / 4X MRHD)*

***NOAEL determination –** toxicity was largely due to metformin with additive effects of alogliptin on some targets. Low doses of metformin ± alogliptin were tolerated but not without toxicity, while higher doses of metformin ± alogliptin caused more marked toxicity (including death of one male). The study was designed to identify potential unexpected toxicity from combined alogliptin and metformin treatment. No unusual synergistic interactions were observed. Combination treatment resulted in toxicity predicted by the individual drugs and the absence of a NOAEL did not affect interpretation of the study results.*

Study Title: Thirteen-week oral gavage toxicity study of SYR-322/Metformin hydrochloride in rats

Study No.:	B-6610 (Takeda code SYR-322MET-10185)
Study report location:	eCTD 4.2.3.7.7 ('Other Toxicity Studies')
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1/9/09
GLP compliance:	Yes (Japan)
QA statement:	Yes
Drug, lot #, and % purity:	SYR-322, Lot No. MA01-001, 100.2%; metformin HCl Lot Nos. OB265 & OB 326, 100.5% & 100.6% (respectively)

Key Study Findings:

- Slight additive effects of alogliptin on certain metformin-induced toxicity were observed but there was no evidence of a synergistic effect of combination

- Toxicity was generally not worsened by alogliptin coadministration
- Difficult to make definitive conclusions about alogliptin-related toxicity because of the absence of any alogliptin only controls

- Metformin-induced toxicity included reduced BW gain, plasma lactic acid accumulation without concomitant increased bicarbonate, increased serum ALT and CK, slight alterations in plasma electrolytes, increased organ weights (heart, liver, kidney, salivary gland, adrenals), and correlative histological lesions.

- Target organs were heart, kidney, liver, and salivary and adrenal glands. Heart cardiomyopathy and myocardial hypertrophy, liver hepatocyte hypertrophy, kidney tubule regeneration and hypertrophy, salivary gland hypertrophy, and adrenal vacuolation and hypertrophy were correlated with organ/tissue weight increases. GI tract was also a target based on stomach gross foci and erosion, duodenum erosion, and cecum hyperplasia.

- Kidney tubules had increased vacuolation and/or droplets, identified as hyaline droplets (possibly α2μ-globulin-containing phagolysosomes) in males and lipid accumulation in females.

- Metformin C_{max}, but not AUC, was decreased 25-30% with alogliptin coadministration. Alogliptin C_{max} and AUC decreased slightly, approximately 25-40%, with increasing metformin coadministration. Alogliptin exposure was approximately 2-fold higher on day 90 than day 1, and male exposures were approximately 20-30% lower than females. Alogliptin metabolite exposure to M-was 10-15% of parent and 2-3% of parent for M-II, consistent with treatment with alogliptin alone in separate studies.

Methods

Doses:	0, 0/300, 0/1000, 100/300, 100/1000 mg/kg alogliptin/metformin
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	Water for injection (JP)
Species/Strain:	Sprague Dawley rat / Crl:CD(SD) SPF
Number/Sex/Group:	10
Age:	8 weeks
Weight:	Male: 275-336 g / Female: 191-245 g
Satellite groups:	4/sex TK, blood bicarbonate ion and lactic acid
Unique study design:	No alogliptin only control and two metformin only controls (300 mg/kg LD and 1000 mg/kg HD); doses calculated as free base of SYR-322Z salt (1.360x) and metformin HCl (1.282x)
Deviation from study protocol:	Minor deviations did not affect integrity or interpretation of study results.

Sponsor's study design summary

Test group	Dosage level [a)] (mg/kg/day)	Concen-tration [a)] (mg/mL)	Dosage volume (mg/kg/day)	Sex	Main group		Satellite group	
					No. of animals	Animal No.	No. of animals	Animal No.
Control	0/0	0/0	10	M	10	1001-1010	4	1201-1204
				F	10	1101-1110	4	1301-1304
Metformin group	0/300	0/30	10	M	10	2001-2010	4	2201-2204
				F	10	2101-2110	4	2301-2304
	0/1000	0/100	10	M	10	3001-3010	4	3201-3204
				F	10	3101-3110	4	3301-3304
SYR-322 /metformin group	100/300	10/30	10	M	10	4001-4010	4	4201-4204
				F	10	4101-4110	4	4301-4304
	100/1000	10/100	10	M	10	5001-5010	4	5201-5204
				F	10	5101-5110	4	5301-5304

M: Male, F: Female
a): As SYR-322Z/metformin, free bases of SYR-322 and metformin hydrochloride

Observations and Results

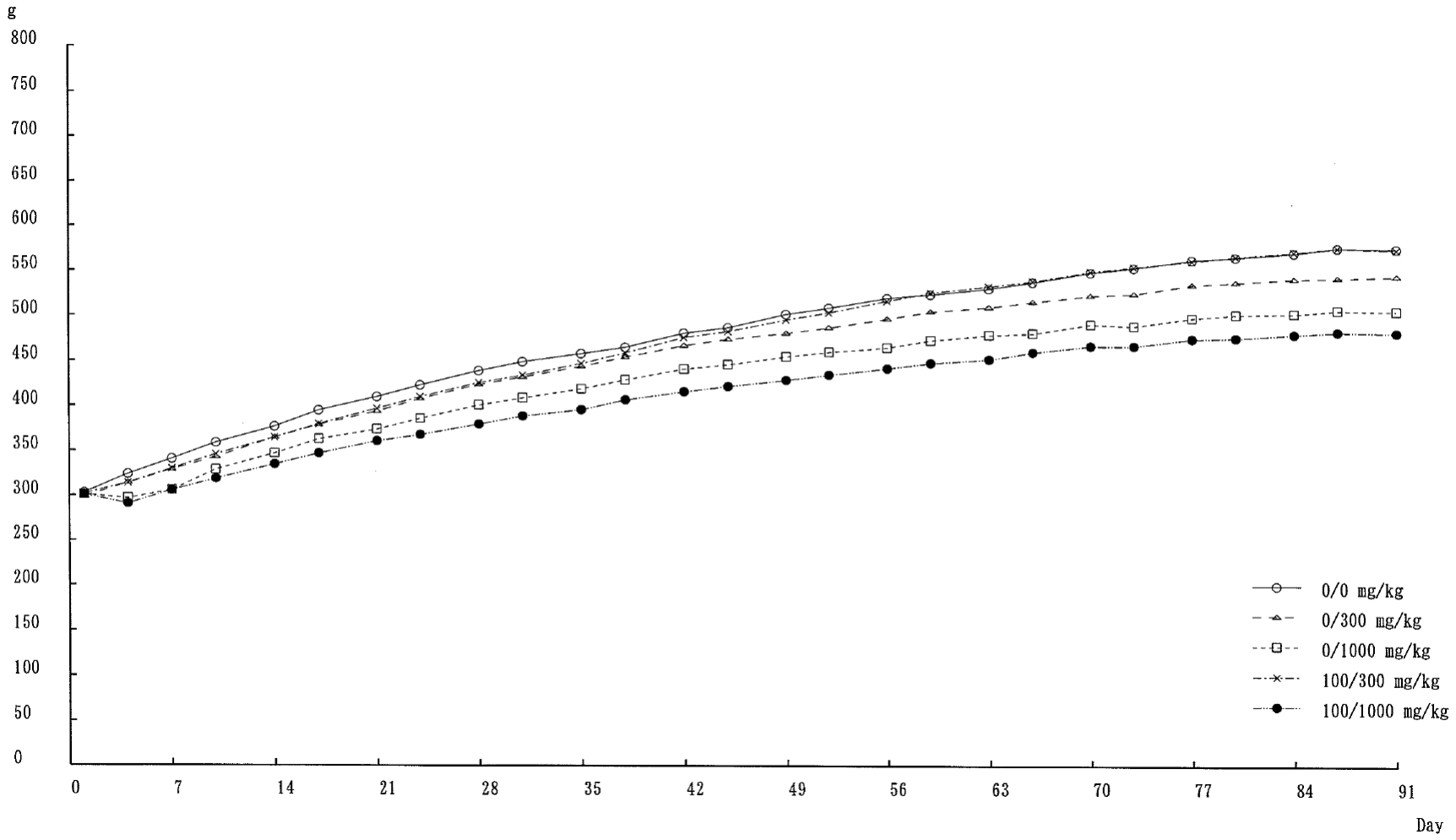
Mortality – A single HD metformin male (1/10) died on day 7 after clinical signs of decreased feces and decreased spontaneous movement. The decedent had very low food consumption, lost weight throughout the dosing period, and was "undernourished" at necropsy. Death was attributed to metformin-related toxicity.

Clinical Signs – Soft feces and diarrhea in HD metformin ± alogliptin starting in week 9. Decreased feces, emaciation, and/or unkempt fur were seen in single male and female HD combination rats during the first two weeks of dosing. Transient salivation was seen in HD metformin ± alogliptin males pre-dose and post-dose.

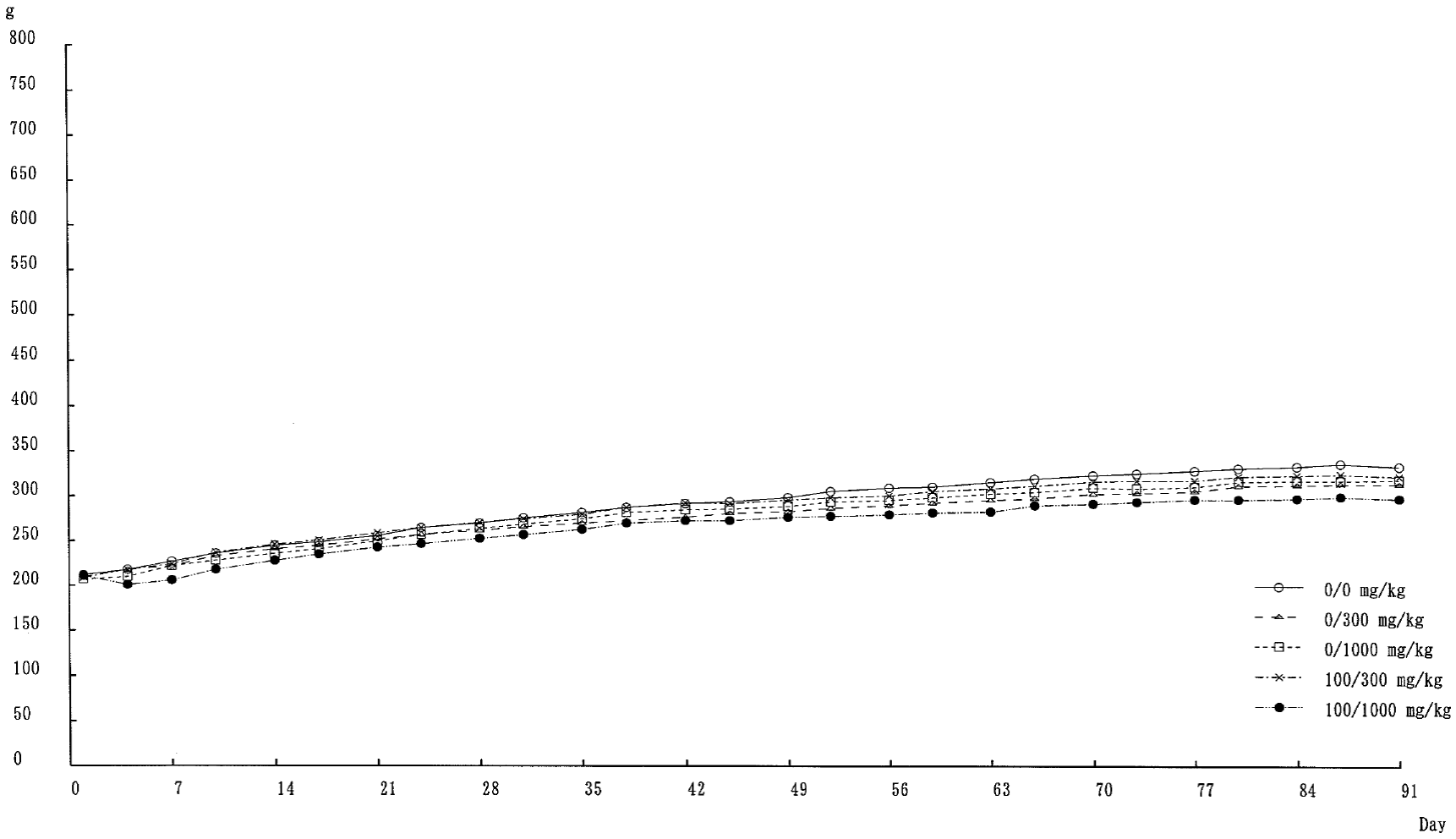
Body Weights (twice weekly) – Final body weights (↓10-20%; ss) and body weight gain (↓29-34%; ss) were reduced in HD metformin + alogliptin male and female groups compared to vehicle controls. Male LD (300 mg/kg) and HD (1000 mg/kg) metformin only groups had decreased body weight (↓5% and 15%) and body weight gain (↓10% and 25%), confirming a dose-related metformin trend and suggesting a slight additive effect of alogliptin on HD metformin BW gain decrement. Body weight trends over time are shown in the Sponsor's data (Figure 6).

Feed Consumption *(weekly)* – Food consumption was transiently decreased during the first week of treatment in HD metformin groups (± alogliptin). Food consumption was increased over controls after a few weeks of treatment in HD metformin groups (± alogliptin) and in 100/300 mg/kg males, confirming that body weight gain decreases were drug-induced and not simply due to decreased food consumption.

Figure 6 – Body weight (13-Week rat combination alogliptin + metformin)



Male


g
800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
0
7
14
21
28
35
42
49
56
63
70
77
84
91
Day
0/0 mg/kg
0/300 mg/kg
0/1000 mg/kg
100/300 mg/kg
100/1000 mg/kg

Female

Ophthalmoscopy (pre-dose and week 13) – Unremarkable.

Urinalysis (week 13) – Urine pH was decreased in HD metformin ± alogliptin male and female groups, as evidenced by median pH 6.5 to 7.5 compared to control and LD metformin ± alogliptin groups.

Hematology (necropsy; EDTA-2K or sodium citrate (PT, APTT) anticoagulant) – Findings were limited to slight 8% to 18% decreased (ss) APTT clotting time in 100/300 and HD metformin ± alogliptin groups, which were not considered biologically meaningful in the absence of PT or other signs of hematologic toxicity.

Clinical Chemistry (serum or heparin anticoagulant for lactic acid, AST, ALT, γ-GTP, CK) –
Plasma lactic acid levels were increased (ss) in HD metformin (2-3X) and HD metformin + alogliptin (3-5X) males and females, suggesting a slight additive effect of alogliptin on metformin-induced lactic acid increases. The lower doses of metformin (300 mg/kg) did not alter lactic acid levels with or without alogliptin co-treatment. Plasma bicarbonate levels were unchanged in HD metformin groups (satellite animals), suggesting the absence of ketoacidosis in animals with elevated lactic acid.

ALT increased with metformin treatment, only slightly in females (≤+30% HD metformin; ss) and up to 2X in males (all groups; ss). CK increased up to +40-50% (ss) in HD metformin ± alogliptin in males and females. Slight to modest electrolytes changes were seen in HD metformin ±alogliptin groups, including Na ↓2%, Cl ↓3% in both sexes and increased Ca ↑5% and P ↑21% in males.

Organ Weights – All trends are discussed as organ weights relative to body weight, due to metformin-induced decreased body weights (mainly in HD metformin groups). Dose-related heart weights increases (+12% to 54% relative to BW; ss) in metformin ± alogliptin groups, with slightly higher weights in HD combination groups compared to HD metformin alone. Relative salivary gland weights also increased in HD metformin ± alogliptin males (+12% to 18%; ss) and females (+25% to 32%; ss), LD metformin ± alogliptin females (+19% and +6%, respectively; ss) with a slight additive effect of alogliptin evident in the HD combination.

Relative liver, kidney, and adrenal weights were elevated in HD metformin ± alogliptin groups in males and females. Weight increases were attributable to metformin treatment and in all cases organ weights were slightly increased in combination with alogliptin compared to metformin alone. Data are summarized below.

Other organ weights showed modest changes in HD metformin-treated groups, but the small magnitude (typically <25% difference from controls) and absence or correlative effects of toxicity suggested findings were not biologically significant and may have been due to maintenance of tissue weight relative to decreased absolute body weight (e.g., male and female reproductive tissues).

Notable organ weight data (relative to vehicle controls) are shown in the abbreviated Sponsor table below (Table 2).

Table 2 – Sponsor's organ weight summary (13-week rat alogliptin + metformin)

Sex	Male				Female			
Dosage (mg/kg/day) [a)]	0/300	0/1000	100/300	100/1000	0/300	0/1000	100/300	100/1000
No. of animals	10	9	10	10	10	10	10	10
Salivary gland								
Absolute	-9%*	N	N	N	N	+15%*	N	+14%*
Relative	N	+12%*	N	+18%*	+19%*	+25%*	+6%#	+32%*
Heart								
Absolute	N	+17%*	N	+23%*	N	+32%*	N	+27%*
Relative	+12%*	+38%*	+12%*	+54%*$$	N	+43%*	N	+47%*
Liver								
Absolute	N	+16%*	+20%*	+16%*	N	+36%*	N	+30%*
Relative	+14%*	+36%*	+21%*	+44%*$$	+8%*	+48%*	+13%*	+51%*
Kidney								
Absolute	N	N	N	N	N	N	N	N
Relative	N	+24%*	+9%*	+33%*$$	N	+11%*	N	+16%*$
Adrenal								
Absolute	N	N	N	N	N	+26%*	N	+19%*
Relative	N	+27%*	N	+36%*	N	+35%*	N	+39%*
Testis								
Absolute	N	N	N	N	NA	NA	NA	NA
Relative	N	+14%*	N	+24%*	NA	NA	NA	NA
Epididymis								
Absolute	N	N	N	N	NA	NA	NA	NA
Relative	N	+12%*	N	+17%*	NA	NA	NA	NA
Seminal vesicle								
Absolute	N	N	N	N	NA	NA	NA	NA
Relative	N	N	N	+21%*	NA	NA	NA	NA
Ovary								
Absolute	NA	NA	NA	NA	N	N	N	N
Relative	NA	NA	NA	NA	N	N	N	+30%*$

a): As SYR-322Z/metformin
Values in the table indicate percentage of change against the control mean (+: increase, -: decrease).
N: No remarkable changes compared to the control group, NA: Not applicable
*: $p \leq 0.05$ (significantly different from the control group)
#: $p \leq 0.05$ (significantly different from the 0/300 mg/kg group)
$/$$: $p \leq 0.05/0.01$ (significantly different from the 0/1000 mg/kg group)

Gross Pathology – Slight increase in incidence of dark red foci in glandular stomach of HD combination males (1/10, 1/10, 1/10, 1/10, 4/10, respectively) and in all female treatment groups (0/10, 1/10, 2/10, 1/10, 2/10, respectively). Gross foci correlated with slightly increased incidence and severity (minimal to mild) glandular stomach erosion histologically.

Histopathology *Adequate Battery* – Yes
Peer Review – Yes

Major target organs identified by histopathology were heart, kidney, liver, and salivary glands. Histopathology findings were consistent with increased organ weights (heart,

kidney, liver, salivary glands, adrenals) and gross pathology findings of glandular stomach foci. Additional findings were seen in adrenals (minimal vacuolation and hypertrophy), intestine (minimal mucosal hyperplasia), pancreas (minimal decreased zymogen granules), and glandular stomach (minimal to mild erosion). Alogliptin coadministration generally did not exacerbate findings in metformin only controls, consistent with toxicity findings driven by metformin treatment. Target organ findings are discussed in more detail below and notable findings are summarized in Table 3.

Cardiomyopathy (minimal to mild) and myocardial hypertrophy (minimal) was evident in most metformin (± alogliptin) groups and incidence and severity increased with metformin dose. Myocardial hypertrophy was more prevalent in males, although relative heart weight increases were similar (+38-54%) in males and females.

Liver hypertrophy ('hepatocytic, midlobular', minimal to mild) was evident in nearly all HD metformin rats, in the absence or presence of alogliptin. There was no clear additive effect of alogliptin coadministration. Hepatocyte hypertrophy was further examined by electron microscopy in a small sub-sample of HD combination and control males (n=2/sex/group). No morphologic anomalies in any sub-cellular organelles were seen on electron microscopy, suggesting mild liver hypertrophy may have been an exaggerated pharmacology effect (e.g., increased glucose metabolism from increased insulin sensitivity) or secondary to drug metabolizing enzyme induction.

Kidney findings included slightly increased incidence of minimal tubular regeneration and focal tubular cell hypertrophy in HD metformin groups ± alogliptin. More pronounced metformin-induced renal tubular cell findings seen at all doses, and with dose-related increased incidence and severity, were hyaline droplets in males and vacuolation in females. A subset of kidney tissues (n=3/sex/group) were subjected to PAS and Oil Red O staining to investigate droplets/vacuolation. PAS staining was negative in males and positive in females, and Oil Red O staining was positive in females. Negative PAS staining of Hyaline droplets in males led the Sponsor to conclude droplets were enlarged phagolysosomes containing α2μ-globulin (reportedly specific to male rats). Positive PAS staining in females (n=2, HD metformin) confirmed glycoprotein accumulation in phagolysosomes (in contrast to males negative for glycoprotein). Oil Red O staining also confirmed 'vacuoles' in females were lipid droplets. The Sponsor considered hyaline droplets consistent with adaptive changes related to high metformin concentrations in kidney, since kidney is the major route of metformin elimination. Nevertheless, lipid accumulation in female kidney tubules may represent a change in lipid metabolism due to metformin ± alogliptin effects on systemic glucose and energy metabolism.

Table 3 – Target organ histopathology (13-week combination alogliptin + metformin) †

Tissue	Finding	Severity	Male (n=10/group[a])					Female (n=10/group)				
			0	0/ 300	0/ 1000	100/ 300	100/ 1000	0	0/ 300	0/ 1000	100/ 300	100/ 1000
Adrenal	Vacuolation, fasciculate cell	minimal	--	--	2	--	5	--	--	1	--	1
	Hypertrophy, fasciculate cell	minimal	--	1	3	3	7	--	--	3	--	5
Heart	Cardiomyopathy	minimal	1	3	6	7	4	--	2	--	3	5
		mild	--	--	--	--	1	--	--	--	--	--
	Hypertrophy, myocardial	minimal	--	--	5	--	10	--	--	2	--	3
	Degeneration, myocardial	minimal	--	--	--	--	--	--	--	1	--	1
		mild	--	--	--	--	--	--	--	1	--	--
Intestine, cecum	Hyperplasia, mucosal, diffuse	minimial	--	--	2	--	4	--	--	3	--	4
duodenum	Erosion	minimal	--	--	1	1	1	--	--	3	1	2
		mild						--	--	1	--	--
Kidney	Regeneration, tubular	minimal	4	2	7	2	8	2	1	4	3	1
		mild	--	1	--	--	--	--	--	--	--	--
	Vacuolation, tubular cell	minimal	--	--	--	--	--	--	5	6	6	7
		mild	--	--	--	--	--	--	--	3	--	3
	Hyaline droplet, tubular cell	minimal	1	8	4	4	8	--	--	2	--	--
		mild	--	--	4	2	2	--	--	--	--	--
	Hypertrophy, tubular cell, focal	minimal	--	--	2	--	1	--	1	6	2	7
Liver	Hypertrophy, hepatocytic, midlobular	minimal	--	--	7	3	8	--	--	9	--	9
		mild	--	--	--	--	2	--	--	--	--	1

Pancreas	Decreased zymogen granule	minimal	--	--	--	--	3	2	--	2	1	5
Salivary gland, submandibular	Decreased granule, granular duct	minimal	--	3	2	6	--	--	5	6	2	3
		mild	--	--	7	--	10	--	--	4	--	7
	Hypertrophy, epithelial, ductal	minimal	--	--	1	--	--	--	--	3	--	--
		mild	--	--	8	--	10	--	--	7	--	10
	Hypertrophy, acinar cell	minimal	--	--	--	--	5	--	--	4	--	2
Salivary gland, sublingual	Hypertrophy, epithelial, ductal	minimal	--	8	--	6	--	--	8	3	9	0
		mild	--	2	9	4	10	--	--	7	0	10
Stomach	Erosion, glandular stomach	minimal	1	1	2	--	2	--	1	3	1	2
		mild	--	--	--	--	1	--	--	1	--	--

† Salient findings at terminal necropsy (not including single 0/1000 male found dead); doses represent mg/kg/day
-- represents no finding
[a] 0/1000 (n=9) due to one early decedent

Toxicokinetics (day 1, day 90; pre-dose, 1, 2, 4, 8, 24 h post-dose for alogliptin (SYR-322Z) and metabolites M-I and M-II, and metformin; blood bicarbonate and lactic acid on day 90 prior to dosing) –

TK trends were examined for metformin ± alogliptin coadministration and potential effects of metformin on alogliptin exposure were estimated by comparing low dose (300 mg/kg) and high dose (1000 mg/kg) coadministration with alogliptin. No marked drug interactions were found.

Metformin maximum plasma exposure (C_{max}) was slightly lower with alogliptin coadministration, with approximately 25-30% decreases, but metformin total exposure (AUC_{0-24h}) was not affected by alogliptin coadministration. There were no gender differences in metformin exposure.

Alogliptin maximum and total plasma exposure (C_{max} and AUC) decreased slightly, approximately 25-40%, with increasing metformin coadministration. Alogliptin exposure was increased approximately 2-fold on day 90 compared to initial exposure on day 1, independent of metformin dose. Alogliptin exposure was slightly lower, approximately 20-30%, in males than females.

Trends for alogliptin metabolites M-I and M-II were similar to parent alogliptin exposures. M-I exposures were approximately 10-15% and M-II exposures were approximately 2-3% parent alogliptin exposures. There were no gender differences in M-I exposures, whereas M-II exposure was lower in males than females (similar to alogliptin parent).

Table 4 – TK Summary (13-week rat combination alogliptin + metformin)

Sex	Male (n=3)				Female (n=3)			
Dosage (mg/kg/day)[a)]	0/300	0/1000	100/300	100/1000	0/300	0/1000	100/300	100/1000
Metformin								
T_{max} (h)								
Day 1	2.0	2.0	1.7	1.3	2.0	2.0	1.3	2.0
Week 13 (90th dose)	2.0	1.7	2.0	3.7	1.0	2.0	2.0	2.3
C_{max} (ng/mL)								
Day 1	13766	21800	10994	15313	13171	27713	10854	19696
Week 13 (90th dose)	14530	20822	10622	15670	16077	26106	12333	23896
AUC_{0-24h} (ng·h/mL)								
Day 1	69107	180646	83267	173836	69882	177378	78411	189980
Week 13 (90th dose)	89185	212059	103260	223659	78909	247803	117589	259462
SYR-322Z								
T_{max} (h)								
Day 1	NE	NE	2.0	2.0	NE	NE	1.7	2.0
Week 13 (90th dose)	NE	NE	1.7	1.3	NE	NE	1.0	1.7
C_{max} (ng/mL)								
Day 1	NE	NE	4475	2105	NE	NE	6306	2798
Week 13 (90th dose)	NE	NE	5954	3312	NE	NE	8458	5792
AUC_{0-24h} (ng·h/mL)								
Day 1	NE	NE	23277	13150	NE	NE	30042	21833
Week 13 (90th dose)	NE	NE	42262	31114	NE	NE	51845	36389
SYR-322 M-I								
T_{max} (h)								
Day 1	NE	NE	2.0	2.0	NE	NE	2.0	2.0
Week 13 (90th dose)	NE	NE	2.0	3.7	NE	NE	1.7	1.7
C_{max} (ng/mL)								
Day 1	NE	NE	721	349	NE	NE	499	286
Week 13 (90th dose)	NE	NE	596	357	NE	NE	595	545
AUC_{0-24h} (ng·h/mL)								
Day 1	NE	NE	5378	3503	NE	NE	3990	3665
Week 13 (90th dose)	NE	NE	5965	4767	NE	NE	6133	5177
SYR-322 M-II								
T_{max} (h)								
Day 1	NE	NE	2.0	2.0	NE	NE	2.0	2.0
Week 13 (90th dose)	NE	NE	2.0	1.3	NE	NE	1.3	1.7
C_{max} (ng/mL)								
Day 1	NE	NE	96	47	NE	NE	155	64
Week 13 (90th dose)	NE	NE	129	51	NE	NE	223	112
AUC_{0-24h} (ng·h/mL)								
Day 1	NE	NE	527	323	NE	NE	876	655
Week 13 (90th dose)	NE	NE	895	613	NE	NE	1489	939

Values in the table indicate the mean. NE: Not examined a): As SYR-322Z/metformin

Stability and Homogeneity – Stability of dosing solutions was confirmed. Homogeneity was not assessed. Since drugs were freely soluble in the aqueous dosing solution and no precipitate was observed, homogeneity could be assumed (i.e., true gavage solution, not a suspension).

9 Reproductive and Developmental Toxicology

9.2 Embryonic Fetal Development

Range-finding alogliptin + metformin combination embryofetal rat study
Non-GLP, signed 8/6/10

Study Title: Range-finding study for effects of SYR-322/metformin hydrochloride on embryo-fetal development in rats (Study No. 09-160/te; Doc. No. SYR-322MET-11134)

Doses (mg/kg/d) *0/0 (vehicle control)*
100/50, 100/150, 100/500, 100/1000, 100/2000 (Alogliptin / Metformin)

NOAEL (maternal) *= 100/150 (Alogliptin / Metformin)*

NOAEL (fetal) *= 100/150 (Alogliptin / Metformin)*

NOAEL determination *– Metformin doses ≥ 500 mg/kg plus alogliptin caused decreased maternal BW gain during the treatment period. Toxicity in dams treated with ≥ 1000 mg/kg metformin exceeded the MTD, including moribund sacrifice and death, clinical signs (abnormal feces, vaginal discharge in survivors), and limited BW gain until the post-treatment period.*

Key study findings:

- Oral gavage range-finding study of alogliptin + metformin coadministration to pregnant rats (n=6/group). Increasing doses of metformin (50 to 2000 mg/kg) coadministered to fixed 100 mg/kg alogliptin dose. Doses of metformin ≥ 1000 mg/kg plus alogliptin were not well tolerated as evidenced by markedly reduced BW gain, abnormal clinical signs, and death or moribund sacrifice of dams.
- Transient decreased BW gain and decreased food consumption with 500 mg/kg metformin plus alogliptin, leading to overall 20% decreased BW gain during treatment with rapid compensatory weight gain post-treatment.
- Signs of lactic acidosis in 2000 mg/kg metformin dams sacrificed in moribund condition included increased lactic acid, decreased bicarbonate ion (HCO_3^-), and increased anion gap.
- There was no apparent effect on pregnancy success or fetal development with up to 1000 mg/kg metformin plus alogliptin treatment. No fetal malformations were observed but skeletal variations seemed to increase with ≥ 500 mg/kg metformin treatment (↑ total skeletal variations (nss), ↑ bipartite ossification thoracic centrum (nss), ↑ short supernumerary rib (nss), ↓ number of ossified sacro-caudal vertebrae (ss)).
- Results are summarized in the Sponsor's summary table (Table 5).

Table 5 – Embryofetal development range-finding (alo + met) Sponsor summary

Animals	Crl:CD(SD) rats, 20-week-old males, 12-week-old females					
Test article	Control	SYR-322/metformin				
Dosage level (mg/kg/day) [1]	0/0	100/50	100/150	100/500	100/1000	100/2000
Dosage volume (mL/kg/day)	10	10	10	10	10	10
No. of pregnant animals	6	6	6	6	6	6
No. of deaths/sacrifices	0	0	0	0	1[2]#/0	3[3]#/3[3]#
Dams Clinical signs	–	–	–	–	SF (1)# DF (6)# SV (2) LS (1)# SDV (3)#	SF (6)# DF (6)# PP (1)# DLA (1)# HP (2)# PE (2)# DH (4)#
Body weight gain	–	–	–	↓ (GD 6-12)#	↓ (GD 6-16)#	↓ (GD 6-8)#
Food consumption	–	–	–	↓ (GD 6)#	↓ (GD 6-16)#	↓ (GD 6-8)#
Lactic acid (mg/dL)	33.5±9.1[4]	NE				↑ 45.8-163.0[5]#
HCO_3^- (mmol/L)	23.7±0.8[4]					↓ 6.5-18.6[5]#
Anion gap (mmol/L)	11.8±1.9[4]					↑ 20.4-22.5[5]#
Necropsy findings	–	–	–	–	WCI (1)[6]# WCS (1)[6]# PS (1)[6]# LAG (1)[6]#	WCI (6)[6]# WCS (6)[6]# PS (1)[6]#
No. of corpora lutea	15.2±1.5	15.3±2.2	14.8±0.8	15.3±0.8	14.6±1.5	NE
No. of implants	14.2±1.5	14.0±2.4	13.8±0.8	14.8±0.8	14.2±1.6	
Placentae Macroscopic abnormalities (%) Main type (%)	0.0±0.0	2.4±5.8 EP (2.4)	0.0±0.0	0.0±0.0	0.0±0.0	NE

Control: Distilled water, -: No treatment-related effects, #: Adverse effects, ↓: Decreased/suppressed, ↑: Increased,
GD: Gestation day, NE: Not examined, SF: Soiled fur, DF: Decrease in feces, SV: Salivation, LS: Loose stool,
SDV: Sanguineous discharge from vagina, PP: Prone position, DLA: Decrease in locomotor activity,
HP: Hypothermia, PE: Piloerection, DH: Diarrhea, WCI: watery contents in intestine,
WCS: watery contents in stomach, PS: petechia in stomach, LAG: large adrenal gland, EP: Enlarged placenta,
1): As free base (SYR-322Z/metformin)
2): Found dead on GD 18
3): Found dead or sacrificed moribund on GD 8 or 9
4): Blood samples were collected from 6 dams at necropsy on GD 20.
5): Blood samples were collected from 3 dams sacrificed moribund on GD 8 or 9.
6): All findings were noted in the animals found dead or sacrificed moribund.
( ): Number of dams showing clinical signs or necropsy findings

Animals		Crl:CD(SD) rats, 20-week-old males, 12-week-old females					
Test article		Control	SYR-322/metformin				
Dosage level (mg/kg/day) [1)]		0/0	100/50	100/150	100/500	100/1000	100/2000
No. of pregnant animals		6	6	6	6	6	6
No. of deaths/sacrifices		0	0	0	0	1[2)]#/0	3[3)]#/3[3)]#
Fetuses							
Post-implantation loss (%)		3.5±3.9	10.5±13.5	4.7±3.6	6.7±4.2	4.0±5.8	
No. of live fetuses		13.7±1.5	12.7±3.2	13.2±0.4	13.8±0.8	13.6±1.5	
Sex ratio [M/(M+F)x100] (%)		42.0±12.5	42.5±8.9	44.4±16.0	43.1±16.4	47.6±7.1	
Body weight (g)	Male	3.58±0.18	3.65±0.26	3.69±0.28	3.59±0.11	3.46±0.25	NE
	Female	3.52±0.25	3.44±0.29	3.55±0.25	3.37±0.08	3.27±0.37	
External abnormalities (%)		0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	
Visceral abnormalities (%)		0.0±0.0	NE			0.0±0.0	
Visceral variations (%)		0.0±0.0				0.0±0.0	
Skeletal abnormalities (%)		0.0±0.0	NE	0.0±0.0	0.0±0.0	0.0±0.0	NE
Skeletal variations (%)		13.5±19.4		22.2±20.2	27.8±22.8	28.5±19.3	
Main type (%)		BOTC (2.4)		BOTC (2.8)	BOTC (8.3)	BOTC (11.9)	
		SSR (11.1)		SSR (16.7)	SSR (19.4)	SSR (22.8)	
No. of ossified SCV		8.2±0.3		8.0±0.4	7.6±0.4w#	7.3±0.5w#	
Conclusion		The NOAEL of SYR-322/metformin was 100/150 mg/kg/day for dams and fetuses under the present experimental conditions and 100/1000 mg/kg/day and higher were not recommended as the highest dosage level of the definitive study because of high probabilities of mortality, moribundity and marked decreases in body weight gain and food consumption in the dams.					

Control: Distilled water, #: Adverse effects, M: Male, F: Female, NE: Not examined,
BOTC: Bipartite ossification thoracic centrum, SSR: Short supernumerary rib, SCV: Sacro-caudal vertebrae
w: Significantly different from the control group (Williams, $p \leq 0.05$)
1): As free base (SYR-322Z/metformin)
2): Found dead on GD 18
3): Found dead or sacrificed moribund on GD 8 or 9
NOAEL: No-observed-adverse-effect level

Alogliptin + metformin combination embryofetal rat study (Seg 2 rat)

GLP study, signed 9/16/10

Doses (mg/kg/d)	*0/0 (vehicle control)* *100/150, 100/500 (Alogliptin / Metformin)* *100/0 (Alogliptin only control)* *0/150, 0/500 (Metformin only controls)*
*Exposures: (µg*h/ml)*	*51 / 70, 35 / 163 (alogliptin / met GD 17)* *44 / 0 (alogliptin)* *0 / 40, 0 / 149 (LD and HD metformin)*

NOAEL (maternal) *= 100 / 150 mg/kg (Alogliptin / Metformin) (34X alo / 3X MRHD)*

NOAEL (fetal) = *100 / 150 (Alogliptin / Metformin) (no exposure data)*

NOAEL determination – *No treatment-related fetal findings were seen in metformin controls or in 100/150 mg/kg alogliptin/metformin dams. The high dose of 100/500 mg/kg alogliptin/metformin caused reduced maternal body weight and eye and vertebral malformations in 4 fetuses from 2/20 dams. A relationship to treatment could not be ruled out for deformed fetuses, but findings were limited to microphthalmia in fetuses from a dam with markedly reduced BW gain and a single fetus with multiple abnormalities in a separate dam.*

Key study findings:

- There was no evidence of an unexpected, synergistic increase in embryofetal toxicity with alogliptin and metformin coadministration.
- Transient reduced BW gain and reduced food consumption at the beginning of treatment led to overall reduced BW gain in HD combination dams (↓ 13%) indicating maternal toxicity.
- A single HD combination dam had 3 fetuses with microphthalmia and 1 fetus with absent sacral vertebrae malformations. The dam had markedly reduced BW gain compared to other dams in the HD combination group and compared to controls, suggestive of marked maternal toxicity.
- A single HD combination fetus (from a different dam) had several malformations: microphthalmia, cleft palate, microglossia, and mandibular micrognathia.
- There were no other treatment-related malformations and no apparent treatment-related external, visceral, or skeletal variations.
- There were no apparent treatment-related effects on pregnancy success or other pregnancy-related or fetal outcomes.
- Fetal exposure was not measured but maternal transfer to developing fetuses has been previously established for alogliptin and metformin.

Study Title: Effects of SYR-322/metformin hydrochloride on embryo-fetal development in rats

Study No.	09-260/TE (Doc. No. SYR-322MET-11346)
Study report location:	eCTD 4.2.3.7.7
Conducting laboratory and location:	Development Research Center / PRD Takeda Pharmaceutical Company Limited 17-85, Jusohonmachi 2-chome, Yodogawa-ku Osaka 532-8686, Japan
Date of study initiation:	2/15/10
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SYR-322, Lot No. MA01-013, 99.6% purity; Metformin HCl, Lot No. OB387, 98.9% purity

Methods

Doses:	0/0, 100/0, 0/150, 0/500, 100/150, 100/500 mg/kg alogliptin/metformin
Frequency of dosing:	Daily
Dose volume:	10 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Distilled water
Species/Strain:	Sprague Dawley rat / Crl:CD(SD)
Number Dams/Group:	20 (presumed pregnant females)
Satellite groups:	4/group TK 12/group Blood chemistry
Study design:	Standard rat embryofetal development study design. Doses were chosen based on the alogliptin/ metformin combination MTD identified in a range-finding study. Presumed pregnant females treated QD by oral gavage GD 6-17, necropsy and C-section GD 20. Pregnancy parameters determined macroscopically, fetuses examined for external, visceral, and skeletal variations and malformations (“abnormalities”). Blood for TK analyses GD 6 & 17 pre-dose, 1, 2, 4, 8, 24 h post-dose. Blood for plasma chemistry GD 10 & 18 for markers of neural tube defect and/or microphthalmia (glucose, homocysteine, folic acid, vitamin B12, methionine, 5-MT, SAM, SAH).
Deviation from study protocol:	Various deviations were reported, related to fetal sampling and tissue retention/observations, which were not considered to affect the integrity of the study or interpretation of the study observations.

Sponsor's study design summary

Group	Test article	Dosage level (mg/kg/day)*	Dosage volume (mL/kg/day)	Concentration (w/v%)*	Number of animals (Animal number)
1	Control	0/0	10	0/0	20 (1F01-20)
2	SYR-322/Met	100/0	10	1/0	20 (2F01-20)
3	SYR-322/Met	0/150	10	0/1.5	20 (3F01-20)
4	SYR-322/Met	0/500	10	0/5	20 (4F01-20)
5	SYR-322/Met	100/150	10	1/1.5	20 (5F01-20)
6	SYR-322/Met	100/500	10	1/5	20 (6F01-20)
7[1)]	SYR-322/Met	100/0	10	1/0	4 (7F01-04)
8[1)]	SYR-322/Met	0/150	10	0/1.5	4 (8F01-04)
9[1)]	SYR-322/Met	0/500	10	0/5	4 (9F01-04)
10[1)]	SYR-322/Met	100/150	10	1/1.5	4 (10F01-04)
11[1)]	SYR-322/Met	100/500	10	1/5	4 (11F01-04)
12[2)]	Control	0/0	10	0/0	12 (12F01-12)
13[2)]	SYR-322/Met	100/0	10	1/0	12 (13F01-12)
14[2)]	SYR-322/Met	0/150	10	0/1.5	12 (14F01-12)
15[2)]	SYR-322/Met	0/500	10	0/5	12 (15F01-12)
16[2)]	SYR-322/Met	100/150	10	1/1.5	12 (16F01-12)
17[2)]	SYR-322/Met	100/500	10	1/5	12 (17F01-12)

Control: distilled water, Met: metformin hydrochloride,

*: as free base (SYR-322Z/metformin)

1): satellite group for toxicokinetics

2): satellite group for blood chemistry

Observations and Results:

Mortality – No maternal deaths or abortions/premature deliveries.

Clinical Signs – No treatment-related findings.

Body Weight – BW gain decreased in 500 mg/kg metformin ± alogliptin dams, primarily at the beginning of treatment (through GD 10; ss). BW gain generally recovered after GD 10 but the HD metformin + alogliptin dams still had decreased BW throughout the treatment period through GD 18 (-13%; ss). LD 150 mg/kg metformin ± alogliptin dams had reduced BW gain at various intervals during treatment, resulting in a trend (nss) of slightly reduced BW (≤ -4%) at the end of treatment on GD 18 and study termination on GD 20.

Feed Consumption – Transiently decreased food consumption was seen at the beginning of treatment in HD metformin ± alogliptin, on GD 6 (HD metformin) or through GD 10 (+ alogliptin), and in the LD metformin + alogliptin group (GD 6 and GD 10). Decreased food consumption was correlated with trends in reduced BW gain.

Body weight and food consumption trends are shown in the Sponsor's summary table, below.

Sponsor's body weight and food consumption summary

Animals	Crl:CD(SD) rats (14-week-old males at the first mating and 12 or 13-week-old females at allocation)					
Test article	Control	SYR-322/metformin hydrochloride				
Dosage level (mg/kg/day)[1)]	0/0	100/0	0/150	0/500	100/150	100/500
No. of pregnant females	20	20	18	19	20	20
No. of deaths	0	0	0	0	0	0
Dams Clinical signs	–	–	–	–	–	–
Body weight	–	–	–	–	–	↓ (GD 12-18)w^1# ↓ (GD 8-18)w^2#
Body weight gain	–	–	–	↓ (GD 6-10)w^1#	↓ (GD 8-10)w^1#	↓ (GD 6-10, 14-16)w^1# ↓ (GD 6-8)w^2# ↓ (GD 6-8^{**4}, 14-16^{*4})#
Food consumption	–	–	–	↓ (GD 6)w^1# ↑ (GD 18)w^1	↓ (GD 6)w^1# ↓ (GD 6, 10)w^2# ↑ (GD 18)^{*3}	↓ (GD 6-10)$w^{1,2}$# ↓ (GD 6)^{*4}# ↑ (GD 18)w^2

Control: Distilled water, -: No treatment-related effects, #: Adverse effects, ↓: Decreased, ↑: Increased, GD: Gestation day,
1): As free base (SYR-322Z/metformin), 5-MT: 5-methyltetrahydrofolic acid, SAM: S-(5'-adenosyl)-L-methionine,
SAH: S-(5'-adenosyl)-L-homocysteine
w^1: Significantly different from group 1 (Williams test for groups 1, 3 and 4 or for groups 1, 5 and 6, $p \leq 0.05$)
s^1: Significantly different from group 1 (Shirley-Williams test for groups 1, 3 and 4, $p \leq 0.05$)
w^2: Significantly different from group 2 (Williams test for groups 2, 5 and 6, $p \leq 0.05$)
$*^1$, $*^3$, $*^4$, $**^4$: Significantly different from groups 1, 3 or 4 (parametric, *: $p \leq 0.05$, **: $p \leq 0.01$)

Necropsy

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.) – Pregnancy and maternal necropsy findings were unremarkable. There were no apparent effects of treatment on pregnancy outcome or fetal parameters (BW, live births, sex ratio). Pregnancy data are summarized in the Sponsor's summary table, below (Table 6).

Table 6 – Alogliptin + metformin embryofetal rat pregnancy and fetal success summary

Test article		Control	SYR-322/metformin hydrochloride				
Dosage level (mg/kg/day)[1)]		0/0	100/0	0/150	0/500	100/150	100/500
No. of pregnant females		20	20	18	19	20	20
Dams Necropsy findings		–	–	–	Ovary (focus) (2 dams)	–	–
No. of corpora lutea		14.5±1.2	15.0±0.9	13.9±1.9	15.1±1.5	14.7±1.4	14.5±1.4
No. of implants		13.5±2.0	14.6±1.2*[1]	12.8±3.2	14.3±1.2	14.1±1.8	14.0±1.3
Pre-implantation loss (%)		7.2±8.9	2.4±5.5$$[1]	9.4±20.6	5.4±4.6	3.9±5.3	3.3±3.9
Placentae Macroscopic abnormalities (%) Main type (%)		0.5±2.2 Fused (0.5)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Fetuses Post-implantation loss (%)		1.8±4.0	4.4±7.0	5.7±8.8	5.4±6.8	6.0±7.4	3.8±7.7
No. of dead implants		0.3±0.6	0.7±1.0	0.8±1.2	0.8±1.0	0.9±1.1$[1]	0.6±1.1
No. of live fetuses		13.3±2.1	14.0±1.5	12.0±3.2	13.5±1.4	13.2±1.6	13.5±1.6
Sex ratio [M/(M+F)x100] (%)		49.9±15.9	49.2±11.4	58.5±15.3	52.0±13.8	52.5±14.0	54.2±15.5
Body weight (g)	Male	3.69±0.33	3.66±0.21	3.81±0.33	3.68±0.29	3.71±0.20	3.63±0.30
	Female	3.46±0.24	3.45±0.22	3.51±0.22	3.43±0.24	3.48±0.22	3.37±0.32

Control: Distilled water, -: No treatment-related effects, #: Adverse effects, M: Male, F: Female
1): As free base (SYR-322Z/metformin), *[1]: Significantly different from group 1 (parametric, $p \leq 0.05$)
$[1]$,$$[1]$: Significantly different from group 1 (nonparametric, $[1]$: $p \leq 0.05$, $$[1]$: $p \leq 0.01$)

Offspring (Malformations, Variations, etc.)

A few external and corresponding visceral malformations ("abnormalities") were seen in the HD combination that were not seen in concurrent controls above historical laboratory controls. Small eye bulge externally corresponding to microphthalmia viscerally was seen in 3 fetuses from one litter (6F08) and in 1 fetus from a separate litter (6F12) that also had cleft palate, microglossia, and mandibular micrognathia. Another single fetus from dam 6F08 had misshapen tail, corresponding to a skeletal malformation of absent sacral vertebra, neither of which have been seen in historical controls.

Since the malformations observed in the HD combination group were outside the historical control range they were considered by the Sponsor to be due to treatment. However, the single dam with three fetuses with microphthalmia and a single fetus with missing sacral vertebrae had the least amount of weight gain of any dam in the group (73g/104g vs. mean 107g/140g at GD 18/GD20 respectively), which was 39g/70g (GD18/GD20) lower than control mean BW gain. The other malformations were from a single fetus from a separate dam. Thus, malformations were isolated to two dams, one of whose low body weight gain suggested marked maternal toxicity.

Membranous ventricular septal defect was seen in fetuses from all groups, including controls, with no relationship to dose and incidence was within the historical control range.

Total visceral variations were increased in the HD combination group but the difference between controls was not statistically significant and no individual visceral variations were different from concurrent controls (nss) or outside the historical control range.

No skeletal variations were considered treatment related based on incidence within historical control ranges and/or absence of dose-related findings.

Data are summarized in the Sponsor's table, below (Table 7).

Table 7 – Alogliptin + metformin fetal malformation and variation summary

Dosage level (mg/kg/day)[1)]	0/0	100/0	0/150	0/500	100/150	100/500
No. of pregnant females	20	20	18	19	20	20
External abnormalities Main type (%)	1.0±3.1 Hypospadias (0.4) Umbilical hernia (0.6)	0.0±0.0	0.0±0.0	0.0±0.0	0.4±1.7 Hypospadias (0.4)	1.9±7.0 Small eye bulge (1.5)# Cleft palate (0.4)# Microglossia (0.4)# Mandibular micrognathia (0.4)# Misshapen tail (0.4)#

Control: Distilled water, -: No treatment-related effects, #: Adverse effects, M: Male, F: Female
1): As free base (SYR-322Z/metformin).

Test article	Control	SYR-322/metformin hydrochloride				
Dosage level (mg/kg/day)[1)]	0/0	100/0	0/150	0/500	100/150	100/500
No. of pregnant females	20	20	18	19	20	20
Fetuses						
Visceral abnormalities (%)	0.6±2.5	1.3±3.8	0.0±0.0	0.8±3.3	2.4±8.0	3.5±10.1
Main type (%)	DL (0.6)	MVSD (1.3)		MVSD (0.8)	MVSD (2.4)	MVSD (1.3) Microphthalmia (2.9)#
Visceral variations (%)	1.5±4.8	0.0±0.0	2.5±8.2	1.5±4.5	1.4±6.4	3.6±6.4s[2]
Main type (%)	LUA (1.5)		LUA (2.5)	LUA (0.8) DA (0.8)	LUA (1.4)	LUA (2.1) Dilated renal pelvis (0.7) Dilated ureter (0.8)
Skeletal abnormalities (%)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.7±1.5
Main type (%)						SLV (0.8) ASV (0.8)#
Skeletal variations (%)	12.9±18.2	22.6±20.7	17.8±23.8	24.7±21.0	23.9±23.9	14.3±22.0
Main type (%)	BOTC (1.0) SSR (12.9) FSR (0.7)	BOTC (6.1)$[1] SSR (15.3) Wavy rib (3.2) UP (0.7)	BOTC (1.8) SSR (16.8)	BOTC (2.5) SSR (21.4) DOCB (0.9) CR (0.9) AS (0.7)	BOTC (1.8) SSR (21.5) BOLC (1.7) CR (0.7)	BOTC (1.7) SSR (12.6) FSR (0.8)
No. of ossified SCV	8.1±0.4	8.0±0.2	7.9±0.4	7.9±0.4	8.1±0.3	7.7±0.4w[1]s[2]#

Control: Distilled water, #: Adverse effects, M: Male, 1): As free base (SYR-322Z or metformin)
DL: Discolored liver, MVSD: Membranous ventricular septal defect, LUA: Left umbilical artery, DA: Discolored adrenal,
SLV: Supernumerary lumbar vertebra, ASV: Absent sacral vertebra, BOTC: Bipartite ossification of thoracic centrum,
SSR: Short supernumerary rib, FSR: Full supernumerary rib, BOLC: Bipartite ossification of lumbar centrum, CR: Cervical rib,
DOCB: Delayed ossification of cranial bones, AS: Asymmetric sternebra, UP: Unossified pubis, SCV: Sacro-caudal vertebra
w[1]: Significantly different from group 1 (Williams test for groups 1, 5 and 6, $p \leq 0.05$),
s[2]: Significantly different from group 2 (Shirley-Williams test for groups 2, 5 and 6, $p \leq 0.05$)
$[1]: Significantly different from group 1 (nonparametric, $p \leq 0.05$),

Blood Chemistry – Modest decreases in folic acid (HD metformin) and SAM/SAH in both LD and HD metformin controls were observed on a single day. Vitamin B12 was increased slightly in some groups with no apparent relationship to treatment. None of the changes in specific markers for birth defects were considered remarkable or correlated to any fetal findings (e.g., neural tube defects) in respective treatment groups. Data are shown in the Sponsor's summary table, below.

Sponsor's blood chemistry summary

Animals	Crl:CD(SD) rats (14-week-old males at the first mating and 12 or 13-week-old females at allocation)					
Test article	Control	SYR-322/metformin hydrochloride				
Dosage level (mg/kg/day)[1)]	0/0	100/0	0/150	0/500	100/150	100/500
No. of pregnant females	20	20	18	19	20	20
Blood chemistry (n=5 or 6, GD 10/18)						
Glucose (mg/dL)	155/122	158/123	153/123	166/119	159/122	149*[4]/127
Homocysteine (μL/mL)	6.6/5.0	6.0/4.7	6.3/4.4	6.4/4.2	5.6/4.2	6.3/4.3
Methionine (μg/mL)	12.1/12.4	11.5/12.3	11.7/12.5	12.0/12.6	11.8/11.7	12.6/11.2
Vitamin B12 (pg/mL)	1902/1288	2302*[1]/1378	2252/1477*[1]	2445w[1]/1175	2422*[1]/1462	2058/1357*[4]
Folic acid (ng/mL)	47.1/31.6	44.6/35.4	44.0/39.5	37.0s[1]/30.7	46.0/40.3	42.7/34.8
SAM (ng/mL)	82.5/113.6	81.2/121.6	83.5/100.5	85.9/102.4	86.2/106.5	113.5/99.5
SAH (ng/mL)	9.23/5.15	5.46/7.52	8.05/7.85	6.81/7.50	6.84/5.37	7.97/5.86
SAM/SAH ratio	13.7/23.1	16.7/18.9	12.0/14.8w[1]	16.1/14.2w[1]	17.6/24.3	23.3/19.8
5-MT (ng/mL)	42.2/29.9	40.5/36.0	44.5/34.0	38.5/29.0	41.7/33.0	43.5/29.3

Control: Distilled water, -: No treatment-related effects, #: Adverse effects, GD: Gestation day,
1): As free base (SYR-322Z/metformin), 5-MT: 5-methyltetrahydrofolic acid, SAM: S-(5'-adenosyl)-L-methionine,
SAH: S-(5'-adenosyl)-L-homocysteine
w[1]: Significantly different from group 1 (Williams test for groups 1, 3 and 4 or for groups 1, 5 and 6, $p \leq 0.05$)
s[1]: Significantly different from group 1 (Shirley-Williams test for groups 1, 3 and 4, $p \leq 0.05$)
w[2]: Significantly different from group 2 (Williams test for groups 2, 5 and 6, $p \leq 0.05$)
*[1], *[3], *[4], **[4]: Significantly different from groups 1, 3 or 4 (parametric, *: $p \leq 0.05$, **: $p \leq 0.01$)

Toxicokinetics – Plasma alogliptin and metformin exposure determined from satellite TK animals are shown in the Sponsor's summary table, below.

Sponsor's TK summary

Group number		1	2	3	4	5	6
Test article		Control	SYR-322/metformin hydrochloride				
Dosage level (mg/kg/day)[1)]		0/0	100/0	0/150	0/500	100/150	100/500
Dosage volume (mL/kg/day)		10	10	10	10	10	10
Dams Toxicokinetics (n=3, GD 6/17)							
SYR-322Z	Tmax (h)		1.3/1.3	NE	NE	2.0/3.7	2.0/1.3
	Cmax (ng/mL)		7715/5280	NE	NE	7870/4759	5611/4796
	$AUC_{0\text{-}24h}$ (ng·h/mL)		53226/44078	NE	NE	49686/50507	34998/34978
SYR-322 M-I	Tmax (h)		2.3/3.0	NE	NE	2.0/4.0	2.0/1.7
	Cmax (ng/mL)		603/470	NE	NE	569/493	342/377
	$AUC_{0\text{-}24h}$ (ng·h/mL)		6341/5435	NE	NE	5658/6478	3703/4499
SYR-322 M-II	Tmax (h)		1.7/2.3	NE	NE	2.0/4.0	2.0/1.7
	Cmax (ng/mL)		235/195	NE	NE	245/201	137/157
	$AUC_{0\text{-}24h}$ (ng·h/mL)		1929/1994	NE	NE	1792/2224	1057/1361
Metformin	Tmax (h)		NE	2.0/2.0	2.0/2.0	1.7/3.3	2.0/2.0
	Cmax (ng/mL)		NE	9237/7542	17591/19127	7111/5976	12905/15557
	$AUC_{0\text{-}24h}$ (ng·h/mL)		NE	49280/40321	109694/149015	55816/70160	128557/162623

Control: Distilled water, NE: Not examined, 1): As free base (SYR-322Z/metformin)

9.4 Juvenile Development

The Sponsor conducted two juvenile animal studies, one general 4-week study in males and females and a separate 8-week study investigating male reproductive system development. The Division did not require juvenile animal studies and there was no discussion between DMEP pharmacology/toxicology and the Sponsor about study design. Male reproductive tissue toxicity in adult males was observed at high doses in chronic toxicity studies, which may have prompted investigations of juvenile male reproductive system development. However, no rationale was given for juvenile study investigations or study designs, so any suggestion of motive or rationale for the studies by this reviewer is merely speculative.

Alogliptin toxicity study in juvenile rats

GLP study, signed 1/20/09

Doses: 0, 30, 100, 300 mg/kg alogliptin
*Exposure: 5, 27, 115 µg*h/ml*

NOAEL = *300 mg/kg (77X MRHD)*

NOAEL determination – *The high dose of 300 mg/kg was considered a NOAEL for effects on growth and development in juvenile rats. Potential signs of toxicity in high dose animals occurred in low incidence and low severity and were unlikely to be drug-related when all data were considered.*

Key study findings:

- No remarkable toxicity. Juvenile animals tolerated alogliptin up to the high dose of 300 mg/kg and there were no apparent effects on juvenile growth and development. Results are summarized in the Sponsor's summary table (Table 8).

- Modest liver weight increases (females only) and isolated cases of hepatocyte hypertrophy (2/10 males, minimal severity) suggest the possibility of high dose alogliptin-induced liver toxicity in sensitive individuals.

Reviewer Comments: *This study provides evidence that the short-term toxicology profile of alogliptin is similar in adults and juvenile rats. However, the short 4 week treatment duration and the limited endpoints evaluated did not address potential effects of alogliptin on pubertal onset, behavioral development, or fertility.*

Study Title: Four-week oral gavage toxicity study of SYR-322 in 4 week old rats

Study no:	B-6504 (Code No. SYR-322/00610)
Study report location:	eCTD 4.2.3.5.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8/4/08
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SYR-322, Lot No. MA01-001, 100.2% purity

Methods

Doses:	0, 30, 100, 300 mg/kg
Frequency of dosing:	Daily
Dose volume:	10 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) methylcellulose (MC)
Species/Strain:	Sprague Dawley rat / Crl:CD(SD) SPF (b) (4)
Number/Sex/Group:	10
Satellite groups:	4/sex/group
Study design:	Four-week old rats were treated for 4-weeks to assess alogliptin effects on juvenile development. Satellite rats (n=4/sex) were administered a single dose of 300 mg/kg alo at 8-weeks for comparison in case of an age-related difference in drug exposure (i.e., between dosing at 4-weeks old and 8-weeks old at the end of the study).
Deviation from study protocol:	Minor deviations did not affect integrity or interpretation of study results.

Sponsor's Study Design Summary

Group composition in the main groups

Test group	Dosage[a)] (mg/kg/day)	Concentration[a)] (mg/mL)	Dosage volume (mL/kg/day)	Sex	Main group	
					No. of animals	Animal No.
Control[b)]	0	0	10	M	10	1001-1010
				F	10	1101-1110
Low	30	3	10	M	10	2001-2010
				F	10	2101-2110
Middle	100	10	10	M	10	3001-3010
				F	10	3101-3110
High	300	30	10	M	10	5001-5010
				F	10	5101-5110

Group composition in the satellite group

Test group	Dosage[a)] (mg/kg/day)	Concentration[a)] (mg/mL)	Dosage volume (mL/kg/day)	Sex	Satellite group	
					No. of animals	Animal No.
Low	30	3	10	M	4	2201-2204
				F	4	2301-2304
Middle	100	10	10	M	4	3201-3204
				F	4	3301-3304
High	300	30	10	M	4	4201-4204
				F	4	4301-4304
Single-dose at 8 weeks of age	300	30	10	M	4	5201-5204
				F	4	5301-5304

a): Dosage levels or concentrations as SYR-322Z (conversion factor: 1.360).
b): 0.5 w/v% MC solution
M: Male, F: Female

Table 8 – Juvenile rat alogliptin toxicity tabulated summary (Sponsor's summary)

Four-week oral gavage toxicity study of SYR-322 in 4 weeks old rats (B-6504)

Animal			Crl:CD(SD) rats, 4 weeks of age			
Test article			Control[a)]	SYR-322		
Dosage (mg/kg/day)[b)]			0	30	100	300
Dosage volume (mL/kg/day)			10	10	10	10
No. of Animals (M:F)[c)]			10:10	10:10	10:10	10:10
Mortality (M:F)			0:0	0:0	0:0	0:0
Clinical signs			–	–	–	–
Body weight			–	–	–	–
Food consumption			–	–	–	–
Ophthalmology			–	–	–	–
Urinalysis			–	–	Occult blood (F), RBC (F)	
Hematology			–	↓PT (M)		
Blood chemistry			–	–	↑TP (F), ↑Globulin (F)	
						↓AST (M),↑T-CHO (M), ↑GLU (M), ↑Ca, ↑P (F), ↑TP (M), ↑Globulin (M)
Organ weights			–	–	↑Liver (F), ↑Ovary (F)	
Necropsy			–	–	–	–
Histopathology			–	–	–	Liver: hypertrophy of centrilobular hepatocyte (M)
Toxicokinetics (M:F, mean values, n=3)						
SYR-322Z						
T_{max} (h)	Day 1	1st dose	1.3:1.0	1.0:2.0	2.0:1.0	
	Week 4	27th dose	2.7:1.0	1.0:1.0	4.3:1.7	
C_{max} (ng/mL)	Day 1	1st dose	957:1003	3970:3792	9935:9657	
	Week 4	27th dose	884:1906	4261:6398	8864:10053	
AUC_{0-24h} (ng·h/mL)	Day 1	1st dose	3970:4174	23226:24061	106621:103990	
	Week 4	27th dose	4275:5644	24737:28710	120444:110412	
M-I						
T_{max} (h)	Day 1	1st dose	2.3:1.0	2.0:2.0	4.0:2.7	
	Week 4	27th dose	2.7:1.0	1.3:2.3	4.3:2.7	
C_{max} (ng/mL)	Day 1	1st dose	138:141	342:219	531:596	
	Week 4	27th dose	287:271	465:321	653:600	
AUC_{0-24h} (ng·h/mL)	Day 1	1st dose	805:862	2869:2042	7232:7478	
	Week 4	27th dose	1730:1187	5084:2487	9584:8113	
M-II						
T_{max} (h)	Day 1	1st dose	2.3:1.0	1.0:2.0	1.0:1.0	
	Week 4	27th dose	2.7:1.0	1.0:1.0	4.3:2.3	
C_{max} (ng/mL)	Day 1	1st dose	16:22	67:84	180:160	
	Week 4	27th dose	28:64	110:197	238:290	
AUC_{0-24h} (ng·h/mL)	Day 1	1st dose	84:113	440:579	2075:1942	
	Week 4	27th dose	153:227	735:1070	3249:3627	
Conclusion			No effects of age on toxicokinetic parameters Non-toxic dosage level: 300 mg/kg/day and above for both sexes			

a): 0.5 w/v% methylcellulose solution
b): As SYR-322Z (SYR-322 free base, conversion factor: 1.360)
c): Additional 4 animals/sex/treated group were used as satellite groups for toxicokinetics.
M: Male, F: Female, -: No treatment-related effects, ↑: Increase, ↓: Decrease

Observations and Results:

Mortality – None.

Clinical Signs – Unremarkable.

Body Weight – No treatment-related findings.

Feed Consumption – No treatment-related findings.

Ophthalmology – No treatment-related findings.

Urinalysis – No remarkable treatment-related findings. Slightly increased incidences of occult blood and urinary sediments were observed in some treatment groups but findings were not considered biologically significant, particularly with respect to juvenile growth and development, in the absence of correlative toxicity (see Sponsor's summary table, below).

Summary of urinalysis

Sex		Male				Female			
Dosage (mg/kg/day)		0	30	100	300	0	30	100	300
No. of animals		10	10	10	10	10	10	10	10
Occult blood	(±)	0	0	0	0	0	0	0	1
	(+)	1	0	1	0	0	0	1	1
	(++)	0	0	0	0	0	0	1	0
Urinary sediments									
Red blood cell	(±)	1	0	1	0	0	0	2	1
Crystal calcium oxalate	(±)	0	0	3	3	0	0	1	0

Values indicate the number of animals.

Hematology – No remarkable treatment-related findings. A very small decrease in PT was seen in males, which was statistically significant but not considered biologically significant due to low magnitude and absence of delay or prolongation in clotting time. Modest, non dose-related decreases in male eosinophils were observed but not considered biologically significant in the absence of other signs of toxicity (see Sponsor's summary table, below).

Summary of hematology

Sex		Male			Female		
Dosage (mg/kg/day)		30	100	300	30	100	300
No. of animals		10	10	10	10	10	10
Eosinophil	ratio	N	-43%**	N	N	N	N
	count	-29%*	-43%**	N	N	N	N
PT		-5%*	-6%*	-9%*	N	N	N

Values in the table indicate percentage of change against the control mean (-: decrease).
N: No remarkable changes
*(**): p≤0.05 (0.01) (significantly different from the control group)

Clinical chemistry – Slight dose-related changes in various serum chemistry biomarkers were seen in HD males and MD and HD females. None of the changes were considered biologically significant in the absence of other signs of toxicity correlated with the various endpoints (see Sponsor's summary table, below).

Summary of blood chemistry

Sex	Male			Female		
Dosage (mg/kg/day)	30	100	300	30	100	300
No. of animals	10	10	10	10	10	10
AST	N	N	-10%*	N	N	N
ALT	N	N	+12%*	N	N	N
Total cholesterol	N	N	+25%*	N	N	N
Glucose	N	N	+13%*	N	N	N
Calcium	N	N	+4%*	N	N	+3%*
Inorganic phosphorus	N	N	N	N	N	+12%*
Total protein	N	N	+3%*	N	+5%*	+5%*
Globulin	N	N	+7%*	N	+7%*	+7%*

Values in the table indicate percentage of change against the control mean (+: increase, -: decrease).
N: No remarkable changes
*: $p \leq 0.05$ (significantly different from the control group)

Organ weights – Modest changes in absolute and/or relative weights were seen for various organs in MD or HD groups. In MD and HD females, mean absolute and relative liver and ovary weights increased 7-11% and 15-19%, respectively, compared to controls. The absence of dose-related differences between MD and HD groups, absence of similar liver weight increases in males, and absence of correlative histological or other signs of toxicity suggest modest statistically significant weight increases were not biologically significant. Other statistically significant organ weight changes were considered incidental (not drug-related) and/or not biologically significant. Organ weight data are shown in the Sponsor's summary table, below.

Summary of organ weights

Sex	Male			Female		
Dosage (mg/kg/day)	30	100	300	30	100	300
No. of animals	10	10	10	10	10	10
Body weight at necropsy	N	N	N	N	N	N
Brain						
Absolute	N	-3%*	-3%*	N	N	N
Relative	N	N	N	N	N	N
Thyroid						
Absolute	N	N	N	N	N	+15%*
Relative	N	N	N	N	N	N
Salivary gland						
Absolute	N	N	N	N	+9%*	+8%*
Relative	N	N	N	+11%*	N	N
Liver						
Absolute	N	N	N	N	+11%*	+11%*
Relative	N	N	+10%*	N	+7%*	+8%*
Kidney						
Absolute	N	N	N	N	+6%*	+4%*
Relative	N	N	N	N	N	N
Epididymis						
Absolute	N	N	-8%*	/	/	/
Relative	N	N	N	/	/	/
Ovary						
Absolute	/	/	/	N	+19%*	+17%*
Relative	/	/	/	+18%*	+16%*	+15%*
Uterus						
Absolute	/	/	/	-23%*	N	N
Relative	/	/	/	N	N	N

Values in the table indicate percentage of change against the control mean (+: increase, -: decrease).
N: No remarkable changes, /: Not applicable
* $p \leq 0.05$ (significantly different from the control group)

Histopathology (control and HD tissues examined; liver and male reproductive tissues examined from all groups) –
Adequate Battery – Yes
Peer Review – Yes

Histopathology findings were generally unremarkable. Findings noted by the pathologist but not discussed below were considered incidental and unrelated to alogliptin treatment.

Minimal centrilobular hepatocyte hypertrophy was seen in liver of 2/10 HD males but not in any other animals (including HD females). Low incidence, absence of serum biomarkers of liver injury, and absence of clear male liver weight increases or histologic findings in females (which had slight liver weight increases) suggest findings were incidental and unrelated to alogliptin treatment. However, the two males with hepatocyte hypertrophy did have increased liver weights compared to the control mean.

One of the HD males with hepatocyte hypertrophy and increased liver weight also had unilateral moderate seminiferous tubule atrophy and moderate hypospermia. The

animal had no other remarkable histopathology findings. It is possible the animal was more sensitive to alogliptin-induced toxicity and/or had unusually high drug exposure but it was not possible to make those conclusions from the available data.

Kidney tubule minimal regeneration was seen in 2/10 male and 2/10 female HD rats but not in control rats. The findings were considerable unremarkable due to low incidence, low severity, and absence of other signs of kidney toxicity.

Considering all of the histopathology data and absence of other signs of drug-induced toxicity, the histopathology findings were not considered indicative of predictable drug-related toxicity.

Stability and Homogeneity – Stability and homogeneity of dosing solutions were confirmed within ±3% of nominal doses.

Toxicokinetics (day 1, week 4; pre-dose, 1, 2, 4, 8, 24 h post-dose) –

Alogliptin was readily bioavailable in juvenile rats after oral dosing and exposure to parent and metabolites M-I and M-II increased with increasing dose. Alogliptin T_{max} was delayed slightly in the HD (1.5-3 h) compared to LD and MD (1-2 h). There was no remarkable difference in alogliptin exposure or metabolism (i.e., exposure to metabolites) after dosing to 4-week old rats (day 1) or 8-week old rats (day 27), although C_{max} and $AUC_{0\text{-}24\ h}$ tended to increase slightly on day 27 (consistent with slightly increased exposure in adults after repeated dosing). The increased exposure to metabolite M-II (up to approximately 2-fold higher) was greater than increased exposure to alogliptin and M-I (generally ≤40%) after repeated dosing. TK trends are shown in the Sponsor's summary table, below (Table 9).

Table 9 – Juvenile rat TK summary

Toxicokinetic parameters for SYR-322Z, SYR-322 M-I and SYR-322 M-II

Dose* (mg/kg/day)	Analyte	No. of dosing	Male (N=3)			Female (N=3)			Total (N=6)		
			Tmax (h)	Cmax (ng/mL)	$AUC_{0\text{-}24h}$ (ng•h/mL)	Tmax (h)	Cmax (ng/mL)	$AUC_{0\text{-}24h}$ (ng•h/mL)	Tmax (h)	Cmax (ng/mL)	$AUC_{0\text{-}24h}$ (ng•h/mL)
30	SYR-322Z	1st	1.3 (0.6)	957 (257)	3970 (670)	1.0 (0.0)	1008 (80)	4174 (195)	1.2 (0.4)	980 (172)	4072 (455)
		27th	2.7 (1.2)	884 (115)	4275 (1281)	1.0 (0.0)	1906 (348)	5644 (250)	1.8 (1.2)	1395 (606)	4960 (1115)
	SYR-322 M-I	1st	2.3 (1.5)	138 (15)	805 (216)	1.0 (0.0)	141 (29)	862 (214)	1.7 (1.2)	140 (21)	834 (195)
		27th	2.7 (1.2)	287 (37)	1730 (392)	1.0 (0.0)	271 (102)	1187 (197)	1.8 (1.2)	279 (69)	1458 (407)
	SYR-322 M-II	1st	2.3 (1.5)	16 (6)	84 (11)	1.0 (0.0)	22 (2)	113 (17)	1.7 (1.2)	19 (5)	98 (20)
		27th	2.7 (1.2)	28 (3)	153 (38)	1.0 (0.0)	64 (19)	227 (17)	1.8 (1.2)	46 (23)	190 (49)
100	SYR-322Z	1st	1.0 (0.0)	3970 (536)	23226 (7577)	2.0 (1.7)	3792 (1095)	24061 (4425)	1.5 (1.2)	3881 (777)	23644 (5569)
		27th	1.0 (0.0)	4261 (1100)	24737 (3525)	1.0 (0.0)	6398 (1770)	28710 (4277)	1.0 (0.0)	5329 (1762)	26724 (4059)
	SYR-322 M-I	1st	2.0 (1.7)	342 (52)	2869 (269)	2.0 (1.7)	219 (34)	2042 (213)	2.0 (1.5)	280 (78)	2456 (491)
		27th	1.3 (0.6)	465 (19)	5084 (460)	2.3 (1.5)	321 (15)	2487 (451)	1.8 (1.2)	393 (80)	3786 (1480)
	SYR-322 M-II	1st	1.0 (0.0)	67 (3)	440 (148)	2.0 (1.7)	84 (22)	579 (125)	1.5 (1.2)	76 (17)	510 (145)
		27th	1.0 (0.0)	110 (29)	735 (107)	1.0 (0.0)	197 (80)	1070 (129)	1.0 (0.0)	154 (72)	903 (212)
300	SYR-322Z	1st	2.0 (1.7)	9935 (1649)	106621 (9914)	1.0 (0.0)	9657 (1207)	103990 (12854)	1.5 (1.2)	9796 (1301)	105306 (10367)
		27th	4.3 (3.5)	8864 (1215)	120444 (23568)	1.7 (0.6)	10053 (1377)	110412 (23236)	3.0 (2.7)	9458 (1331)	115428 (21641)
	SYR-322 M-I	1st	4.0 (0.0)	531 (18)	7232 (882)	2.7 (1.2)	596 (167)	7478 (1538)	3.3 (1.0)	564 (112)	7355 (1130)
		27th	4.3 (3.5)	653 (68)	9584 (650)	2.7 (1.2)	600 (79)	8113 (870)	3.5 (2.5)	627 (72)	8848 (1059)
	SYR-322 M-II	1st	1.0 (0.0)	180 (65)	2075 (450)	1.0 (0.0)	160 (9)	1942 (332)	1.0 (0.0)	170 (43)	2009 (361)
		27th	4.3 (3.5)	238 (92)	3249 (1237)	2.3 (1.5)	290 (83)	3627 (871)	3.3 (2.7)	264 (84)	3438 (979)

Mean (S.D.)
*: As SYR-322Z

Alogliptin juvenile male reproductive development toxicity study in rats

GLP study, signed 9/6/10

Doses: 0, 30, 100, 300 mg/kg alogliptin
*Exposure: 7, 38, 132 µg*h/ml*

***NOAEL** = 300 mg/kg (88X MRHD)*

***NOAEL determination –** There were no signs of general toxicity and there were no apparent effects on male reproductive tissues/organs up to the highest dose of 300 mg/kg alogliptin.*

Key study findings:

- No remarkable toxicity. Juvenile males tolerated alogliptin up to the high dose of 300 mg/kg with no apparent effects on male reproductive system growth and development. Results are summarized in the Sponsor's summary table (Table 10).
- Thought not evaluated directly, the absence of effects on the male reproductive system implies that fertility would not be altered by exposure to alogliptin during sexual development. This assumes that mating behavior would not be adversely affected (another endpoint which was not evaluated in this study).

Study Title: Eight-week oral gavage toxicity study of SYR-322 in 4 weeks old male rats

Study no:	B-6812 (Code No. SYR-322/18040)
Study report location:	eCTD 4.2.3.5.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1/8/10
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SYR-322, Lot No. MA01-013, 99.6% purity

Methods

Doses:	0, 30, 100, 300 mg/kg
Frequency of dosing:	Daily
Dose volume:	10 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) methylcellulose (MC)
Species/Strain:	Male Sprague Dawley rat / Crl:CD(SD) SPF (b) (4)
Number/Sex/Group:	10 males
Satellite groups:	4/group (males)
Study design:	Alogliptin effects on male reproductive system toxicity were investigated in 4-week old juvenile male rats. Rats were treated for 8-weeks to cover the period of male sexual development and maturation. Organ weight and histopathology analyses were limited to male reproductive tissues/organs.
Deviation from study protocol:	None.

Sponsor's Study Design Summary

Group composition

Test group	Dosage [a)] (mg/kg/day)	Concentration (mg/mL)	Dosage volume (mL/kg/day)	Sex	Main group		Satellite group	
					No. of animals	Animal No.	No. of animals	Animal No.
Control [b)]	0	0	10	M	10	1001-1010	-	-
Low	30	3	10	M	10	2001-2010	4	2201-2204
Middle	100	10	10	M	10	3001-3010	4	3201-3204
High	300	30	10	M	10	4001-4010	4	4201-4204

a): Dosage levels or concentrations as SYR-322Z (conversion factor: 1.360)
b): 0.5 w/v% MC solution
M: Male

Table 10 – Juvenile male rat reproductive development (Sponsor's summary)

Eight-week oral gavage toxicity study of SYR-322 in 4 weeks old male rats (B-6812)

Animal		Crl:CD(SD) rats, 4 weeks of age, male			
Test article		Control [a]	SYR-322		
Dosage level (mg/kg/day) [b]		0	30	100	300
Dosage volume (mL/kg/day)		10	10	10	10
No. of animals [c]		10	10	10	10
Mortality		0	0	0	0
Clinical signs		–	–	–	–
Body weight		–	–	–	–
Food consumption		–	–	–	–
Organ weights (Testis, Epididymis, Prostate, Seminal vesicle)		–	–	–	–
Necropsy		–	–	–	–
Histopathology (Testis, Epididymis, Prostate, Seminal vesicle)		–	NE	NE	–
Toxicokinetics (mean values, n=3)					
SYR-322Z					
T_{max} (h)	Day 1		1.0	1.0	1.3
	Week 8		1.0	1.0	6.7
C_{max} (ng/mL)	Day 1		1214	6297	12294
	Week 8		1364	4419	9363
AUC_{0-24h} (ng·h/mL)	Day 1		5007	22922	103206
	Week 8		6928	37679	132327
M-I					
T_{max} (h)	Day 1		2.0	1.0	3.3
	Week 8		1.0	2.3	5.3
C_{max} (ng/mL)	Day 1		183	374	573
	Week 8		264	442	565
AUC_{0-24h} (ng·h/mL)	Day 1		1068	2551	6311
	Week 8		2190	5468	8639
M-II					
T_{max} (h)	Day 1		1.0	1.0	1.3
	Week 8		1.0	1.0	4.3
C_{max} (ng/mL)	Day 1		21	103	176
	Week 8		51	155	231
AUC_{0-24h} (ng·h/mL)	Day 1		83	417	1582
	Week 8		246	1289	3054
Conclusion		Non-toxic dosage level for juvenile male reproductive organs: 300 mg/kg/day and above			

a): 0.5 w/v% methylcellulose solution
b): As SYR-322Z (SYR-322 free base, conversion factor: 1.360)
c): Additional 4 animals were provided for determining toxicokinetics in each group except for the control group.
–: No treatment-related effects, NE: Not examined, Day 1: 1st dose, Week 8: 55th dose

Observations and Results:

Mortality – None.

Clinical Signs – Unremarkable.

Body Weight and Food Consumption – No treatment-related findings.

Organ weights (testes, epididymids, prostate, seminal vesicles) – No treatment-related findings.

Histopathology (complete tissue battery collected and fixed; only control and HD testes, epididymids, prostate, and seminal vesicles examined) –
Adequate Battery – Yes
Peer Review – Yes

Histopathology findings in reproductive tissues of HD males were limited to low incidence and severity of prostate interstitial cell infiltration which was seen at higher incidence in control males. LD and MD male reproductive tissues were not examined due to absence of findings in HD males. Data are summarized in the Sponsor's table, below (Table 11).

Table 11 – Juvenile male rat reproductive tissue histopathology summary

Organs	Sex:	M	M	M	M
	Dose(mg/kg/day):	0	30	100	300
Findings	Number:	10	10	10	10
Epididymis					
Number examined		10	0	0	10
Not remarkable		8	0	0	10
Cell infiltration,interstitial		2	0	0	0
minimal		2	0	0	0
Epididymis(PAS stain)					
Number examined		10	0	0	10
Not remarkable		10	0	0	10
Prostate					
Number examined		10	0	0	10
Not remarkable		4	0	0	8
Cell infiltration,interstitial		6	0	0	2
minimal		4	0	0	2
mild		2	0	0	0
Seminal vesicle					
Number examined		10	0	0	10
Not remarkable		10	0	0	10
Testis					
Number examined		10	0	0	10
Not remarkable		9	0	0	10
Hypoplasia		1	0	0	0
severe		1	0	0	0
Testis(PAS stain)					
Number examined		10	0	0	10
Not remarkable		10	0	0	10

M : Male

Toxicokinetics – Alogliptin was readily absorbed and exposure to parent and metabolites increased with increasing dose. Exposure to alogliptin and metabolites was slightly greater after repeated dosing, consistent with previous studies in juvenile and adult rats. Alogliptin exposures in 12-week old male rats at the end of 8-weeks of dosing were slightly higher than in 8-week old males (at the end of 4-weeks of dosing), with 75%, 52%, and 15% increases in 30, 100, and 300 mg/kg groups. Increased exposures may be due to further drug accumulation or represent modest differences in male rats of different ages. TK data shown in the Sponsor's summary table, below (Table 12).

Table 12 – Juvenile male rat TK summary (8-weeks treatment)

Summary of TK parameters

Sex	Male (n=3)		
Dosage (mg/kg/day)	30	100	300
SYR-322Z			
T_{max} (h)			
Day 1	1.0	1.0	1.3
Week 8 (55th dose)	1.0	1.0	6.7
C_{max} (ng/mL)			
Day 1	1214	6297	12294
Week 8 (55th dose)	1364	4419	9363
AUC_{0-24h} (ng·h/mL)			
Day 1	5007	22922	103206
Week 8 (55th dose)	6928	37679	132327
M-I			
T_{max} (h)			
Day 1	2.0	1.0	3.3
Week 8 (55th dose)	1.0	2.3	5.3
C_{max} (ng/mL)			
Day 1	183	374	573
Week 8 (55th dose)	264	442	565
AUC_{0-24h} (ng·h/mL)			
Day 1	1068	2551	6311
Week 8 (55th dose)	2190	5468	8639
M-II			
T_{max} (h)			
Day 1	1.0	1.0	1.3
Week 8 (55th dose)	1.0	1.0	4.3
C_{max} (ng/mL)			
Day 1	21	103	176
Week 8 (55th dose)	51	155	231
AUC_{0-24h} (ng·h/mL)			
Day 1	83	417	1582
Week 8 (55th dose)	246	1289	3054

Values in the table indicate the mean.
SYR-322Z: SYR-322 free base

Stability and Homogeneity – Stability and homogeneity of dosing solutions were confirmed within ±2% of nominal doses.

10 Special Toxicology Studies

Several environmental assessment studies were submitted and listed below with brief synopses (or Sponsor's conclusions). The Sponsor requested and was granted an environmental assessment waiver so the environmental studies were not extensively reviewed.

Fish early life stage alogliptin toxicity test (OECD Test 210; NOTOX Project 489436)

No effects on fathead minnow embryo growth, hatching time or success, or larval survival and growth up to the regulatory limit concentration of 10 mg/l.

'Ready' biodegradability CO_2 evolution test (OECD Test 301 B; NOTOX Project 489437)

Neither alogliptin nor the free benzoate (from the alogliptin benzoate salt) were readily biodegradable and there was no inhibition of microbial activity.

Activated sludge respiration inhibition (OECD Test 209; NOTOX Project 489438)

Alogliptin benzoate was not toxic to activated sludge bacteria in waste water up to a nominal concentration of 100 mg/l (73.5 mg/l alogliptin).

Fresh water algal growth inhibition (OECD Test 201; NOTOX Project 490325)

Alogliptin benzoate inhibited growth and yield of fresh water algae (*Pseudokirchneriella subcapitata*) at 100 mg/l. The EC_{50} was > 100 mg/l for growth rate reduction and 80 mg/l for yield inhibition. The NOEC was 56 mg/l alogliptin for growth rate reduction and yield inhibition.

***Daphnia magna* reproduction (OECD Test 211; NOTOX Project 489434)**

Alogliptin benzoate exposure to *Daphnia magna* did not affect survival, growth, reproduction, or offspring growth up to the limit concentration of 10 mg/l.

Alogliptin sludge adsorption/desorption (OECD Test 106; NOTOX Project 489439)

Alogliptin adsorption and desorption to wastewater sludge was assessed with samples from two different wastewater treatment plants. Alogliptin was stable in sludges with calculated adsorption equilibrium of 3-5 h and desorption equilibrium of 6 h. The Sponsor concluded "alogliptin isotherms could be described by the Freundlich equation ($K_{f,oc}^{ads}$) values" of 25.2 ml/g and 18.7 ml/kg for the two sludges.

Alogliptin aerobic degradation (OECD Test 308; NOTOX Project 490438)

Alogliptin degradation under aerobic conditions in two wastewater samples showed limited metabolism with partitioning into sediment (≥ 51-77% after 28 d equilibration), negligible mineralization to CO_2, and 27-36% bound to wastewater components.

11 Integrated Summary and Safety Evaluation

The proposed alogliptin tablet (Nesina™ – proposed) and alogliptin plus pioglitazone FDC tablet (Oseni™ – proposed) drug products were submitted in accordance with 21 USC 505(b)(1) for treatment of type 2 diabetes mellitus as adjunct to diet and exercise. The Sponsor owns all of the drug substances in the two proposed drug products. All pivotal studies previously reviewed and the toxicology studies in the Complete Response submissions were conducted in compliance with current GLP standards.

Pharmacology

Several pharmacology studies were submitted and reviewed. Studies generally confirmed information known about primary pharmacology and alogliptin mechanism of action. A combination alogliptin plus metformin study showed metformin, but not alogliptin, slowed intestinal sugar absorption. Results suggested improved glucose tolerance in diabetic rats (Wistar Fatty) treated with alogliptin plus metformin may involve independent and/or complementary mechanisms of the two drugs, consistent with improved glucose control with alogliptin treatment on background metformin therapy. When diabetic rats (Wistar Fatty) were pre-treated with pioglitazone followed by alogliptin plus metformin, glucose excursion was improved compared to treatment with dual combination treatments (pio + alo, pio + met, or alo + met). Triple combination treatment resulted in maximal effects on active plasma GLP-1 but did not further improve insulin response expected from pioglitazone ± metformin. In a different diabetic rat model (N-STZ-1.5), combination treatment of alogliptin plus glibenclamide (a sulfonylurea) showed additive improvement in glucose excursion and insulin secretion, with earlier maximum insulin response, compared to individual drugs alone.

PK/ADME

No pivotal PK/ADME studies were included in the CR submission. Studies submitted and reviewed were consistent with studies that have been previously reviewed. Discussion and conclusions have not changed from the original NDA reviews.

Toxicology

Toxicology studies were submitted to support safe use of alogliptin on anticipated background metformin therapy and in combination with pioglitazone. The GLP studies focused on alogliptin and metformin combination treatment in healthy rats (13-week combination toxicity study) and pregnant rats (combination embryofetal development study), and alogliptin treatment in juvenile rats (general juvenile growth study and male reproductive system development study). Alogliptin plus pioglitazone combination toxicity studies in healthy (13-week) and pregnant rats (embryofetal development) were previously reviewed for NDA 22-426.

There was no unexpected toxicity and no apparent synergistic increase in toxicity in combination alogliptin and metformin studies in rats. Toxicity was generally driven by metformin treatment due to lower metformin exposure margins at the respective MTDs for alogliptin and metformin in rats. Toxicity studies were designed to identify additive or synergistic effects of well tolerated alogliptin doses in combination with MTD metformin doses.

A standard three month rat alogliptin plus metformin toxicity study showed slight additive effects of alogliptin on certain metformin-induced toxicity but toxicity was generally not worsened by combination treatment. There was no evidence of a synergistic effect of alogliptin and metformin combination treatment but the absence of an alogliptin only control made interpretation of any alogliptin-induced toxicity difficult. Consistent with predicted effects, metformin-induced toxicity included reduced BW gain, plasma lactic acid accumulation without concomitant increased bicarbonate, increased serum ALT and CK, slight alterations in plasma electrolytes, and increased organ weights (heart, liver, kidney, salivary gland, adrenals) with correlative histological lesions. Heart cardiomyopathy and myocardial hypertrophy, liver hepatocyte hypertrophy, kidney tubule regeneration and hypertrophy, salivary gland hypertrophy, and adrenal vacuolation and hypertrophy were correlated with organ/tissue weight increases. GI tract was also a target based on stomach gross foci and erosion, duodenum erosion, and cecum hyperplasia. Alogliptin exposure (C_{max} and $AUC_{0\text{-}24\ h}$) decreased slightly, approximately 25-40% with increasing metformin coadministration. Metformin co-treatment did not affect alogliptin metabolism.

DMEP required a combination alogliptin plus metformin rat embryofetal development study with the CR submission because of potential class-related effects of DPP4 inhibitors and metformin. There were no treatment related fetal findings in alogliptin or metformin controls or in the low dose combination treatment (34X alogliptin/ 3X metformin MRHD). In the high dose combination treatment group there was evidence of maternal toxicity based on reduced BW gain. Eye and vertebral malformations were seen in 4 fetuses from 2 (of 20) dams in the HD combination (23X alogliptin/ 6X metformin MRHD). A relationship to treatment could not be ruled out for deformed fetuses, but findings were limited to microphthalmia in three fetuses from one dam with markedly reduced BW gain and a single fetus (microphthalmia, cleft palate, microglossia, and mandibular micrognathia) with multiple abnormalities in a separate dam. There were no other treatment-related malformations and no apparent treatment-related external, visceral, or skeletal variations. There were no apparent treatment-related effects on pregnancy success or other pregnancy-related or fetal outcomes in any group. With the exception of fetal malformations from two HD combination dams but none in dams treated only with alogliptin or metformin, there was no clear evidence of unexpected or synergistic increase in maternal or embryofetal toxicity.

Juvenile toxicity studies have not been required for the DPP4 inhibitor class prior to conducting pediatric clinical trials, based on the absence of a clearly increased risk of children and adolescents to treatment. Pediatric trials had not been planned prior to submission of the CR and juvenile toxicity studies were not required by DMEP prior to

CR submission. However, evidence of alogliptin-induced testicular toxicity at high multiples of expected clinical exposures in monkeys (>30X) and rats (>200X) suggested potential risk to developing male reproductive system in male children. The Sponsor conducted and submitted separate studies in juvenile male and female rats and a longer duration study dedicated to male reproductive system development in rats. There was no evidence of alogliptin-induced toxicity in male and female rats treated from age 4-weeks to age 8-weeks with up to 77-times expected clinical exposures. In the dedicated male reproductive system development study, male rats treated from age 4-weeks to age 12-weeks had no apparent general toxicity or any effects on male reproductive system growth and development at up to 88-times expected clinical exposures.

While results of the juvenile animal studies suggest a similar toxicity profile in adults and juveniles after short term exposures, there are several issues that limit conclusions about juvenile and/or male reproductive toxicity. The Sponsor provided no rationale for the conduct and design of juvenile animal studies and there were no discussions with DMEP pharmacology/toxicology about study endpoints. The combined sex study was a short duration, limited to 4 weeks of treatment, and lacked standard endpoints such as pubertal onset, behavioral maturation, and fertility. Similarly, the dedicated juvenile male study was limited to treatment post-weaning and to standard toxicity assessment of male reproductive tissues (e.g., organ weight, gross pathology, histopathology) but did not directly assess sperm development (e.g., sperm morphology and motility) or male fertility. As conducted, study results did not identify any clear risk to alogliptin exposure in juvenile rats. Considering high exposure multiples in juvenile animal studies and absence of findings in standard reproductive and developmental toxicity studies (male and female fertility and early embryonic development, embryofetal development, pre- and post-natal development), risks to developing animals are considered minimal.

Environmental toxicity assessment was not required by the FDA but several environmental toxicity studies were submitted. The studies were likely conducted to support regulatory requirements for other countries. The studies were not comprehensively reviewed but a cursory review was done for each study and no major environmental issues were identified. A study on early life stage development in fish showed no effects on fathead minnow embryo growth, hatching time or success, or larval survival and growth. Studies on bacterial and algal growth showed: alogliptin was not readily biodegradable and did not inhibit microbial activity; alogliptin was not toxic to activated sludge in waste water; alogliptin did not affect *Daphnia* survival, growth, or reproduction or offspring survival; and, alogliptin did not affect algal growth up to 56 mg/l but high aqueous alogliptin concentrations inhibited algal growth (EC_{50} > 100 mg/l) and algal yield (EC_{50} = 80 mg/l).

A brief tabulated summary of alogliptin toxicity trends and written summaries of pivotal combination alogliptin plus pioglitazone toxicity study reviews are included here for reference purposes.[4]

[4] D. Carlson, Pharmacology/Toxicology Review NDA 22-271 (8/27/08) and NDA 22-426 (6/8/09)

Alogliptin target organ summary

- **Kidney** (≥ 32X MRHD at NOAEL; > 200X MRHD at LOAEL in chronic studies)
  - Rat: mortality, tubular degeneration/regeneration, transitional cell hyperplasia, increased female chronic progressive nephropathy background disease rate (~50% background incidence)
  - Rabbit: mortality
- **Lung** (≥ 32X MRHD at NOAEL; > 200X MRHD at LOAEL in chronic studies)
  - Rat: gross discoloration and focus/foci, histological histiocytosis
- **Liver** (≥ 32X MRHD at NOAEL; > 200X MRHD at LOAEL in chronic studies)
  - Rat: hypertrophy, periportal vacuolation, basophilic 'focus of cell. alteration'
- **Testes** (≥ 32X MRHD at NOAEL; > 200X MRHD at LOAEL)
  - Rat: testes atrophy/degeneration, small seminal vesicles, oligospermia and abnormal sperm, epididymids germ cell debris
  - Monkey: possible, unconfirmed decreased testes weight
- **Urinary bladder** (≥ 32X MRHD at NOAEL; > 200X MRHD at LOAEL)
  - Rat: gross calculus/calculi, simple transitional cell hyperplasia, treatment-related mortality
- **Immune/skin** (hypersensitivity/pseudoallergy) (~10-20X MRHD at NOAEL; ~20-30X MRHD at LOAEL)
  - Dog: flushing, swelling, hypersensitivity/pseudoallergy (as early as single dose)
  - No skin lesions any species
  - No phototoxicity

Combination alogliptin and pioglitazone toxicity summary

Three-month subchronic toxicity in rat: Alogliptin plus pioglitazone combination treatment in rats was assessed in subchronic toxicity studies up to 13-weeks. Alogliptin co-treatment had minimal effects on pioglitazone exposure and pioglitazone-mediated toxicity in rats. Target organs were consistent with known pioglitazone effects and included **heart** (cardiomyopathy), **spleen** (extramedullary hematopoiesis), **bone marrow** (adipocyte hypertrophy), **brown** and **white fat** (adipocyte hypertrophy/hyperplasia), and **adrenals** (cortical cell hypertrophy). Alogliptin co-treatment increased pioglitazone M-II plasma maximum (C_{max}) and total (AUC) exposure approximately 2- to 3-fold and slightly increased pioglitazone T_{max} approximately 0.4 to 3 h.

Embryofetal rat development: Embryofetal development was assessed in rats with the combination of alogliptin plus pioglitazone. Alogliptin co-administered with pioglitazone did not show any teratogenic signal in fetuses. Consistent with previous experience, pioglitazone treatment did show some effect on embryofetal development, including increased placental weights (16-30%) and various visceral variations (left umbilical artery, renal pelvis dilatation, and convoluted/dilatation

ureter). Co-treatment with 100 mg/kg alogliptin caused slightly decreased fetal weights (4-5%) and potentiated effects of pioglitazone alone on total visceral variations (number of fetuses and number of dams with fetal variations), most notably seen as increased supernumerary coronary ostium and renal pelvis dilatation.

12 Appendix/Attachments

Table 13 – Tabulated summary of toxicity studies reviewed

Toxicity Studies in Rat			
Study	**NOAEL**	**MRHD Multiple[a]**	**Findings**
13-Week Combo. Tox. GLP (#B-6610) Alogliptin / Metformin 100/300, 100/1000 0/300, 0/1000 mg/kg *47/110, 34/242* *0/84, 0/230 µg*h/ml*	< 100/300 mg/kg	< 31X Alo / 4X Met	Slight additive effect of alogliptin on metformin-induced toxicity Metformin toxicity: ↓ BW gain, ↑ lactic acid, ↑ ALT, ↑ CK, ↑ organ weights (heart, liver, kidney, sal. gland, adrenals), cardiomyopathy + myocardial hypertrophy, hepatocyte hypertrophy, kidney tubule regen./hypertrophy, sal. gland hypertrophy, adrenal vacuolation & hypertrophy
Embryofetal Develop. GLP (#09-260/TE) Alogliptin / Metformin 100/150, 100/500, 100/0, 0/150, 0/500 *51/70, 35/163, 44/0, 0/40,* *0/149 µg*h/ml*	100/150 mg/kg (Maternal & Fetal)	34X Alo/ 3X Met	No synergistic toxicity 100/500 – maternal toxicity (↓ BW gain; fetal malformations (2 dams, 4 fetuses) – microphthalmia, etc.; no fetal variations or pregnancy effects
Juvenile tox. (alogliptin) 4-Week (♂ & ♀) GLP (#B-6504) 30, 100, 300 mg/kg 5, 27, 115 µg*h/ml	300 mg/kg	77X Alo	No treatment-related findings Did not evaluate pubertal onset, behavioral development, or fertility
Juvenile tox. (alogliptin) 8-Wk ♂ Repro. organs GLP (#B-6812) 30, 100, 300 mg/kg 7, 38, 132 µg*h/ml	300 mg/kg	88X Alo	No treatment-related findings Did not evaluate sperm development (morphology/motility), fertility, or mating

[a] Exposure multiple estimates based on daily MRHD clinical exposures from 25 mg alogliptin ($AUC_{0\text{-}24\ h}$ = 1.5 µg*h/ml) and 2000 mg metformin ($AUC_{0\text{-}24\ h}$ = 26 µg*h/ml)

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/s/

DAVID B CARLSON
01/18/2012
Approval recommendation -- Alogliptin tablets and alogliptin + pioglitazone FDC tablets CR

TODD M BOURCIER
01/18/2012
I concur

Tertiary Pharmacology/Toxicology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology
OND IO
NDA: 22-426
Submission date: 9/19/2008
Drug: alogliptin/pioglitazone
Sponsor: Takeda Pharmaceuticals
Indication: treatment of type 2 diabetes mellitus

Reviewing Division: Division of Metabolism and Endocrinology Products

Comments:
The pharm/tox reviewer and supervisor found the nonclinical information adequate to support approval of the alogliptin/pioglitazone combination product for the intended indication.

The toxicity of the combination of alogliptin and pioglitazone was assessed in a 3 month study in rats and a rat embryofetal toxicity study. These studies did not reveal any new toxicity compared to that identified with the individual agents.

The reviewer and supervisor recommended pregnancy category C for the combination product. Pioglitazone is pregnancy category C while the pharm/tox recommendation for alogliptin alone is for pregnancy category B.

Conclusions:
I read the pharm/tox review and supervisory memorandum and I agree that the information is adequate from a pharm/tox perspective to support approval of this NDA. No additional nonclinical studies are recommended at this time. Final labeling has not been discussed at this time in the pharm/tox review. However, I agree with the pharm/tox review which notes that pregnancy category C appears to be appropriate. Specific wording can be addressed at a later time and may depend on labeling for alogliptin alone.

Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
NDA 22426	ORIG 1	TAKEDA GLOBAL RESEARCH DEVELOPMENT CENTER INC	ALOGLIPTIN/PIOGLITAZONE TABLET

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

/s/

PAUL C BROWN
08/26/2009

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:	**22-426**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**09/19/08**
PRODUCT:	(b) (4) **(Alogliptin/Pioglitazone Fixed-Dose Combination Tablets)**
INTENDED CLINICAL POPULATION:	**Treatment of type 2 diabetes mellitus**
SPONSOR:	**Takeda Pharmaceuticals**
DOCUMENTS REVIEWED:	**eCTD**
REVIEW DIVISION:	**Division of Metabolism and Endocrinology Products**
PHARM/TOX REVIEWER:	**David B. Carlson, Ph.D.**
PHARM/TOX SUPERVISOR:	**Todd Bourcier, Ph.D.**
DIVISION DIRECTOR:	**Mary Parks, M.D.**
PROJECT MANAGER:	**Julie Marchick**

Date of review submission to Division File System (DFS): 8 June, 2009

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

Approval. Pharmacology/Toxicology recommends approval of NDA 22-426 (b) (4)

B. Recommendation for nonclinical studies

No additional nonclinical studies needed.

C. Recommendations on labeling

Pharmacology/toxicology agrees with Pregnancy Category C designation by the sponsor. The proposed labeling language relevant to pharmacology/toxicology accurately represents the approved ACTOS (pioglitazone) label. Final label wording will need to incorporate any changes to the final Nesina (alogliptin) label recommended by pharmacology/toxicology for Sections 8 and 13 (pregnancy/reproductive toxicity, lactation, carcinogenicity).

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Alogliptin benzoate (Nesina) was recommended for approval by pharmacology/toxicology based on nonclinical data, with exposure multiples based on the maximum recommended human dose (MRHD) of 25 mg QD ($AUC_{0\text{-}24} \approx 1.5$ µg*h/ml, $C_{max} \approx 140$ ng/ml).

Pioglitazone HCl is currently marketed as ACTOS (15, 30, or 45 mg QD) and MRHD multiples are based on prior pharmacology/toxicology reviews and $AUC_{0\text{-}24} \approx 10$ µg*h/ml.

Alogliptin plus pioglitazone combination treatment in rats was assessed in subchronic toxicity studies up to 13-weeks. Rats are an acceptable model for combination treatment because both alogliptin and pioglitazone are active in rats, human metabolites are produced in rats, and rats are sensitive to the hallmark TZD-induced cardiac toxicity. Monkey studies are not required for the combination because no skin lesions were seen with alogliptin. Dogs are not an appropriate species for the combination because dogs are exquisitely sensitive to thiazolidinedione-induced cardiac toxicity. Alogliptin co-treatment had minimal effects on pioglitazone-mediated toxicity in rats. Target organs were consistent with known pioglitazone effects and included **heart** (cardiomyopathy), **spleen** (extramedullary hematopoiesis), **bone marrow** (adipocyte hypertrophy), **brown**

and **white fat** (adipocyte hypertrophy/hyperplasia), and **adrenals** (cortical cell hypertrophy).

Alogliptin co-treatment increased maximum (C_{max}) and total (AUC) plasma exposure to pioglitazone active metabolite M-II by approximately 2- to 3-fold and slightly increased pioglitazone T_{max} approximately 0.5 h. Increases in pioglitazone T_{max} were minimal and not considered toxicologically or biologically significant. The altered metabolism finding in rats seems to have little biologic significance to humans because M-II is not considered a relevant pioglitazone metabolite in humans— it has apparently been identified at very low levels in human plasma but it is not noted on the ACTOS label and it was not measured in the alogliptin plus pioglitazone clinical drug-drug interaction study.

Alogliptin alone was well tolerated in animals up to doses on the order of tens- to hundreds-fold higher than expected human exposure. Major alogliptin target organs identified at high multiples of human exposure are testes, kidney, liver, urinary bladder, and lung. Kidney and bladder toxicity were the only toxicities that contributed to mortality in animals (mice, rats, rabbits), which occurred only at high exposures (generally > 50X MRHD). Dogs showed consistent but intermittent clinical signs of reddened/flushing of the ears and face, along with body and facial swelling, consistent with a pseudoallergy response.

Notable toxicity in animals treated with pioglitazone alone includes left atrial thrombosis, hydrothorax, cardiac hypertrophy, and elevation of hepatic enzymes. Increased heart weight and cardiac hypertrophy occur at exposure margins of 1, 2, and 11 in rat, dog, and mice, respectively, based on body surface area extrapolations (as noted in the ACTOS label).

Alogliptin co-administered with pioglitazone in a rat embryofetal development study did not show any teratogenic signal in fetuses. Consistent with previous experience, pioglitazone treatment did show some effect on embryofetal development, including increased placental weights (16-30%) and various visceral variations that did not affect fetal survival. Co-treatment with 100 mg/kg (estimated 33-67X MRHD) alogliptin caused slightly decreased fetal weights (4-5%) and potentiated effects of pioglitazone alone on total visceral variations (number of fetuses and number of dams with fetal variations), most notably seen as increased supernumerary coronary ostium and renal pelvis dilatation.

Alogliptin plus pioglitazone combination carcinogenicity studies were not conducted. Alogliptin as monotherapy poses minimal carcinogenic risk to humans based on an absence of drug-related tumors in mice (60X MRHD), very high exposure multiples in male rats (≥ 288X) at doses that increased thyroid C-cell tumors (adenomas, carcinomas), and high exposure multiples at the rat NOAEL (32X) (see Carlson, 8/20/08, NDA 22-271 pharmacology/toxicology review). Chronic pioglitazone treatment caused benign and/or malignant urinary bladder transitional cell neoplasms that occurred at approximate human equivalent doses

in male rats. No pioglitazone-induced tumors were seen in female rats or in mice. Pioglitazone carcinogenicity findings are present in the current ACTOS label and have been reproduced in the proposed (b) (4) label.

B. Pharmacologic activity

(b) (4) is a fixed-dose combination of a DPP4 inhibitor, alogliptin, and a thiazolidinedione (TZD), pioglitazone. The mechanisms of action are expected to be complementary and result in improved blood glucose control compared to either treatment alone. Neither treatment alone significantly increases hypoglycemia in healthy animals, consistent with a glucose-dependent mechanism for DPP4 inhibitors and an insulin-sensitization (rather than insulin secretagogue) mechanism for TZDs. In addition, absence of hypoglycemia in combination treatment in healthy rats supports an absence of significantly increased clinical hypoglycemia risk.

DPP4 inactivates glucagon-like peptide 1 (GLP-1) by N-terminal cleavage. GLP-1 is released from the L-cells in the gut after meals, which potentiates glucose-dependent insulin secretion from pancreatic β cells, leading to increased hepatic glucose metabolism. GLP-1 also suppresses glucagon secretion, which delays gastric emptying and independently contributes to reduced blood glucose concentrations. DPP4 inhibition has been shown to reduce blood sugar and glycated hemoglobin ($HbA1_c$) *in vivo* in healthy and diabetic animal models and in diabetic patients. Nonclinical experience with DPP4 inhibitors has shown negligible hypoglycemia risk because the target incretin, GLP-1, is only released after meals or other exogenous glucose loading. One DPP4 inhibitor, sitagliptin (Januvia), is currently marketed in the U.S. and globally for treatment of type 2 diabetes. A second DPP4 inhibitor, vildagliptin, is currently marketed in various countries outside the U.S. In addition, exenatide, a GLP-1 analog and incretin mimetic, is also marketed in the U.S. The intended pharmacodynamic effect of increased GLP-1 activity is similar for exenatide and DPP4 inhibitors.

Pioglitazone and other TZDs lower circulating glucose levels in an insulin-dependent manner. TZDs do not increase insulin secretion but increase peripheral insulin sensitivity and insulin-dependent hepatic glucose disposal and decrease hepatic glucose production, leading to overall decreased insulin resistance. The exact mechanism of action of TZDs is not clear, but much of their activity is thought to be due to activation of peroxisome proliferator-activated receptor gamma (PPARγ). PPARγ is present in a wide variety of tissues including adipose, skeletal muscle, and liver, where PPARγ-mediated transcription alters expression of insulin responsive genes. Beneficial lipid effects of pioglitazone are thought to occur via activation of PPARα. Two TZDs, pioglitazone and rosiglitazone, are currently marketed in the U.S. and have been shown efficacious in diabetic and insulin-resistant animal models and in type 2 diabetic patients.

C. Nonclinical safety issues relevant to clinical use

1. Clinical risks of pioglitazone (included on current labels) are well known from years of clinical use and previous nonclinical reviews. To reiterate, cardiac hypertrophy and increased heart weights with concomitant physiologic sequelae are the major risks identified in pioglitazone-treated animals at low multiples of human exposure. Nonclinical cardiac changes may be adaptive in nature due to PPARγ-mediated (or other unknown mechanism) plasma volume expansion and changes in hemodynamic parameters and water distribution. Nonclinical data show no apparent risk of alogliptin on cardiac toxicity and, importantly, establish there is no apparent additive or synergistic effect of alogliptin co-treatment on pioglitazone-mediated cardiac or other toxicity.

2. Combination treatment in rats did not cause an increase in hypoglycemia compared to alogliptin or pioglitazone monotherapy. Hypoglycemia risk is expected to be low due to glucose-dependent and insulin-dependent mechanisms of the two drugs. Nevertheless, because of the independent, complementary mechanisms for glucose lowering, clinical hypoglycemia risks may be higher compared to either monotherapy.

3. Hypersensitivity and/or pseudoallergy are predicted in sensitive individuals in the clinical population based on findings in dogs administered alogliptin. The reactions in dogs include facial swelling/edema at high multiples of human exposure (≥ 32X MRHD) which is also notable since edema is listed in the 'precautions' section of the ACTOS label. The reaction in dogs seemed to be separate from DPP4-inibitor induction of necrotic skin lesions in monkeys. The risk of skin lesions from prolonged alogliptin treatment cannot be ruled out, but there was no evidence of skin lesions in any species in the non-clinical program.

4. Since DPP4 cleaves substrates other than the targeted incretin hormones, inhibition of DPP4 may have unintended consequences with prolonged dosing. In particular, antigen-mediated responses and immune cell trafficking may be affected by DPP4 inhibition. Immune-related effects remain an unresolved, unavoidable risk with DPP4 inhibitor drugs.

5. Both alogliptin and pioglitazone are secreted in milk of nursing rats and combination treatment in rats showed alogliptin potentiated some pioglitazone-mediated fetal effects including low fetal weights and increased non-teratogenic visceral variations. The ACTOS label and proposed (b) (4) label accurately note fetal toxicity in animals and recommend against drug use in pregnant women or nursing mothers.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-426
Review number: 1
Sequence number/date/type of submission: N-000/19 September, 2008/Original submission
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Takeda Global Research and Development Center, One Takeda Parkway, Deerfield, IL 60015
Manufacturer for drug substance: Takeda Pharmaceutical Company Ltd, 17-85, Juso-hommachi 2-chome, Yodogawa-ku, Osaka, 532-8686 Japan

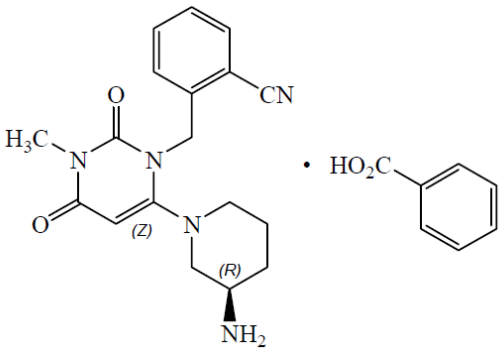
Reviewer name: David B. Carlson, Ph.D.
Division name: Metabolism and Endocrinology Products (DMEP)
HFD #: 510
Review completion date: 29 June, 2009

Drug:

Trade name: (b) (4)
Generic name: Alogliptin/Pioglitazone fixed dose combination (FDC)
Code name: SYR-322-4833

Alogliptin benzoate

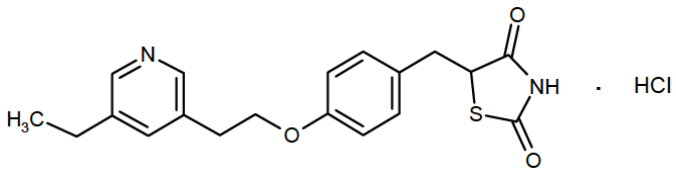
Code name: SYR-322 (SYR-322S; SYR110322; SYR110322S; SYR110322 benzoate)
Chemical name: 2-({6-[(3*R*)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl}methyl)benzonitrile monobenzoate
CAS registry number: 850649-62-6
Molecular formula/molecular weight: $C_{18}H_{21}N_5O_2 \cdot \bullet C_7H_6O_2$ / 461.51 g/mol (benzoate salt); 339.30 g/mol (free base)
Structure:



Alogliptin benzoate

Pioglitazone HCl

Code name: AD-4833 (HCl); U-72,107A
Chemical name: (±)-5[[4-[2-(5-ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-2,4-thiazolidinedione monohydrochloride
CAS registry number: 112529-15-4
Molecular formula/molecular weight: $C_{19}H_{20}N_2O_3S \bullet HCl$ / 392.90 g/mol (HCl salt); 356.43 (free base)
Structure:



Pioglitazone HCl

Relevant INDs/NDAs/DMFs: NDA 22-271 – Nesina® (alogliptin), INDs 69,707 (alo.) and 73,193 (alo. + pio.); NDA 21-073 – Actos® (pioglitazone HCl)

Drug class: Dipeptidyl peptidase IV inhibitor (DPP4 inhibitor) and thiazolidinedione (TZD)

Intended clinical population: Treatment of type 2 diabetes mellitus (T2DM)

Clinical formulation: Immediate release tablets (12.5 or 25 mg alogliptin + 15, 30, or 45 mg pioglitazone), distinguished by film color and printing ink color and markings, in the following combinations –
12.5 mg + 15 mg; 12.5 mg + 30 mg; 12.5 mg + 45 mg
25 mg + 15 mg; 25 mg + 30 mg; 25 mg + 45 mg

Inactive ingredients (see sponsor's Table 1, below) conform to USP/NF monographs, all have been previously included at higher concentrations as inactive ingredients in oral drugs, and printing ink is food-grade. No safety concerns from impurities in the drug substance or drug product were identified.

Sponsor's Inactive Ingredients Summary Table

Table 1 Inactive Components of SYR-322-4833 Tablets

Ph.Eur., 95/45/EC Designation	USP/NF Designation	Quality Standards
Mannitol	Mannitol	Ph.Eur., USP
Cellulose, microcrystalline	Microcrystalline cellulose	Ph.Eur., NF
Lactose monohydrate	Lactose monohydrate	Ph.Eur., NF
Hydroxypropylcellulose	Hydroxypropyl cellulose	Ph.Eur., NF
Croscarmellose sodium	Croscarmellose sodium	Ph.Eur., NF
Magnesium stearate	Magnesium stearate	Ph.Eur., NF
Hypromellose (b) (4)	Hypromellose (b) (4)	Ph.Eur., USP
Talc	Talc	Ph.Eur., USP
Titanium dioxide	Titanium dioxide	Ph.Eur., USP
Iron oxide yellow (b) (4)	Ferric oxide, yellow	95/45/EC, NF
Iron oxide red (b) (4)	Ferric oxide, red	95/45/EC, NF
(b) (4)		

Route of administration: Oral

Disclaimer/Key: Some tabular and graphical information are copied directly from sponsor's electronic NDA submission; any difference of opinion from the sponsor's data are noted in the text or as footnotes to figures and tables. Drug-related trends are discussed in relation to vehicle only controls and any single drug controls (e.g. pioglitazone or alogliptin alone) to determine any effects of co-treatment of alogliptin and pioglitazone. Sprague-Dawley rats used in alogliptin plus pioglitazone combination toxicity studies are the same strain as used in alogliptin toxicity studies.

Key: alo. (alogliptin), pio. (pioglitazone), LD (low dose), MD (mid dose), HD (high dose), mkd (mg/kg/day), ss (statistically significant), nss (not statistically significant), GD (gestation day), MRHD (maximum recommended human dose), NOAEL (no observed adverse effect level), LOAEL (lowest observed adverse effect level).

Studies reviewed within this submission: See Table of Contents, above. Reference is made throughout the text to studies reviewed in IND and NDA reviews for alogliptin and pioglitazone monotherapy.

Studies <u>not</u> reviewed within this submission: None.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Pharmacology of alogliptin (NDA 22-271) and pioglitazone (NDA 21-073) have been previously described in pharmacology/toxicology reviews. Pharmacodynamic drug interactions were assessed in NDA 22-271 based on co-treatment in various diabetic rodent models.

Descriptions of pharmacodynamic interactions from the alogliptin NDA (Carlson, 8/20/08) are reproduced here:

Effects of chronic administration of SYR-322 in combination with pioglitazone on diabetic indices in *db/db* mice (SYR-322/00078.001R)

> "Dietary treatment of diabetic *db/db* mice for 3-weeks with high dose SYR-322 (55-75 mg/kg) ± pioglitazone improved various diabetes-related effects compared to either drug alone… Combination treatment effectively preserved pancreatic islet morphology, with increased insulin expression in pancreatic islets, normal β-cell and α-cell distribution, and preserved pdx-1 expression compared to controls or either pioglitazone or SYR-322 treatment alone.
>
> In a separate study in *db/db* mice, twenty three days of SYR-322 treatment did not affect adiponectin levels. In contrast, similar treatment with either pioglitazone or a combination of pioglitazone plus SYR-322 increased plasma adiponectin levels greater than 2-fold. The sponsor hypothesized that increased plasma adiponectin induced by pioglitazone may contribute to the efficacy of pioglitazone plus alogliptin combination treatment on diabetic parameters in *db/db* mice."

Effects of combined treatment with SYR-322 and pioglitazone in *ob/ob* mice (Study SD1AD2006-KT-041; Takeda No. SYR-322/00126.002R)

> "A SYR-322 plus pioglitazone combination treatment of hyperinsulinemic, hyperglucagonemic *ob/ob* mice for 4-weeks improved various diabetes-related parameters compared to either drug alone. Improvements were modest overall, but effects of combination treatment were improved compared to each individual drug alone …"

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Standard toxicokinetic parameters have been assessed in several studies in rats co-treated with alogliptin and pioglitazone. Combination treatment effects have not been assessed in other animal models. Alogliptin parent, M-I, and M-II metabolite exposure are not affected by pioglitazone co-treatment in rats. In addition, alogliptin co-treatment has no apparent effect on pioglitazone or pioglitazone M-III or M-IV metabolite exposure (Cmax or AUC). High doses of alogliptin (e.g. 100 mg/kg) did slightly increase pioglitazone T_{max}, approximately 0.4 to 3 h, in some repeat dose combination toxicity studies in rats.

Alogliptin does seem to alter rat plasma exposure to pioglitazone M-II, a metabolite that is produced at higher levels in rats than humans. Pioglitazone M-II exposure (as C_{max} or AUC) consistently increased 3- to 6-fold with alogliptin co-treatment in rat combination toxicity studies. M-II exposure accounted for approximately 6-12% of pioglitazone exposure in combination studies compared to 2-3% in pioglitazone alone treatments. The finding seems to have little or no relevance to humans because M-II is not considered a relevant pioglitazone metabolite in humans. While M-II apparently has been identified at low levels in human plasma, the ACTOS® label lists only M-III and M-IV as notable human pioglitazone metabolites. In addition, pioglitazone M-II was not measured in an open-label clinical drug-drug interaction study with alogliptin plus pioglitazone co-treatment. Oxidative metabolism is responsible for formation of six pioglitazone metabolites (M-I through M-VI), with CYP2C9, CYP3A4 and CYP2C8 involved in M-II metabolism. The ACTOS® label notes the potential for drug-drug interactions with CYP2C8 inducers or inhibitors, which may affect M-II metabolism. Nevertheless, any potential increase in human pioglitazone M-II with alogliptin co-treatment is not expected to significantly affect human pioglitazone pharmacokinetics.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: Alogliptin co-treatment had minimal effects on pioglitazone-mediated toxicity in 3-month combination toxicity studies in rats. Rats are an acceptable model for combination treatment because both alogliptin and pioglitazone are active in rats, human metabolites are produced in rats, and rats are sensitive to the hallmark TZD-induced cardiac toxicity.

Alogliptin alone was well tolerated in animals up to doses on the order of tens- to hundreds-fold higher than expected human exposure. Major alogliptin target organs identified at high multiples of human exposure are testes, kidney, liver, urinary bladder, and lung. Kidney and bladder toxicity were the only toxicities that contributed to mortality in animals (mice, rats, rabbits) at high exposures, generally greater than 50-fold higher than expected human exposures. Dogs showed consistent but intermittent clinical signs of reddened/flushing ears and face, along with body and facial swelling, consistent with a pseudoallergy response.

Notable toxicity in animals treated with pioglitazone alone includes left atrial thrombosis, hydrothorax, cardiac hypertrophy, and elevation of hepatic enzymes. Increased heart weight and cardiac hypertrophy occur at exposure margins of 1, 2, and 11 in rat, dog, and mice, respectively, based on body surface area extrapolations.

Genetic toxicology: Alogliptin was considered negative for genotoxic potential based on a series of genetic toxicity assays that included adequate assessment of metabolites. Genotoxicity assessment of the proposed FDC was not required or considered necessary.

Carcinogenicity: Alogliptin carcinogenicity was assessed in two-year mouse and rat carcinogenicity assays. Alogliptin poses minimal carcinogenic risk to humans based on an absence of drug-related tumors in mice (60X MRHD), very high exposure multiples in male rats ($\geq$ 288X) at doses that increased thyroid C-cell tumors (adenomas, carcinomas), and high exposures multiples at the rat NOAEL (32X) (see Carlson, 8/20/08, NDA 22-271 pharmacology/toxicology review). Benign and/or malignant urinary bladder transitional cell neoplasms occurred at approximate human equivalent pioglitazone doses in male rats treated for two years. No pioglitazone-induced tumors were seen in a two-year mouse carcinogenicity study.

Carcinogenicity studies with the alogliptin plus pioglitazone combination were not conducted.

Reproductive toxicology: Embryofetal development was assessed in rats with the combination of alogliptin plus pioglitazone. Alogliptin co-administered with pioglitazone did not show any teratogenic signal in fetuses. Consistent with previous experience,

pioglitazone treatment did show some effect on embryofetal development, including increased placental weights (16-30%) and various visceral variations (left umbilical artery, renal pelvis dilatation, and convoluted/dilatation ureter). Co-treatment with 100 mg/kg alogliptin caused slightly decreased fetal weights (4-5%) and potentiated effects of pioglitazone alone on total visceral variations (number of fetuses and number of dams with fetal variations), most notably seen as increased supernumerary coronary ostium and renal pelvis dilatation.

Toxicology of the alogliptin/pioglitazone combination: Alogliptin plus pioglitazone combination treatment in rats was assessed in subchronic toxicity studies up to 13-weeks. Alogliptin co-treatment had minimal effects on pioglitazone exposure and pioglitazone-mediated toxicity in rats. Target organs were consistent with known pioglitazone effects and included **heart** (cardiomyopathy), **spleen** (extramedullary hematopoiesis), **bone marrow** (adipocyte hypertrophy), **brown** and **white fat** (adipocyte hypertrophy/hyperplasia), and **adrenals** (cortical cell hypertrophy). Alogliptin co-treatment increased pioglitazone M-II plasma maximum (C_{max}) and total (AUC) exposure approximately 2- to 3-fold and slightly increased pioglitazone T_{max} approximately 0.4 to 3 h.

2.6.6.2 Single-dose toxicity

Single oral gavage TK study of alogliptin + pioglitazone

Single oral gavage toxicokinetic study of SYR-322/AD-4833(HCl) in rats (Study No. 36-090/tk; Takeda No. SYR-322-4833/00026.001A)
Doses (mg/kg): 0/3.6, 0/14.5 (AD-4833 only)
30/0, 100/0 (SYR-322 only)
100/3.6, 30/14.5 (SYR-322/AD-4833)
NOAEL = None determined (no toxicity endpoints monitored)

Key study findings:

- **Pioglitazone M-II exposure (as C_{max} or AUC) increased 3- to 6-fold with alogliptin co-treatment, accounting for approximately 6-12% of pioglitazone exposure compared to 2-3% in pioglitazone alone treatment.**

This was a single dose non-GLP study to investigate toxicokinetics in Sprague-Dawley rats after individual and combination oral gavage doses of alogliptin (SYR-322) and pioglitazone (AD-4833 HCl). No toxicity endpoints were investigated. **Alogliptin parent and M-I and M-II metabolites were not affected by pioglitazone co-treatment.** Pioglitazone and metabolites M-III and M-IV were also unaffected by co-treatment. Pioglitazone M-II plasma maximum (C_{max}) and total (AUC) exposure increased 3- to 6-fold with alogliptin co-treatment. Percent of pioglitazone M-II exposure (as C_{max} or AUC) compared to parent (pioglitazone) increased from approximately 2-3% in pioglitazone treatment to 6-12% in alogliptin + pioglitazone combination treatment. Reviewer and Sponsor summary tables are shown below.

Single oral gavage toxicokinetic study of SYR-322/AD-4833(HCl) in rats †	
Species	Crl:CD(SD) rat (Study 36-090/tk; Takeda No. SYR-322-4833/00026.001A)
GLP Study?	No – *TK study*
No/Sex/Group	3 males/group
Route & Volume (Vehicle)	Gavage, 10 ml/kg (water for injection vehicle)
Dose (mg/kg)[a] – SYR-322/ AD-4833(HCl)	0/3.6, 0/14.5 (AD-4833 only) 30/0, 100/0 (SYR-322 only) 100/3.6, 30/14.5 (SYR-322/AD-4833)
Duration	Single dose
Follow-up	None
Toxicokinetics	3 males/group, day 1 @ 1, 2, 4, 8, 24 h postdose
Summary	**No TK interactions between SYR-322 and AD-4833 (pioglitazone) in rats predictive of significant human clinical interactions.**
† No toxicology endpoints or observations [a] SYR-322 = alogliptin; AD-4833 = pioglitazone	

Sponsor's Tabulated Summary

Single oral gavage toxicokinetic study of SYR-322/AD-4833(HCl) in rats

Animal	Crl:CD(SD) rats, male, 6 weeks of age at dosing					
Test article*[1]	SYR-322/AD-4833(HCl)*[2]					
Dosage level*[3] (mg/kg)	0/3.6	0/14.5	30/0	100/0	100/3.6	30/14.5
Dosage volume (mL/kg)	10	10	10	10	10	10
No. of animals	3	3	3	3	3	3
Plasma SYR-322Z concentrations (mean values, n=3)						
Tmax (h)			1.0	1.0	1.0	1.0
Cmax (μg/mL)			0.881	4.271	5.215	0.874
AUC0-24h (μg·h/mL)			3.925	22.102	23.194	3.983
Plasma SYR-322 M-I concentrations (mean values, n=3)						
Tmax (h)			2.0	1.7	1.0	2.0
Cmax (μg/mL)			0.330	0.878	0.744	0.299
AUC0-24h (μg·h/mL)			2.399	8.117	6.391	2.235
Plasma SYR-322 M-II concentrations (mean values, n=3)						
Tmax (h)			1.0	1.0	1.0	1.0
Cmax (μg/mL)			0.015	0.084	0.096	0.019
AUC0-24h (μg·h/mL)			0.074	0.509	0.475	0.103
Plasma AD-4833 concentrations (mean values, n=3)						
Tmax (h)	1.3	2.0			1.3	2.0
Cmax (μg/mL)	6.04	17.58			4.24	16.44
AUC0-24h (μg·h/mL)	52.8	177.0			49.0	178.8
Plasma AD-4833 M-II concentrations (mean values, n=3)						
Tmax (h)	8.0	6.7			8.0	8.0
Cmax (μg/mL)	0.12	0.18			0.39	1.05
AUC0-24h (μg·h/mL)	1.7	2.4			5.7	15.4
Plasma AD-4833 M-III concentrations (mean values, n=3)						
Tmax (h)	5.3	5.3			8.0	8.0
Cmax (μg/mL)	0.51	1.71			0.46	1.76
AUC0-24h (μg·h/mL)	7.4	25.0			6.2	24.9
Plasma AD-4833 M-IV concentrations (mean values, n=3)						
Tmax (h)	4.0	5.3			8.0	6.7
Cmax (μg/mL)	0.47	1.56			0.42	1.59
AUC0-24h (μg·h/mL)	5.6	21.8			5.7	22.3
Conclusion	No toxicokinetic interactions between SYR-322 and AD-4833(HCl)					

*1: Dissolved or suspended in distilled water for injection, *2: Dosed as AD-4833 (b) (4) *3: As free base (SYR-322Z/AD-4833)

2.6.6.3 Repeat-dose toxicity

Four-week rat alogliptin + pioglitazone

Preliminary, Non-GLP study (no statement of GLP compliance)

Preliminary four-week oral gavage toxicity study of SYR-322 administered to rats concurrently with AD-4833(HCl) (Study No. 06-209/su; Takeda No. SYR-322-4833/00027.001A)

Doses (mg/kg): 0/0 (vehicle control)
0/14.5 (AD-4833 only)
30/14.5, 100/14.5, 30/3.6 (SYR-322/AD-4833)
n.b. no SYR-322 only control
NOAEL = Not determined (range-finding study, MTD not exceeded)

Key study findings:

- No remarkable novel or additive toxicity identified in this exploratory alogliptin plus pioglitazone co-treatment study.

- Slightly increased salivation with high dose alogliptin and pioglitazone combination treatment, considered unremarkable in the absence of histological findings.

- Slightly increased heart weights (~10-20%) with no corresponding histological lesions. Slightly decreased spleen weights in pioglitazone treatment groups. Findings consistent with pioglitazone toxicity, with no additional effects of alogliptin co-treatment.

NOAEL Determination

No NOAEL was estimated for this exploratory range-finding study. Increased heart weights, even in the absence of histological lesions, are consistent with known cardiotoxicity of high dose and extended duration pioglitazone treatment in rats and clinical congestive heart failure risks.

This was a non-GLP, preliminary, non-pivotal 4-week combination toxicity study in rats. The FDA reviewer agrees with the Sponsor's conclusions unless otherwise noted. Toxicity was consistent with pioglitazone treatment and there were no notable effects of alogliptin co-treatment on any toxicity endpoints. Consistent with single dose combination toxicity study results, alogliptin parent, M-I, and M-II metabolite exposure were not affected by pioglitazone co-treatment but pioglitazone M-II plasma maximum (C_{max}) and total (AUC) exposure increased approximately 3-fold with alogliptin co-treatment. Study design (including materials and methods) and tabulated summary tables are reproduced below from the Sponsor's study report.

Sponsor's Tabulated Summary

Preliminary four-week oral gavage toxicity study of SYR-322 administered to rats concurrently with AD-4833(HCl)

Animal	Crl:CD(SD) rats, 6 weeks of age at start of dosing				
Test article	Cont.	SYR-322/AD-4833(HCl)*1			
Dosage level*2 (mg/kg/day)	0	0/14.5	30/14.5	100/14.5	30/3.6
Dosage vol. (mL/kg/day)	10	10	10	10	10
No. of animals (M:F)	4:4	4*3:4*3	4*3:4*3	4*3:4*3	4*3:4*3
Mortality (M:F)	0:0	0:0	0:0	0:0	0:0
Clinical signs (M:F)	–	–	–	Salivation (4:2)	–
Body weights	–	–	–	–	–
Food consumption	–	–	–	–	–
Hematology	–	–	–	–	–
Blood chemistry	–	–	–	–	–
Gross pathology	–	–	–	–	–
Organ weights	–	↓Ovary#	–	↓Ovary#	–
Histopathology (M:F)	–	Brown adipose tissue: Hypertrophy			
		(4:4)	(4:4)	(4:4)	(2:2)
		Bone marrow: Hypertrophy of adipocytes			
		(2:0)	(4:1)	(4:0)	(2:1)
		White adipose tissue Hyperplasia			
		(1:0)	(2:0)	(1:0)	(0:0)

Cont.: 0.5 w/v% methylcellulose solution, M: Male, F: Female, –: No abnormalities, ↓: Decrease, *1: Dosed as AD-4833 (b) (4) *2: As free base (SYR-322Z/AD-4833), *3: Additional rats (2/sex/group) used for toxicokinetics, #: Toxicologically significant findings

Animal		Crl:CD(SD) rats, 6 weeks of age at start of dosing				
Test article		Cont.	SYR-322/AD-4833(HCl)*1			
Dosage level*2 (mg/kg/day)		0	0/14.5	30/14.5	100/14.5	30/3.6
Dosage vol. (mL/kg/day)		10	10	10	10	10
No. of animals (M:F)		4:4	4*3:4*3	4*3:4*3	4*3:4*3	4*3:4*3
Mortality (M:F)		0:0	0:0	0:0	0:0	0:0
Plasma SYR-322Z concentrations (M:F, mean values, n=2)						
Tmax (h)	1st			1.5:1.5	1.0:1.0	1.0:1.0
	29th			1.0:1.0	1.0:1.5	1.5:1.0
Cmax (μg/mL)	1st			0.725:1.372	6.553:6.100	0.889:1.518
	29th			1.482:2.972	7.051:9.342	1.406:2.958
AUC0-24h (μg·h/mL)	1st			3.688:5.017	24.445:33.972	3.472:5.014
	29th			5.908:7.066	30.102:40.207	5.266:6.685
Plasma SYR-322 M-I concentrations (M:F, mean values, n=2)						
Tmax (h)	1st			2.0:1.5	1.0:1.5	1.0:1.0
	29th			3.0:1.0	1.5:2.0	2.0:1.0
Cmax (μg/mL)	1st			0.396:0.372	0.942:0.623	0.373:0.374
	29th			0.476:0.484	0.759:0.710	0.449:0.433
AUC0-24h (μg·h/mL)	1st			2.699:2.065	6.588:5.443	2.302:1.581
	29th			2.827:1.950	6.432:5.655	2.355:1.600
Plasma SYR-322 M-II concentrations (M:F, mean values, n=2)						
Tmax (h)	1st			1.5:1.5	1.0:1.5	1.0:1.0
	29th			1.0:1.0	1.0:1.5	1.0:1.0
Cmax (μg/mL)	1st			0.015:0.032	0.111:0.140	0.018:0.041
	29th			0.032:0.068	0.116:0.214	0.032:0.071
AUC0-24h (μg·h/mL)	1st			0.088:0.139	0.466:0.887	0.081:0.132
	29th			0.121:0.210	0.530:1.137	0.117:0.218

Cont.: 0.5 w/v% methylcellulose solution, M: Male, F: Female, *1: Dosed as AD-4833 (b) (4) *2: As free base (SYR-322Z/AD-4833), *3 Additional rats (2/sex/group) used for toxicokinetics

Animal		Crl:CD(SD) rats, 6 weeks of age at start of dosing			
Test article		Cont.	SYR-322/AD-4833(HCl)*1		
Dosage level*2 (mg/kg/day)		0	0/14.5	30/14.5	100/14.5	30/3.6
Dosage vol. (mL/kg/day)		10	10	10	10	10
No. of animals (M:F)		4:4	4*3:4*3	4*3:4*3	4*3:4*3	4*3:4*3
Mortality (M:F)		0:0	0:0	0:0	0:0	0:0
Plasma AD-4833 concentrations (M:F, mean values, n=2)						
Tmax (h)	1st		1.0:1.5	2.0:2.0	3.0:3.0	1.5:1.0
	29th		1.5:1.0	1.5:1.0	4.5:1.5	1.5:1.0
Cmax (μg/mL)	1st		15.32:23.38	13.54:13.11	12.89:11.62	3.69:6.17
	29th		17.81:16.22	9.84:10.71	13.09:10.71	3.69:6.00
AUC0-24h (μg·h/mL)	1st		134.9:125.5	140.3:161.3	152.6:135.2	28.4:31.0
	29th		139.6:69.0	95.5:80.7	161.2:114.4	32.2:30.8
Plasma AD-4833 M-II concentrations (M:F, mean values, n=2)						
Tmax (h)	1st		8.0:3.0	8.0:8.0	8.0:6.0	6.0:2.0*4
	29th		6.0:2.0	8.0:4.0	8.0:8.0	8.0*4:2.0*4
Cmax (μg/mL)	1st		0.47:1.71	0.52:0.58	1.52:0.68	0.12:0.04
	29th		0.56:0.92	0.40:0.33	1.55:0.48	0.16:0.05
AUC0-24h (μg·h/mL)	1st		6.6:18.6	7.8:7.7	20.5:8.5	1.5:0.5
	29th		9.0:10.0	6.6:4.1	25.5:6.7	2.8:0.6
Plasma AD-4833 M-III concentrations (M:F, mean values, n=2)						
Tmax (h)	1st		4.0:4.0	6.0:6.0	8.0:6.0	3.0:3.0
	29th		4.0:3.0	6.0:4.0	6.0:6.0	5.0:3.0
Cmax (μg/mL)	1st		1.50:2.44	1.38:1.91	1.73:2.05	0.39:0.71
	29th		1.29:1.67	0.95:1.27	1.11:1.50	0.31:0.58
AUC0-24h (μg·h/mL)	1st		20.6:28.7	20.2:26.3	22.7:27.3	5.1:8.8
	29th		17.0:18.3	14.1:15.6	15.9:22.9	4.4:6.9
Plasma AD-4833 M-IV concentrations (M:F, mean values, n=2)						
Tmax (h)	1st		4.0:3.0	4.0:8.0	8.0:6.0	6.0:4.0
	29th		4.0:3.0	6.0:4.0	6.0:8.0	5.0:3.0
Cmax (μg/mL)	1st		1.64:2.76	1.08:1.74	1.67:2.35	0.34:0.56
	29th		1.19:1.32	0.63:0.97	0.98:1.64	0.23:0.41
AUC0-24h (μg·h/mL)	1st		22.1:31.8	15.3:24.5	21.7:29.9	4.4:6.9
	29th		14.7:13.8	9.0:12.5	13.5:23.3	2.9:5.1
Conclusion			The combination of SYR-322 and AD-4833(HCl) did not alter the nature of any findings or show any evidence of exacerbation of findings known to be induced by AD-4833(HCl).			

Cont.: 0.5 w/v% methylcellulose solution, M: Male, F: Female, *1: Dosed as AD-4833 (b) (4) *2: As free base (SYR-322Z/AD-4833), *3: Additional rats (2/sex/group) used for toxicokinetics, *4: N=1

Sponsor's Materials and Methods Summary

Animals	Crl:CD(SD) rats, 4 males and 4 females per group (total 56 animals including additional rats, 2/sex/group, used for toxicokinetics), 6 weeks of age at start of dosing, males: 199-236 g, females: 141-174 g
Test article	SYR-322 (Lot No. MA01-001) AD-4833(HCl), dosed as AD-4833 (b) (4) (Lot No. Z509Z01) Dissolved or suspended in distilled water for injection
Dosage levels (SYR-322Z/ AD-4833)	0/0, 0/14.5, 30/14.5, 100/14.5 and 30/3.6 mg/kg as free base *Concentrations of SYR-322Z or AD-4833 in dosing preparations*: The actual values for the concentrations of SYR-322Z or AD-4833 were 100% to 106% of the target values in the dosing preparations (Appendices 8 and 9).
Dosage volume	10 mL/kg/day
Dosing method	Oral by gavage
Dosing days	4 weeks (July 6 to August 3, 2006)
Observation and Examinations	*Clinical signs*: 2 or 3 times daily *Body weight*: 2 or 3 times a week *Food consumption*: Once a week *Hematology*: Erythrocyte counts, hematocrit value, hemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet counts, leukocyte counts, reticulocyte counts, differential leukocyte counts *Blood chemistry*: Total protein, albumin, A/G ratio, glucose, total cholesterol, triglyceride, urea nitrogen, creatinine, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatine kinase (CK), sodium, potassium, chloride, calcium, inorganic phosphorus *Gross pathology* *Organ weights*: Body weight (to calculate body weight ratio), brain, heart, lungs, liver, kidneys, spleen, thymus, adrenal glands, testes, ovaries, ventral prostate, pituitary gland *Histopathology*: Liver, kidney, heart, spleen, lung, tongue, stomach, duodenum, jejunum, ileum, cecum, colon, mesenteric lymph node, thymus, pancreas, submandibular gland, sublingual gland, submandibular lymph node, eye, Harderian gland, brain, spinal cord, thyroid gland, parathyroid gland, trachea, esophagus, aorta, pituitary gland, adrenal gland, sciatic nerve, skeletal muscle, femur, bone marrow, sternum, skin, mammary gland, urinary bladder, prostate, seminal vesicle, epididymis, testis, vagina, ovary, uterus, brown adipose tissue (between scapulas), white adipose tissue (groin). All organs indicated above in the control and 100/14.5 mg/kg groups, and the liver, heart, spleen, kidney, ovary, brown adipose

	tissue (between scapulas), white adipose tissue (groin) and bone marrow in another groups were examined. *Toxicokinetics*: Plasma concentrations for SYR-322Z and its metabolites, M-I and M-II, and/or AD-4833 and its metabolites, M-II, M-III and M-IV, levels on the 1st 28th days of dosing were determined using LC/MS/MS before dosing (except for the 1st day of dosing) and at 1, 2, 4, 8 and 24 hours after dosing. *Statistics*: 1. The effects of SYR-322 in the rats treated with AD-4833(HCl): 1.1 The interval scale data were tested by the F test for homogeneity of variance between the control and 0/14.5 mg/kg groups. When the variances were homogeneous, Student's t test was used, and when the variances were heterogeneous, the Aspin & Welch t test was performed to compare the mean in the control group with that in the dosage group. The F test was conducted at the significance level of 0.20, and the other tests were conducted at the two-tailed significance levels of 0.05 and 0.01. 1.2 The data in the 0/14.5 (as control), 30/14.5 and 100/14.5 mg/kg groups were tested by Bartlett's test for homogeneity of variance. When the variances were homogeneous, Williams' test assuming a dose-related trend was performed. If there were no significant differences with Williams' test, Dunnett's test was performed to compare the mean in the control group with that in each dosage group. When the variances were heterogeneous, the Shirley-Williams test assuming a dose-related trend, was performed. If there were no significant differences with the Shirley-Williams test, the Steel test was performed to compare the mean rank in the control group with that in each dosage group. Bartlett's test was conducted at the significance level of 0.05, the Williams and Shirley-Williams tests were conducted at the two-tailed significance level of 0.05, and the other tests were conducted at the two-tailed significance levels of 0.05 and 0.01. 2. The effects of the treatment at 30/3.6 mg/kg: 2.1 The statistical analysis was conducted according to the method described above (see 1.1) between the control and 30/3.6 mg/kg groups.

Thirteen-week rat alogliptin + pioglitazone

Signed GLP and QA statements

Thirteen-week oral gavage toxicity study of SYR-322 administered to rats concurrently with AD-4833(HCl) (Study No. B060832; Takeda No. SYR-322-4833/00041.001A)

Doses (mg/kg): *0/0 (vehicle control)*
0/14.5 (AD-4833 only)
30/14.5, 100/14.5, 30/3.6 (SYR-322/AD-4833)
n.b. no SYR-322 only control

NOAEL = 100 mg/kg (33X MRHD) alogliptin (on pioglitazone co-treatment)
30 mg/kg alogliptin (6X) + 3.6 mg/kg pioglitazone (7X) co-treatment

Key study findings:

- Alogliptin co-treatment had minimal effects on pioglitazone exposure and pioglitazone-mediated toxicity in rats.

- Pioglitazone doses were low and pioglitazone-mediated toxicity was modest up to the maximum 14.5 mg/kg/day pioglitazone treatment. Target organs were consistent with known pioglitazone effects and included **heart** (cardiomyopathy), **spleen** (extramedullary hematopoiesis), **bone marrow** (adipocyte hypertrophy), **brown** and **white fat** (adipocyte hypertrophy/hyperplasia), and **adrenals** (cortical cell hypertrophy).

- Alogliptin co-treatment increased pioglitazone M-II plasma maximum (C_{max}) and total (AUC) exposure approximately 2- to 3-fold and slightly increased pioglitazone T_{max} approximately 0.4 to 3 h.

NOAEL Determination

Estimated NOAELs were 100 mg/kg/day alogliptin and 30 mg/kg/day alogliptin plus 3.6 mg/kg/day pioglitazone co-treatment. While treatment-related findings were largely unremarkable, due to absence of interactions between alogliptin and low pioglitazone doses, findings in the 14.5 mg/kg pioglitazone groups were consistent with known toxicity. Slight increases in heart weight and minimal cardiomyopathy in all 14.5 mg/kg/day pioglitazone treatments were used to estimate the LOAEL for the high dose pioglitazone treatments.

Study no.: B060832
Volume #, and page #: eCTD
Conducting laboratory and location: (b) (4)

Date of study initiation: 8/25/06
GLP compliance: Yes
QA report: yes (X) no ( )

Drug, lot #, and % purity: SYR-322 (alogliptin), Lot No. MA01-001, 99.9% purity / AD-4833 (HCl) (pioglitazone; AD-4833 [redacted]), Lot No. Z509Z01, 98.2% purity

13-Week Repeat Dose Combination Toxicity in Rats — Summary

SPECIES **DOSES AND ADMINISTRATION** **# ANIMALS** **FOLLOW-UP**	**NOAEL = 100 MG/KG/DAY (ALOGLIPTIN);** **33X MRHD (ALOGLIPTIN)**
SD rat (Crl:CD) 13-week, Oral gavage (10 ml/kg, water for injection vehicle) *0/0 (vehicle control)* *0/14.5 (pio. only)* *30/14.5, 100/14.5, 30/3.6 (alo./pio.)* *n.b. no SYR-322 only control* Main: 10/sex/group TK: 4/sex/group (1, 2, 4, 8, 24 h post-dose)	**$AUC_{0\text{-}24h}$ (µg*h/ml) †** Alo/Pio Dose (mg/kg) / SYR-322 (Alogliptin) Day 1 / SYR-322 (Alogliptin) Day 91 / AD-4833 (Pioglitazone) Day 1 / AD-4833 (Pioglitazone) Day 91 0/14.5 -- -- 140 165 30/14.5 5 9 115 143 100/14.5 33 50 147 169 30/3.6 5 9 45 69 † Exposure data from both sexes combined; MRHD estimates based on estimated clinical exposures of 1.5 µg*h/ml alogliptin and 10 µg*h/ml pioglitazone
Mortality: None.	
Clinical Signs: Unremarkable.	
Body Weight: Trend of increased overall body weight gain in all male pioglitazone groups, ranging from 8-13%. Weight gain was greatest in the low dose pioglitazone group, thus the trend was independent of dose. Magnitude of the response was modest and slight weight gain is consistent with pioglitazone pharmacologic effect and body weight increases seen in some toxicity studies. Body weight trends were unaffected in females. ***Food consumption*** – male body weight trends were concomitant with slight, consistently increased food consumption throughout the study (not statistically significant for weekly consumption and no statistical analysis for consumption over the course of the study). ***Water consumption*** – no consistent dose-related trends.	
Hematology: Various statistically significant findings were considered unremarkable based on small magnitude changes (e.g. <10% difference from control), absence of dose-related alogliptin effect compared to either control or pioglitazone control, and/or absolute value compared to the range of historical control data provided by the conducting laboratory. Slight, statistically significant decreases in RBC, hematocrit, and platelet counts in pioglitazone groups were consistent with hemodilution and modest heart weight increases.	

Clinical Chemistry: Triglycerides were decreased approximately 32-44% in all male 14.5 mg/kg/day pioglitazone groups consistent with its pharmacodynamic effect (effect less pronounced in 3.6 mg/kg/day pio. and all female pio. groups). LDH levels were slightly lower in pioglitazone groups compared to untreated controls (maximum ↓ 20%), however, trends were different in males (decreased LDH in alogliptin groups compared to pio. control) and females (similar decreased LDH in all pio. groups, including pio. control). The contribution of alogliptin was considered minimal and not likely biologically significant compared to pioglitazone effects.
Organ Weights: Heart weights (relative and absolute) were increased approximately 10-20% in male and 7-9% in female pioglitazone groups, with no apparent effect of alogliptin co-treatment. Heart weight effects are consistent with known pioglitazone effects.
Gross Pathology: Treatment-related findings limited to increases in brown and white adipose tissue, which were consistent with pioglitazone effects. There was no apparent effect of alogliptin in males but a possible contribution to increased incidence in females (based on absence of increased adipose tissue in female pioglitazone control).
Histopathology: Histopathology findings were largely unremarkable, generally limited to minimal severity, and consistent with known pioglitazone toxicity. Cardiomyopathy and adipocyte hypertrophy in bone marrow and brown and white fat were the most notable findings. There was no evidence that alogliptin co-treatment contributed to the incidence or severity of pioglitazone-mediated effects. Minimal cardiomyopathy findings were consistent with the low doses of pioglitazone and subchronic treatment duration.
Toxicokinetics: Alogliptin parent, M-I, and M-II metabolite exposure were not affected by pioglitazone co-treatment but pioglitazone M-II plasma maximum (C_{max}) and total (AUC) exposure increased approximately 2- to 3-fold with alogliptin co-treatment. High dose alogliptin also slightly increased pioglitazone T_{max} (approximately 0.4 to 3 h). Alogliptin co-treatment had no apparent effect on pioglitazone or pio. M-III or M-IV metabolite exposure (C_{max} or AUC). TK trends were consistent with single dose and four-week rat combination toxicity study results.
Summary: Treatment-related findings were largely unremarkable. Low doses of pioglitazone were chosen to allow detection of any potential additive or synergistic effects of alogliptin combination treatment. Toxicity trends were consistent with those expected from pioglitazone treatment and there was no apparent effect of alogliptin co-treatment on pioglitazone-mediated toxicity. Slight increases in heart weight and minimal cardiomyopathy, concomitant with evidence of slight hemodilution, were the only toxicity suggestive of any adverse treatment effect in the study. All other findings were likely due to exaggerated pharmacological effects of pioglitazone. Estimated NOAELs were 100 mg/kg/day alogliptin and 30 mg/kg/day alogliptin plus 3.6 mg/kg/day pioglitazone co-treatment.

Methods

Doses: 0/0, 0/14.5, 30/14.5, 100/14.5, 30/3.6 mg/kg/day SYR-322/AD-4833(HCl)
Species/strain: Sprague-Dawley / Crl:CD(SD)
Number/sex/group or time point (main study): 10
Route, formulation, volume, and infusion rate: Oral gavage, 10 ml/kg, water for injection vehicle.
Satellite groups used for toxicokinetics or recovery: 4
Age: 6 weeks
Weight: Male – 190 to 224 g / Female – 154 to 189 g

Sampling times: Scheduled necropsy day 91; TK for SYR-322Z + M-I, M-II and AD-4833 + M-II, M-III, M-IV on day 1 and day 91 at 1, 2, 4, 8, 24 h post-dose from satellite TK animals (~ 0.3 ml, sodium heparin anticoagulant)
Unique study design or methodology: No alogliptin only control.

Observations and times:
Mortality: Minimum twice daily
Clinical signs: Twice daily (pre- and 0.5 to 2 h post-dose)
Body weights: Twice weekly through week 4, then weekly.
Food consumption: Weekly
Ophthalmoscopy: Pre-study and day 90 (including peer-review day 90)
EKG: None.
Hematology: Scheduled necropsy day 92/93 – blood (4-5 ml) from posterior vena cava, ~ 1ml EDTA-2K anticoagulant **– RBC, Hb, Hct, MCV, MCH, MCHC, reticulocyte ratio, platelet, WBC, differential leukocyte ratio**
Clinical chemistry: Scheduled necropsy day 92/93 – blood (4-5 ml) from posterior vena cava, ~ 2-3 ml no anticoagulant (~ 1ml, lithium heparin anticoagulant for **LDH, CK**) **– AST, ALT, ALP, total bilirubin, urea nitrogen, creatinine, glucose, total cholesterol, triglyceride, total protein, Ca, P, Na, K, Cl, globulin, albumin, A/G**
Urinalysis: Week 13 (day 85/86, 6/sex/group) in metabolism cages (4 h, pre-dose, fasting; then 20 h post-dose with *ad lib* food and water for urine volume) ***– pH, protein, glucose, ketones, occult blood, urobilinogen, sediments, urine volume***
Gross pathology: Complete exam at necropsy.
Organ weights: See Sponsor table, below.
Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )
note – control and HD examined plus all groups' ***heart, spleen, ovary, femoral*** *and* ***sternal bone marrow, brown fat*** *(back neck area)****, white fat*** *(inguinal area)****, adrenals.***

Sponsor's Necropsy Summary Table

Organs/tissues		Collection	(laterality)	Organ weight	(laterality)	Histopathology	(laterality)
(1) Heart		+	–	+	–	+	–
(2) Lymph node	Submandibular	+	Bilateral	–	–	+	Unilateral
	Mesenteric	+	–	–	–	+	–
(3) Thymus		+	–	+	–	+	–
(4) Spleen		+	–	+	–	+	–
(5) Trachea		+	–	–	–	+	–
(6) Lungs / bronchus		+	–	+	–	+	–
(7) Tongue		+	–	–	–	+	–

(8) Esophagus		+	–	–	–	+	–
(9) Stomach		+	–	–	–	+	–
(10) Duodenum		+	–	–	–	+	–
(11) Jejunum		+	–	–	–	+	–
(12) Ileum	with Peyer's patch	+	–	–	–	+	–
(13) Cecum		+	–	–	–	+	–
(14) Colon		+	–	–	–	+	–
(15) Salivary glands	Parotid	+	Bilateral	–	–	+	Unilateral
	Submandibular	+	Bilateral	–	–	+	Unilateral
	Sublingual	+	Bilateral	–	–	+	Unilateral
(16) Liver		+	–	+	–	+	–
(17) Pancreas		+	–	–	–	+	–
(18) Kidneys		+	Bilateral	+	Total	+	Bilateral
(19) Urinary bladder		+	–	–	–	+	–
(20) Testis		+	Bilateral	+	Total	+	Bilateral
(21) Epididymis		+	Bilateral	–	–	+	Bilateral
(22) Seminal vesicle		+	–	–	–	+	–
(23) Prostate	Ventral lobe	+	–	+	–	+	–
(24) Pituitary		+	–	+	–	+	–
(25) Thyroids / Parathyroids		+	Bilateral	–	–	+	Bilateral*
(26) Adrenals		+	Bilateral	+	Total	+	Bilateral
(27) Femur / Bone marrow		+	Bilateral	–	–	+	Unilateral
(28) Sternum / Bone marrow		+	–	–	–	+	–
(29) Skin / Mammary gland		+	Bilateral	–	–	+	Unilateral
(30) Eyeballs / Harderian gland		+	Bilateral	–	–	+	Bilateral
(31) Optic nerve		+	Bilateral	–	–	+	Bilateral
(32) Brain		+	–	+	–	+	–
(33) Spinal cord	Cervical	+	–	–	–	+	–
	Thoracic	+	–	–	–	+	–
	Lumbar	+	–	–	–	+	–
(34) Ovaries		+	Bilateral	+	Total	+	Bilateral
(35) Oviducts		+	Bilateral	–	–	–	–
(36) Uterus		+	–	–	–	+	–
(37) Vagina		+	–	–	–	+	–
(38) Aorta	Thoracic	+	–	–	–	+	–
(39) Skeletal muscle / Sciatic nerve	Femur	+	Bilateral	–	–	+	Unilateral
(40) Nasal cavity		+	–	–	–	–	–
(41) Zymbal's gland		+	Bilateral	–	–	–	–
(42) Adipose tissue (brown fat)	Dorsal neck region	+	–	–	–	+	–
(43) Adipose tissue (white fat)	Inguinal	+	Bilateral	–	–	+	Bilateral
(44) Others showing gross abnormality		+	–	–	–	+	–

+: Organs/tissues subjected to collection or examination
*: At least, either right or left portion of the parathyroid was examined.

Results (additional data to supplement summary above):

Clinical signs: No dose-related findings. Sporadic findings, each in a single animal and some in controls, included neck erosion/crust formation, mass (right oral region), and teeth loss.

Body weights: Trend of increased overall body weight gain in all male pioglitazone groups, however, body weight gain was greater in the low dose pio. group compared to high dose pio. groups. The Sponsor did not perform a statistical analysis on the overall body weight gain, although weight for weekly intervals were sporadically increased in both male and female groups. Magnitude of body weight gain was modest, ranging from 8-13%. There was no effect in females. Male body weight trends are shown in the reviewer's table, below.

Male Body Weight Gain Summary			
Alogliptin/Pioglitazone Dose (mg/kg/day)	Body weight (g)		Weight Gain (g)
	Day 1	Day 91	
0	207.6 +7.8	494.3 ± 40.5	286.7
0/14.5	208.1 ± 9.7	518.2 ± 39.1	310.1 (+8%)
30/14.5	208.1 ± 8.7	524.2 ± 33.1	316.1 (+10%)
100/14.5	205.6 ± 8.8	522.9 ±39.3	317.3 (+11%)
30/3.6	206.6 ±9.1	531.3 ± 85.4	324.7 (+13%)

Ophthalmoscopy: No treatment related findings.

Urinalysis: No treatment related findings.

Hematology: Pioglitazone alone did not reduce RBC parameters or cause significant hemodilution which is otherwise commonly seen with PPARγ agonists, perhaps reflecting the relatively low dose and short treatment duration in this study. There was evidence of slight hemodilution with the pioglitazone plus alogliptin combination, suggesting alogliptin exacerbated pioglitazone-mediated fluid retention. Heart weight, on the other hand, generally increased similarly in response to pioglitazone with or without coadministration of alogliptin. This suggests that the relatively minor differences in hemodilution across the dose groups were not sufficient to affect the increased heart weight induced by pioglitazone. Considering the hematology and heart weight findings in males and females, this reviewer concluded alogliptin did not clearly potentiate known pioglitazone-mediated fluid retention and cardiomegaly effects. Notable findings are summarized below.

Hematology Summary – Male				
Alogliptin/Pioglitazone Dose (mg/kg/day)	**RBC (10^6/µl)**	**Hematocrit (%)**	**Platelets (10^3/µl)**	**WBC (10^3/µl)**
0	9.04	45.1	1253	9.15
0/14.5	9.10	45.8	1066*	8.81
30/14.5	8.61#	44.4#	963	7.61
100/14.5	8.59#	44.5#	917#	6.95
30/3.6	8.97	45.7	1048*	7.91

* $p < 0.05$ vs. vehicle control
# $p < 0.05$ vs. 14.5 mg/kg pioglitazone control

Hematology Summary – Female				
Alogliptin/Pioglitazone Dose (mg/kg/day)	**RBC (10^6/µl)**	**Hematocrit (%)**	**Platelets (10^3/µl)**	**WBC (10^3/µl)**
0	8.33	44.9	1114	5.65
0/14.5	8.25	43.8	999*	5.49
30/14.5	8.20	43.2	992	4.60
100/14.5	8.15	42.6	954	4.44
30/3.6	8.46	44.3	1121	5.05

* $p < 0.05$ vs. vehicle control
# $p < 0.05$ vs. 14.5 mg/kg pioglitazone control

Clinical chemistry: Triglycerides were decreased approximately 32-44% in all male 14.5 mg/kg/day pioglitazone groups consistent with its pharmacodynamic effect (effect less pronounced in 3.6 mg/kg/day pio. and all female pio. groups). LDH levels were slightly lower in pioglitazone groups compared to untreated controls (maximum ↓ 20%), however, trends were different in males (decreased LDH in alogliptin groups compared to pio. control) and females (similar decreased LDH in all pio. groups, including pio. control). Serum potassium was decreased 6% in the high dose alogliptin plus pio. group, which could exacerbate heart-related effects of pioglitazone. No other female groups or any male groups had any biologically significant K changes, suggesting the female finding was sporadic (or at least isolated).

Urinalysis: No treatment related findings.

Organ weights: Heart weights (relative and absolute) were increased approximately 10-20% in male and 7-9% in female pioglitazone groups, with no apparent effect of alogliptin co-treatment. Heart weight effects are consistent with known pioglitazone effects. Male thymus weights (relative and absolute) were slightly decreased with alogliptin treatment compared to pio. alone (↓ 18-27%, ss only in high dose alogliptin group). Absence of findings in females, plus high variability in male groups and in historical controls, suggest thymus weight changes were incidental and not biologically significant. Male spleen weights (relative and absolute) were decreased slightly (~15%)

in all pioglitazone groups but there was no effect in females and no effect of alogliptin co-treatment.

Heart weight findings are summarized below.

Organ Weight – Male Heart Weight			
Alogliptin/Pioglitazone Dose (mg/kg/day)	**Body weight (day 92)**	**Heart weight (g)**	**Relative heart weight (% body weight)**
0	527.4	1.644	0.312
0/14.5	549.0	1.943*	0.352*
30/14.5	571.3	1.945	0.343
100/14.5	570.2	2.120	0.375
30/3.6	587.5	1.834	0.316

* $p < 0.05$ vs. vehicle control
# $p < 0.05$ vs. 14.5 mg/kg pioglitazone control

Organ Weight – Female Heart Weight			
Alogliptin/Pioglitazone Dose (mg/kg/day)	**Body weight (day 92)**	**Heart weight (g)**	**Relative heart weight (% body weight)**
0	307.9	1.032	0.337
0/14.5	303.3	1.104	0.366*
30/14.5	302.5	1.089	0.361
100/14.5	305.2	1.087	0.359
30/3.6	306.9	1.038	0.340

* $p < 0.05$ vs. vehicle control
# $p < 0.05$ vs. 14.5 mg/kg pioglitazone control

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

Histopathology findings were largely unremarkable and generally corresponded with gross pathology and organ weight findings. Findings were limited to minimal severity with the exception of mild adipocyte hypertrophy in male bone marrow and brown fat. Alogliptin treatment did not appear to have any effect on lesions consistent with pioglitazone treatment. Target organs were **heart** (cardiomyopathy), **spleen** (extramedullary hematopoiesis), **bone marrow** (adipocyte hypertrophy), b**rown and white fat** (adipocyte hypertrophy/hyperplasia), and **adrenals** (cortical cell hypertrophy). Cardiomyopathy consistent with pioglitazone treatment was limited to minimal severity and occurred in 3/10 ♂ and 1/10 ♀ control animals, with notable increased incidence above background only in males. Minimal cardiomyopathy findings were consistent with the low doses of pioglitazone and subchronic treatment duration. Selected Sponsor summary tables are shown below.

Sponsor's Histology Summary (Selected) – Males

Table 13 Histological findings - Summary Week 14

Organ / Findings	Grade	Male Control 0 mg/kg 10	SYR-322/AD-4833 (HCl) 0/14.5 mg/kg 10	SYR-322/AD-4833 (HCl) 30/14.5 mg/kg 10	SYR-322/AD-4833 (HCl) 100/14.5 mg/kg 10	SYR-322/AD-4833 (HCl) 30/3.6 mg/kg 10
Heart		<10>	<10>	<10>	<10>	<10>
Cardiomyopathy	1	3	8	5	9	6
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
Spleen		<10>	<10>	<10>	<10>	<10>
Extramedullary hematopoiesis, erythrocytic	1	0	0	1	2	1
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
Bone marrow, femur		<10>	<10>	<10>	<10>	<10>
Hypertrophy, adipocyte	1	0	7	7	7	7
	2	0	3	2	3	2
	3	0	0	0	0	0
	4	0	0	0	0	0
Bone marrow, sternum		<10>	<10>	<10>	<10>	<10>
Hypertrophy, adipocyte	1	0	10	9	10	2
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
Adrenal		<10>	<10>	<10>	<10>	<10>
Hypertrophy, cortical cell, glomerular zone, diffuse	1	0	2	0	3	1
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
Brown fat		<10>	<10>	<10>	<10>	<10>
Hypertrophy, adipocyte	1	0	6	6	4	7
	2	0	4	4	6	3
	3	0	0	0	0	0
	4	0	0	0	0	0
White fat		<10>	<10>	<10>	<10>	<10>
Hyperplasia, adipocyte	1	0	4	3	4	1
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0

<> , Number of animals examined
1 , Minimal; 2 , Mild; 3 , Moderate; 4 , Severe

Sponsor's Histology Summary (Selected) – Females

Table 13 Histological findings - Summary Week 14

Organ / Findings	Grade	Female Control 0 mg/kg 10	SYR-322/AD-4833 (HCl) 0/14.5 mg/kg 10	SYR-322/AD-4833 (HCl) 30/14.5 mg/kg 10	SYR-322/AD-4833 (HCl) 100/14.5 mg/kg 10	SYR-322/AD-4833 (HCl) 30/3.6 mg/kg 10
Heart		<10>	<10>	<10>	<10>	<10>
Cardiomyopathy	1	1	1	1	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
Spleen		<10>	<10>	<10>	<10>	<10>
Extramedullary hematopoiesis, erythrocytic	1	0	0	0	2	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
Bone marrow, sternum		<10>	<10>	<10>	<10>	<10>
Hypertrophy, adipocyte	1	0	7	9	9	2
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0

Adrenal		<10>	<10>	<10>	<10>	<10>
Hypertrophy, cortical cell, glomerular zone, diffuse	1	0	3	2	4	2
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
Brown fat		<10>	<10>	<10>	<10>	<10>
Cell infiltration, lymphocyte, focal	1	0	0	1	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
Hypertrophy, adipocyte	1	0	10	10	10	4
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
White fat		<10>	<10>	<10>	<10>	<10>
Hyperplasia, adipocyte	1	0	4	2	3	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0

<> , Number of animals examined
1 , Minimal; 2 , Mild; 3 , Moderate; 4 , Severe

Toxicokinetics: Sponsor TK summary tables are shown below.

Sponsor's TK summary tables

13-Week Toxicity Study with SYR-322 and AD-4833(HCl), Day 91 AUC(0-24) (Mean Values)

Dose SYR-322/ AD-4833(HCl) (mg/kg)	Sex	AUC(0-24) µg·hr/mL						
		SYR-322			AD-4833(HCl)			
		SYR-322	M-I	M-II	AD-4833 (HCl)	M-II	M-III	M-IV
0/14.5	M	-	-	-	170	17.6	21.4	16.2
	F	-	-	-	159	3.8	33.9	29.2
30/14.5	M	8.17	3.51	0.222	163	21.9	18.1	12.2
	F	9.42	2.52	0.332	124	8.2	25.9	21.6
100/14.5	M	51.1	9.90	1.03	166	57.9	19.8	14.9
	F	49.1	7.35	1.74	172	5.5	30.8	25.5
30/3.6	M	8.95	3.62	0.219	85.4	4.0	11.5	5.9
	F	8.15	2.41	0.269	53.3	0.8	11.3	7.0

-=not measured.

Table 1 Toxicokinetic parameters for SYR-322Z, M-I and M-II in rats

Dose (mg/kg/day)	Analyte	No. of dosing	Male (N=3)			Female (N=3)			Total (N=6)		
			Tmax (h)	Cmax (ng/mL)	AUC_{0-24h} (ng·h/mL)	Tmax (h)	Cmax (ng/mL)	AUC_{0-24h} (ng·h/mL)	Tmax (h)	Cmax (ng/mL)	AUC_{0-24h} (ng·h/mL)
30/14.5 *	SYR-322Z	1st	1.0 (0.0)	860 (146)	4088 (505)	1.3 (0.6)	1439 (283)	6526 (728)	1.2 (0.4)	1150 (375)	5307 (1448)
		91st	1.3 (0.6)	1828 (377)	8166 (791)	1.0 (0.0)	2849 (771)	9415 (2301)	1.2 (0.4)	2339 (780)	8790 (1684)
	SYR-322 M-I	1st	1.3 (0.6)	363 (45)	2397 (135)	1.7 (0.6)	321 (77)	2066 (728)	1.5 (0.5)	342 (61)	2231 (502)
		91st	2.0 (0.0)	510 (79)	3509 (167)	1.3 (0.6)	500 (104)	2519 (169)	1.7 (0.5)	505 (83)	3014 (563)
	SYR-322 M-II	1st	1.0 (0.0)	17 (5)	106 (12)	1.3 (0.6)	51 (22)	210 (58)	1.2 (0.4)	34 (23)	158 (68)
		91st	1.3 (0.6)	48 (14)	222 (60)	1.3 (0.6)	88 (28)	332 (90)	1.3 (0.5)	68 (29)	277 (91)
100/14.5 *	SYR-322Z	1st	1.3 (0.6)	6834 (2296)	29103 (4846)	1.0 (0.0)	8339 (330)	37236 (2554)	1.2 (0.4)	7587 (1683)	33170 (5643)
		91st	1.7 (0.6)	7858 (749)	51073 (5988)	1.7 (0.6)	10552 (2443)	49102 (5305)	1.7 (0.5)	9205 (2188)	50088 (5174)
	SYR-322 M-I	1st	1.3 (0.6)	1174 (176)	9891 (2641)	2.0 (1.7)	776 (270)	7122 (3329)	1.7 (1.2)	975 (298)	8507 (3086)
		91st	1.3 (0.6)	983 (148)	9897 (1256)	2.0 (0.0)	862 (183)	7347 (2720)	1.7 (0.5)	922 (163)	8622 (2354)
	SYR-322 M-II	1st	2.0 (1.7)	136 (41)	735 (163)	1.0 (0.0)	217 (31)	1326 (63)	1.5 (1.2)	176 (55)	1031 (342)
		91st	1.3 (0.6)	142 (8)	1025 (145)	1.3 (0.6)	325 (35)	1744 (349)	1.3 (0.5)	234 (103)	1385 (460)
30/3.6 *	SYR-322Z	1st	1.0 (0.0)	947 (305)	4101 (604)	2.0 (1.7)	1161 (384)	6791 (1768)	1.5 (1.2)	1054 (332)	5446 (1889)
		91st	1.0 (0.0)	2142 (619)	8954 (1003)	1.0 (0.0)	2290 (438)	8154 (1487)	1.0 (0.0)	2216 (487)	8554 (1216)
	SYR-322 M-I	1st	3.0 (1.7)	326 (13)	2176 (409)	3.0 (1.7)	290 (91)	2488 (318)	3.0 (1.5)	308 (61)	2332 (370)
		91st	2.0 (1.7)	530 (60)	3624 (692)	1.3 (0.6)	431 (52)	2411 (280)	1.7 (1.2)	480 (74)	3018 (815)
	SYR-322 M-II	1st	1.0 (0.0)	16 (6)	82 (29)	2.0 (1.7)	27 (7)	198 (44)	1.5 (1.2)	22 (8)	140 (72)
		91st	1.0 (0.0)	53 (10)	219 (41)	1.0 (0.0)	73 (18)	269 (68)	1.0 (0.0)	63 (17)	244 (57)

*:SYR-322 / AD-4833(HCl)
Mean (S.D.)

Table 1 Toxicokinetic parameters for AD-4833 in rats

Test article	Dose* (mg/kg/day)	No. of dosing	Male (N=3)			Female (N=3)			Total (N=6)		
			Tmax (h)	Cmax (μg/mL)	AUC_{0-24h} (μg·h/mL)	Tmax (h)	Cmax (μg/mL)	AUC_{0-24h} (μg·h/mL)	Tmax (h)	Cmax (μg/mL)	AUC_{0-24h} (μg·h/mL)
SYR-322/ AD-4833(HCl)	0/14.5	1st	1.7 (0.6)	18.81 (1.79)	137.2 (7.2)	1.7 (0.6)	19.64 (2.08)	141.7 (12.1)	1.7 (0.5)	19.22 (1.79)	139.5 (9.2)
		91st	1.3 (0.6)	17.32 (2.24)	170.4 (34.3)	1.3 (0.6)	21.26 (2.67)	158.7 (22.4)	1.3 (0.5)	19.29 (3.08)	164.6 (26.7)
	30/14.5	1st	2.7 (1.2)	11.59 (0.35)	130.4 (10.1)	4.0 (3.5)	10.96 (2.33)	99.9 (28.4)	3.3 (2.4)	11.28 (1.53)	115.2 (25.3)
		91st	3.3 (1.2)	13.09 (0.70)	162.8 (0.1)	1.7 (0.6)	12.57 (1.07)	123.7 (30.0)	2.5 (1.2)	12.83 (0.86)	143.3 (28.6)
	100/14.5	1st	3.3 (1.2)	11.70 (1.17)	156.7 (5.8)	4.0 (3.5)	12.64 (4.83)	137.1 (29.8)	3.7 (2.3)	12.17 (3.19)	146.9 (22.0)
		91st	6.7 (2.3)	12.92 (2.09)	166.1 (31.0)	4.3 (3.5)	17.10 (4.71)	172.3 (54.5)	5.5 (2.9)	15.01 (3.98)	169.2 (39.8)
	30/3.6	1st	2.0 (0.0)	3.89 (0.17)	44.9 (3.0)	2.0 (0.0)	4.12 (0.86)	45.8 (8.8)	2.0 (0.0)	4.00 (0.57)	45.4 (5.9)
		91st	2.7 (1.2)	7.18 (0.19)	85.4 (11.7)	1.7 (0.6)	5.94 (0.86)	53.3 (6.8)	2.2 (1.0)	6.56 (0.88)	69.3 (19.6)

Mean (S.D.)
*: As free base

Table 2 Toxicokinetic parameters for M-II in rats

Test article	Dose*1 (mg/kg/day)	No. of dosing	Male (N=3) Tmax (h)	Male (N=3) Cmax (μg/mL)	Male (N=3) AUC 0-24h (μg·h/mL)	Female (N=3) Tmax (h)	Female (N=3) Cmax (μg/mL)	Female (N=3) AUC 0-24h (μg·h/mL)	Total (N=6) Tmax (h)	Total (N=6) Cmax (μg/mL)	Total (N=6) AUC 0-24h (μg·h/mL)
SYR-322/ AD-4833(HCl)	0/14.5	1st	6.7 (2.3)	0.73 (0.93)	10.6 (13.8)	4.0 (0.0)	0.24 (0.14)	3.3 (2.2)	5.3 (2.1)	0.49 (0.65)	6.9 (9.7)
		91st	8.0 (0.0)	1.04 (1.39)	17.6 (23.6)	5.3 (2.3)	0.27 (0.21)	3.8 (3.4)	6.7 (2.1)	0.66 (0.99)	10.7 (16.9)
	30/14.5	1st	8.0 (0.0)	0.90 (0.88)	12.8 (12.7)	6.7 (2.3)	0.36 (0.10)	4.5 (1.8)	7.3 (1.6)	0.63 (0.63)	8.6 (9.3)
		91st	8.0 (0.0)	1.25 (1.36)	21.9 (23.4)	6.0 (3.5)	0.54 (0.50)	8.2 (7.5)	7.0 (2.4)	0.90 (1.00)	15.0 (17.2)
	100/14.5	1st	8.0 (0.0)	1.95 (0.82)	27.9 (9.8)	8.0 (0.0)	0.30 (0.26)	4.1 (3.9)	8.0 (0.0)	1.13 (1.06)	16.0 (14.6)
		91st	8.0 (0.0)	3.48 (0.71)	57.9 (9.5)	5.3 (2.3)	0.44 (0.47)	5.5 (5.7)	6.7 (2.1)	1.96 (1.75)	31.7 (29.6)
	30/3.6	1st	8.0 (-)*2	0.10 (0.12)	1.2 (1.5)	8.0 (-)*3	0.05 (0.09)	0.7 (1.2)	8.0 (0.0)*4	0.08 (0.10)	1.0 (1.3)
		91st	8.0 (0.0)	0.24 (0.22)	4.0 (3.8)	6.0 (-)*2	0.07 (0.08)	0.8 (1.0)	7.2 (1.8)*5	0.16 (0.18)	2.4 (3.0)

Mean (S.D.)
*1: As free base *2: N=2 *3: N=1 *4: N=3 *5: N=5
-: Not calculated

Table 3 Toxicokinetic parameters for M-III in rats

Test article	Dose* (mg/kg/day)	No. of dosing	Male (N=3) Tmax (h)	Male (N=3) Cmax (μg/mL)	Male (N=3) AUC 0-24h (μg·h/mL)	Female (N=3) Tmax (h)	Female (N=3) Cmax (μg/mL)	Female (N=3) AUC 0-24h (μg·h/mL)	Total (N=6) Tmax (h)	Total (N=6) Cmax (μg/mL)	Total (N=6) AUC 0-24h (μg·h/mL)
SYR-322/ AD-4833(HCl)	0/14.5	1st	4.0 (0.0)	2.01 (0.44)	25.7 (3.1)	4.0 (0.0)	1.97 (0.04)	24.7 (1.6)	4.0 (0.0)	1.99 (0.28)	25.2 (2.3)
		91st	5.3 (2.3)	1.53 (0.18)	21.4 (0.4)	3.3 (1.2)	2.47 (0.44)	33.9 (3.0)	4.3 (2.0)	2.00 (0.60)	27.7 (7.1)
	30/14.5	1st	8.0 (0.0)	1.44 (0.13)	19.8 (1.0)	5.3 (2.3)	1.28 (0.25)	16.2 (3.6)	6.7 (2.1)	1.36 (0.20)	18.0 (3.1)
		91st	8.0 (0.0)	1.19 (0.14)	18.1 (1.4)	5.3 (2.3)	1.70 (0.34)	25.9 (3.1)	6.7 (2.1)	1.44 (0.36)	22.0 (4.8)
	100/14.5	1st	8.0 (0.0)	1.87 (0.59)	24.5 (6.4)	8.0 (0.0)	1.61 (0.46)	22.8 (5.5)	8.0 (0.0)	1.74 (0.50)	23.7 (5.4)
		91st	6.7 (2.3)	1.43 (0.45)	19.8 (5.3)	6.7 (2.3)	2.12 (0.53)	30.8 (11.2)	6.7 (2.1)	1.78 (0.58)	25.3 (9.9)
	30/3.6	1st	8.0 (0.0)	0.55 (0.02)	7.6 (0.4)	8.0 (0.0)	0.58 (0.05)	7.9 (0.5)	8.0 (0.0)	0.56 (0.04)	7.8 (0.5)
		91st	8.0 (0.0)	0.77 (0.18)	11.5 (2.1)	5.3 (2.3)	0.79 (0.13)	11.3 (2.5)	6.7 (2.1)	0.78 (0.14)	11.4 (2.1)

Mean (S.D.)
*: As free base

Table 4 Toxicokinetic parameters for M-IV in rats

Test article	Dose* (mg/kg/day)	No. of dosing	Male (N=3) Tmax (h)	Male (N=3) Cmax (μg/mL)	Male (N=3) AUC 0-24h (μg·h/mL)	Female (N=3) Tmax (h)	Female (N=3) Cmax (μg/mL)	Female (N=3) AUC 0-24h (μg·h/mL)	Total (N=6) Tmax (h)	Total (N=6) Cmax (μg/mL)	Total (N=6) AUC 0-24h (μg·h/mL)
SYR-322/ AD-4833(HCl)	0/14.5	1st	4.7 (3.1)	1.81 (0.22)	23.0 (1.1)	4.0 (0.0)	1.91 (0.34)	24.1 (6.0)	4.3 (2.0)	1.86 (0.26)	23.5 (3.9)
		91st	5.3 (2.3)	1.25 (0.17)	16.2 (1.9)	4.0 (0.0)	2.20 (0.11)	29.2 (3.4)	4.7 (1.6)	1.72 (0.54)	22.7 (7.5)
	30/14.5	1st	8.0 (0.0)	1.12 (0.13)	15.1 (1.8)	6.7 (2.3)	1.25 (0.18)	15.8 (2.8)	7.3 (1.6)	1.19 (0.16)	15.5 (2.1)
		91st	8.0 (0.0)	0.85 (0.06)	12.2 (0.3)	4.0 (0.0)	1.60 (0.51)	21.6 (5.6)	6.0 (2.2)	1.23 (0.52)	16.9 (6.2)
	100/14.5	1st	8.0 (0.0)	1.63 (0.49)	21.1 (5.8)	8.0 (0.0)	1.75 (0.53)	23.9 (7.6)	8.0 (0.0)	1.69 (0.46)	22.5 (6.2)
		91st	6.7 (2.3)	1.13 (0.44)	14.9 (4.8)	5.3 (2.3)	1.85 (0.69)	25.5 (11.4)	6.0 (2.2)	1.49 (0.65)	20.2 (9.7)
	30/3.6	1st	8.0 (0.0)	0.39 (0.07)	5.3 (0.9)	8.0 (0.0)	0.52 (0.13)	7.0 (1.7)	8.0 (0.0)	0.46 (0.12)	6.2 (1.5)
		91st	8.0 (0.0)	0.41 (0.06)	5.9 (0.9)	5.3 (2.3)	0.52 (0.04)	7.0 (0.6)	6.7 (2.1)	0.46 (0.07)	6.5 (0.9)

Mean (S.D.)
*: As free base

Other:

Sponsor's Tabulated Summary

Thirteen-Week Oral Gavage Toxicity Study of SYR-322 Administered to Rats Concurrently with AD-4833(HCl) (Study No. B060832)

Animals	Crl:CD(SD) rats, 6 weeks of age				
Test article	Control [a]	SYR-322/AD-4833(HCl)*			
Dosage level (mg/kg/day)[b]	0	0/14.5	30/14.5	100/14.5	30/3.6
Dosage volume (mL/kg)	10	10	10	10	10
No. of animals (M:F)	10:10	10[c]:10[c]	10[c]:10[c]	10[c]:10[c]	10[c]:10[c]
Mortality (M:F)	0:0	0:0	0:0	0:0	0:0
Clinical signs	–	–	–	–	–
Body weight	–	–	–	–	–
Food consumption	–	–	–	–	–
Water consumption	–	–	–	–	–
Ophthalmology	–	–	–	–	–
Urinalysis	–	–	–	–	–
Hematology	–	–	–	–	–
Blood chemistry	–	–	–	–	–
Necropsy					
Brown fat	–	M5[d]	Increase, adipose tissue M2:F3[d]	M5:F3[d]	M2[d]
White fat	–	M5[d]	Increase, adipose tissue M2[d]	M5:F1[d]	M2[d]
Organ weight	–	↑Heart # (M)	↑Heart # (M)	↑Heart # (M)	–
Histopathology					
Heart	M3:F1[d]	M8#:F1[d]	Cardiomyopathy M5:F1[d]	M9#[d]	M6[d]
Bone marrow			Hypertrophy, adipocyte		
Femur	–	M10:F10[d]	M9:F8[d]	M10:F10[d]	M9:F4[d]
Sternum	–	M10:F7[d]	M9:F9[d]	M10:F9[d]	M2:F2[d]
Brown fat	–	M10:F10[d]	Hypertrophy, adipocyte M10:F10[d]	M10:F10[d]	M10:F4[d]
White fat	–	M4:F4[d]	Hyperplasia, adipocyte M3:F2[d]	M4:F3[d]	M1[d]

a: distilled water for injection, b: as SYR-322Z or AD-4833, free base of each test article, c: 4 additional animals/sex/dose were used as satellite groups for toxicokinetics, d: the number of animals with the findings, –: no treatment-related effect, ↑: increase, #: toxicologically significant findings

*: dosed as AD-4833 (b) (4)

2.6.6.6 Reproductive and developmental toxicology

Embryofetal development

Alogliptin + pioglitazone embryofetal development (Seg II) in rats

Effects of SYR-322, administered concurrently with AD-4833 (HCl), on embryo-fetal development in rats (Study No. B060833; Takeda No. SYR-322-4833/00028.001A)

Doses mg/kg *0/0 (vehicle control)*
0/40 (AD-4833 only)
30/40, 100/40, 30/20 (SYR-322/AD-4833)
n.b. no SYR-322 only controls

NOAEL (maternal) = < 30 mg/kg alogliptin / 20 mg/kg pioglitazone in dams
NOAEL (fetal) = < 40 mg/kg pioglitazone
30 mg/kg alogliptin (potentiation of pio. effects at 100 mg/kg)

Key study findings:

- Alogliptin co-treatment with pioglitazone did not show any teratogenic effect in fetuses. This FDA pharmacology/toxicology reviewer agrees with the Sponsor's conclusions:

  "Combination treatment with alogliptin and pioglitazone in an embryo-fetal development study in rats slightly augmented pioglitazone-related fetal effects of growth retardation and visceral variations, but did not induce embryo-fetal mortality or teratogenicity."

- Maternal body weights were decreased very slightly (max. ~2%) at termination (GD20) in all 40 mg/kg pioglitazone groups. Pioglitazone exacerbated weight gain during the start of the dosing period (GD6-10), but weight gain actually decreased compared to controls during the last part of dosing (GD14-17) and after dosing ended (GD18-20). These changes in body weight gain are indicative of maternal toxicity with pioglitazone treatment. There were no other apparent effects of alogliptin co-treatment on maternal health.

- Pioglitazone treatment (40 mg/kg) did show some effect on embryofetal development, including increased placental weights (16-30%) and various visceral variations (left umbilical artery, renal pelvis dilatation, and convoluted/dilatation ureter). An increased incidence of ventral septal defects in all groups could not be attributed to pioglitazone, however, background rates in controls seemed very high (no laboratory-specific historical control data provided).

- Co-treatment with 100 mg/kg alogliptin caused slightly decreased fetal weights (4-5%) and potentiated effects of pioglitazone alone on total visceral variations (number of fetuses and number of dams with fetal variations), most notably seen as increased

supernumerary coronary ostium and renal pelvis dilatation. Previous embryofetal development studies with pioglitazone showed effects on fetal weight and survival.

- No toxicokinetic analysis was included in the study design. Exposure was assumed based on TK data from various alogliptin, pioglitazone, and co-treatment in subchronic toxicity studies.

NOAEL Determination

Maternal body weight gain was altered in all pioglitazone groups, which confirmed maternal exposure, showed evidence of maternal toxicity, and showed doses were adequate to determine effects of alogliptin co-treatment on embryofetal development. No NOAEL could be determined for fetal effects with pioglitazone treatment, based on modest increases in placental weights and visceral variations in the 40 mg/kg pioglitazone control and alogliptin co-treatment groups. Alogliptin co-treatment increased visceral variations above pioglitazone controls only at the high dose of 100 mg/kg alogliptin (approximately 33-times maximum human exposure). Thus, a NOAEL of 30 mg/kg alogliptin (approximately 6-times maximum human exposure) co-treatment could be estimated for potentiation of pioglitazone-mediated effects.

Study no.: B060833
Volume #, and page #: eCTD
Conducting laboratory and location: (b) (4)

Date of study initiation: 8/22/06
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: SYR-322 (alogliptin), Lot No. MA01-001, 99.9% purity / AD-4833 (HCl) (pioglitazone; AD-4833 (b) (4)), Lot No. Z509Z01, 98.2% purity

Methods

Doses: 0/0 (vehicle control); 0/40 (AD-4833 only); 30/40, 100/40, 30/20 (SYR-322/AD-4833)
Dosing notes – (1) no SYR-322 only controls; (2) doses chosen based on 30X MRHD for alogliptin (< NOAEL) and embryo-/fetotoxic dose of 40 mg/kg pioglitazone in a previous study
Species/strain: Sprague-Dawley rat / Crl:CD(SD)
Number/sex/group: 20 pregnant females/group
Route, formulation, volume, and infusion rate: Oral gavage, 10 ml/kg (water for injection vehicle)
Satellite groups used for toxicokinetics:
Study design: Treatment gestations days 6 to 17 (GD) in pregnant rats after one-to-one mating (copulation confirmed by sperm plug and vaginal smear), necropsy GD 20; ***Clinical observations***- twice daily; ***Body weight & food consumption***- days 0, 6, 8, 10, 12, 14, 16, 18, 20; ***Necropsy***- macroscopic examination, gross lesions/abnormalities fixed and examined;

Parameters and endpoints evaluated:

C-section- GD 20 examined corpora lutea, implantations, live fetuses, dead embryos, placentae exam/weight, fetuses sexed/weighed and external exam (anomalies)

Sponsor's implantation calculation summary

Pre-implantation loss (%):

(Number of corpora lutea - Number of implantations) / Number of corpora lutea × 100

Post-implantation loss (%):

(Number of early embryonic deaths + Number of late embryonic deaths) / Number of implantations × 100

Total implantation loss (%):

$$\frac{\text{Number of corpora lutea} - \text{Number of implantations} + \text{Number of early embryonic deaths} + \text{Number of late embryonic deaths}}{\text{Number of corpora lutea}} \times 100$$

Visceral examination (1/2 fetuses)- visceral abnormalities (anomalies and variations) by Wilson's method (head) and microdissection (cervical, thorax, abdomen)

Skeletal examination (1/2 fetuses)- control and high dose (100/40 mg/kg) skeletal abnormalities (anomalies and variations) and degree of ossification (sacral/caudal vertebral bodies)

Sponsor's Study Design Summary

Groups	Test article	Dosage level (mg/kg/day)	Concentration (mg/mL)	Dosage volume (mL/kg/day)	Number of animals copulated (Animal Nos.)
1	Control [1]	0	0	10	20 (50101 to 50120)
2	AD-4833(HCl) [2]	40 [4]	4 [4]	10	20 (50201 to 50220)
3	SYR-322/AD-4833(HCl)	30 [3]/ 40 [4]	3 [3]/ 4 [4]	10	20 (50301 to 50320)
4	SYR-322/AD-4833(HCl)	100 [3]/ 40 [4]	10 [3]/ 4 [4]	10	20 (50401 to 50420)
5	SYR-322/AD-4833(HCl)	30 [3]/ 20 [4]	3 [3]/ 2 [4]	10	20 (50501 to 50520)

1 Distilled water for injection

2 AD-4833(HCl) (b) (4)

3 SYR-322 free base

4 AD-4833 free base

Results

Mortality (dams): None.

Clinical signs (dams): No treatment related findings.

Body weight (dams): There was no notable effect of alogliptin co-treatment on pioglitazone groups. Maternal body weights were decreased very slightly (max. ~2%) in all 40 mg/kg pioglitazone (± alogliptin) groups at termination. However, trends showed significant increased weight gain in all pioglitazone treated dams during GD 6-10, with weight gain decreases during the last part of dosing (GD 14-18) and post-treatment (GD 18-20) accounting for the slight overall body weight decrements compared to vehicle

controls at termination. Pioglitazone causes modest weight gain increases in subchronic and chronic rat treatment, but that effect was absent in the short term treatments during pregnancy. Nevertheless, body weight changes are consistent with a known pioglitazone-induced effect on body weight and significant body weight changes during pregnancy are indicative of maternal toxicity. The Sponsor's body weight and body weight gain summary tables are shown below.

Table 2 Body weight (F0 gestation) - Summary

Test article Dose	Day	0	6	8	10	12	14	16	18	20
1.										
Control	Mean	273.1	314.0	322.6	330.5	343.5	356.9	374.1	403.9	440.0
0 mg/kg	S.D.	13.9	15.2	15.1	16.6	17.9	18.8	20.9	21.0	23.5
	n	20	20	20	20	20	20	20	20	20
2.										
AD-4833(HCl)	Mean	272.3	309.3	322.7	332.3	344.2	356.8	371.1	396.9	428.9
40 mg/kg	S.D.	13.9	19.7	21.4	21.6	23.1	24.5	24.6	27.3	29.6
	n	20	20	20	20	20	20	20	20	20
3.										
SYR-322/AD-4833(HCl)	Mean	273.6	312.1	324.2	333.9	345.3	360.1	375.3	401.5	433.7
30/40 mg/kg	S.D.	13.5	16.4	17.8	19.9	22.2	22.9	25.5	27.3	27.8
	n	20	20	20	20	20	20	20	20	20
4.										
SYR-322/AD-4833(HCl)	Mean	272.9	310.7	321.9	331.0	345.0	359.2	373.9	397.9	426.6
100/40 mg/kg	S.D.	13.8	18.1	18.6	18.8	21.4	22.6	24.1	24.8	29.3
	n	20	20	20	20	20	20	20	20	20
5.										
SYR-322/AD-4833(HCl)	Mean	273.4	314.5	327.0	335.7	349.4	361.7	378.0	406.3	441.0
30/20 mg/kg	S.D.	16.2	21.4	22.2	24.3	26.3	27.5	28.9	29.4	30.8
	n	20	20	20	20	20	20	20	20	20

Not significant

Table 3 - 1 Body weight gain (F0 gestation) - Summary
Base: Day 6 of gstation

Test article Dose	Day	0	6	8	10	12	14	16	18	20
1.										
Control	Mean	-40.9	0.0	8.6	16.5	29.6	43.0	60.2	90.0	126.1
0 mg/kg	S.D.	5.9	0.0	6.1	5.5	6.8	6.7	9.2	9.6	12.6
	n	20	20	20	20	20	20	20	20	20
2.										
AD-4833(HCl)	Mean	-37.1	0.0	13.4 *	23.0 **	34.9 *	47.5	61.8	87.6	119.6
40 mg/kg	S.D.	8.9	0.0	5.3	6.1	6.3	8.8	10.0	12.6	14.8
	n	20	20	20	20	20	20	20	20	20
3.										
SYR-322/AD-4833(HCl)	Mean	-38.6	0.0	12.1	21.8 *	33.2	48.0	63.2	89.4	121.6
30/40 mg/kg	S.D.	7.7	0.0	6.7	7.4	8.4	9.7	13.0	15.1	17.3
	n	20	20	20	20	20	20	20	20	20
4.										
SYR-322/AD-4833(HCl)	Mean	-37.9	0.0	11.2	20.3 *	34.3 *	48.5 *	63.2	87.2	115.9
100/40 mg/kg	S.D.	7.5	0.0	6.8	5.9	7.2	9.5	11.7	14.4	19.9
	n	20	20	20	20	20	20	20	20	20
5.										
SYR-322/AD-4833(HCl)	Mean	-41.1	0.0	12.6	21.3 *	35.0 *	47.3	63.5	91.8	126.6
30/20 mg/kg	S.D.	8.7	0.0	6.4	7.2	8.6	11.0	13.6	14.4	18.0
	n	20	20	20	20	20	20	20	20	20

Significantly different from control: *: P<0.05; **, P<0.01 (t test, Student or Aspin-Welch) , group 1 vs group 2, 3, 4, 5 (2 group comparison)

Table 3 - 2 Body weight gain (F0 gestation) - Summary
Base: Interval measurement date Unit : g

Test article Dose	Day	0-6	6-8	8-10	10-12	12-14	14-16	16-18	6-18	6-10	10-14	14-18	18-20
1.	Mean	40.9	8.6	7.9	13.1	13.4	17.2	29.8	90.0	16.5	26.5	47.0	36.1
Control	S.D.	5.9	6.1	4.7	5.1	4.3	6.3	5.0	9.6	5.5	4.9	6.1	5.3
0 mg/kg	n	20	20	20	20	20	20	20	20	20	20	20	20
2.	Mean	37.1	13.4 *	9.6	11.9	12.6	14.3	25.9 *	87.6	23.0 **	24.5	40.2 **	32.0 *
AD-4833(HCl)	S.D.	8.9	5.3	4.4	4.0	4.7	4.8	5.5	12.6	6.1	6.5	7.1	4.8
40 mg/kg	n	20	20	20	20	20	20	20	20	20	20	20	20
3.	Mean	38.6	12.1	9.7	11.5	14.8	15.3	26.2 *	89.4	21.8 *	26.2	41.4 *	32.3
SYR-322/AD-4833(HCl)	S.D.	7.7	6.7	4.3	5.5	4.4	4.9	5.7	15.1	7.4	5.3	7.0	8.4
30/40 mg/kg	n	20	20	20	20	20	20	20	20	20	20	20	20
4.	Mean	37.9	11.2	9.1	14.0	14.2	14.7	24.0 **	87.2	20.3 *	28.2	38.7 **	28.7 **
SYR-322/AD-4833(HCl)	S.D.	7.5	6.8	4.3	4.0	5.7	4.5	6.0	14.4	5.9	6.9	7.5	6.7
100/40 mg/kg	n	20	20	20	20	20	20	20	20	20	20	20	20
5.	Mean	41.1	12.6	8.7	13.7	12.3	16.3	28.3	91.8	21.3 *	26.0	44.6	34.8
SYR-322/AD-4833(HCl)	S.D.	8.7	6.4	5.6	5.8	5.8	6.5	5.2	14.4	7.2	6.9	7.0	7.2
30/20 mg/kg	n	20	20	20	20	20	20	20	20	20	20	20	20

Significantly different from control : *,P<0.05; **,P<0.01 (t test, Student or Aspin-Welch), group 1 vs group 2, 3, 4, 5 (2 group comparison)

Food consumption (dams): Food consumption trends were generally consistent with body weight trends; that is, compared to vehicle controls consumption increased slightly in pioglitazone groups at the beginning of dosing and consumption was similar or slightly decreased after the dosing period ended.

Toxicokinetics: None.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

No remarkable dam toxicity based on gross exam at necropsy. Embryo survival, sex ratio, and implantation loss trends were unremarkable. There was a trend of very slightly increased late embryo resorption (i.e. 'dead embryos') in alogliptin plus high dose pioglitazone groups, but differences were not statistically significant or dose-related. High dose alogliptin plus pioglitazone (100/40) fetal weights were slightly lower than pioglitazone controls and vehicle controls (♂ ↓ 5%, ss; ♀ ↓ 4%, nss). Placental weights were consistently increased 16-30% in all pioglitazone groups, but there was no effect of alogliptin co-treatment. Fusion of two placentae in one dam in the low dose combination group (30/20) were considered spontaneous and unrelated to treatment due to incidence in a single dam and absence of a dose-related effect. The Sponsor's summary table is shown below.

Sponsor's C-Section Summary

Table 6 Cesarean section (F0) - Summary

Test article Dose		Number of corpora lutea	Number of total implants	Number of dead embryos			Implantation loss (%)			Number of live fetuses			Live fetal weight(g)		Placental weight(g)	
				Early	Late	Total	Pre-	Post-	Total	M	F	Total	M	F	M	F
1.																
Control	Mean	16.8	16.2	1.3	0.1	1.4	3.28	8.29	11.25	7.2	7.7	14.9	3.41	3.27	0.44	0.43
0 mg/kg	S.D.	0.8	0.9	1.5	0.2	1.6	3.60	9.74	10.21	1.8	2.3	1.7	0.23	0.25	0.05	0.05
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	(M/F)											(143/154)				
2.																
AD-4833(HCl)	Mean	17.0	16.3	1.3	0.0	1.3	4.66	7.43	11.95	7.5	7.6	15.0	3.32	3.23	0.54 **	0.55 **
40 mg/kg	S.D.	2.3	2.7	1.3	0.0	1.3	8.90	7.43	9.34	2.6	1.8	2.6	0.27	0.18	0.09	0.09
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	(M/F)											(149/151)				
3.																
SYR-322/AD-4833(HCl)	Mean	16.4	15.9	1.0	0.4	1.4	2.98	8.16	11.02	6.7	7.8	14.5	3.30	3.18	0.55 **	0.54 **
30/40 mg/kg	S.D.	1.9	1.9	1.1	1.6	1.8	4.22	9.61	8.98	2.8	2.9	1.5	0.24	0.18	0.06	0.06
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	(M/F)											(134/156)				
4.																
SYR-322/AD-4833(HCl)	Mean	16.9	15.9	1.3	0.2	1.5	4.94	8.95	13.31	7.5	7.0	14.5	3.23 *	3.13	0.55 **	0.56 **
100/40 mg/kg	S.D.	1.8	1.9	1.7	0.4	1.7	11.34	9.72	14.18	2.5	2.6	2.1	0.21	0.19	0.08	0.07
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	(M/F)											(149/140)				
5.																
SYR-322/AD-4833(HCl)	Mean	16.6	15.8	1.0	0.0	1.0	4.83	6.00	10.40	7.3	7.6	14.8	3.46	3.25	0.51 **	0.53 **
30/20 mg/kg	S.D.	1.8	1.9	1.1	0.0	1.1	5.05	6.28	9.06	1.3	2.0	2.0	0.30	0.29	0.09	0.07
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	(M/F)											(145/151)				

Significantly different from control: *: P<0.05; **, P<0.01 (t test, Student or Aspin-Welch), group 1 vs group 2, 3, 4, 5 (2 group comparison)
M: Male; F: Female

Offspring (malformations, variations, etc.):

There was no apparent effect of alogliptin co-treatment on any teratogenic endpoint. No external anomalies were observed in any fetuses and there were no skeletal anomalies observed. Skeletal variations were not increased in the high dose alogliptin plus pioglitazone group.

Several visceral and skeletal findings were described. Anomalous ventricular septal defects were observed in all groups including controls. There was no dose-related increase in septal defects and a single increased incidence (from a single additional dam) noted in the high dose combination (100/40) group compared to vehicle control was considered unrelated to treatment. No historical data on ventricular septal defects was provided, however, rates of control and pioglitazone-treated ventricular septal defects were high compared to historical data from studies conducted at (b) (4) (the animal supplier).

Total visceral variations (numbers of fetuses ***and*** number of dams with fetal variations) were increased in high dose alogliptin plus pioglitazone (100/30) compared to either vehicle or pioglitazone controls. Variations increased with high dose alogliptin co-treatment included **supernumerary coronary ostium, dilatation of renal pelvis,** and **dilatation of ureter**. Visceral variation trends in pioglitazone treated groups (albeit not statistically significant) included left umbilical artery dilatation of renal pelvis, and ureter dilatation and convoluted ureter. No laboratory historical control data were provided to assess relevance of pioglitazone visceral variation trends, however, ureter findings were consistent with (b) (4) historical background data.

Notable visceral variations are shown in the Sponsor's summary table, below. The Sponsor's complete tabulated summary is also reproduced below the visceral variations.

Sponsor's Visceral Variations Summary

Table 9 Visceral examination of fetuses (F1) - Summary

		1.	2.	3.	4.	5.
Test article		Control	AD-4833(HCl)	SYR-322/AD-4833(HCl)	SYR-322/AD-4833(HCl)	SYR-322/AD-4833(HCl)
Dose		0	40	30/40	100/40	30/20
Dose unit		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Number of dams		20	20	20	20	20
Number of fetuses		155	156	151	150	153
Number of dams with anomalous fetuses		5 (25.0%)	3 (15.0%)	1 (5.0%)	6 (30.0%)	2 (10.0%)
Number of fetuses with any anomalies		7 (4.5%)	3 (1.9%)	1 (0.6%)	7 (5.2%)	3 (2.1%)
Number of dams with variant fetuses		7 (35.0%)	9 (45.0%)	12 (60.0%)	17 (85.0%) FF	10 (50.0%)
Number of fetuses with any variations		10 (6.7%)	13 (9.5%)	19 (12.7%)	27 (18.7%) $$, S	16 (10.6%)
Anomaly						
Small thymus	a)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean b)	0.6	0.0	0.0	0.0	0.0
	S.D.	2.8	0.0	0.0	0.0	0.0
Lobulation anomaly of lung		1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean	0.6	0.0	0.0	0.0	0.0
	S.D.	2.8	0.0	0.0	0.0	0.0
Ventricular septal defect		6 (5)	3 (3)	1 (1)	7 (6)	3 (2)
	Mean	3.9	1.9	0.6	5.2	2.1
	S.D.	7.4	4.7	2.8	9.9	7.0
Atrial septal defect		1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean	0.6	0.0	0.0	0.0	0.0
	S.D.	2.8	0.0	0.0	0.0	0.0
Aortic arch stenosis		1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean	0.6	0.0	0.0	0.0	0.0
	S.D.	2.8	0.0	0.0	0.0	0.0
Lobulation anomaly of liver		1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean	0.6	0.0	0.0	0.0	0.0
	S.D.	2.8	0.0	0.0	0.0	0.0

a):Number of anomalous or variant fetuses (number of dams with anomalous or variant fetuses)
b):Number of anomalous or variant fetuses / number of fetuses examined x 100(%), on litter basis
Significantly different from control : F,P<0.05; FF,P<0.01 (Fisher test), group 1 vs group 2, 3, 4, 5 (2 group comparison)
Significantly different from control : $,P<0.05; $$,P<0.01 (Wilcoxon test), group 1 vs group 2, 3, 4, 5 (2 group comparison)
Significantly different from control : S,P<0.05 (Shirley-Williams Test), group 2 vs groups 3, 4

Table 9 Visceral examination of fetuses (F1) - Summary

		1. Control	2. AD-4833(HCl)	3. SYR-322/AD-4833(HCl)	4. SYR-322/AD-4833(HCl)	5. SYR-322/AD-4833(HCl)
Test article						
Dose		0	40	30/40	100/40	30/20
Dose unit		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Number of dams		20	20	20	20	20
Number of fetuses		155	156	151	150	153
Number of dams with anomalous fetuses		5 (25.0%)	3 (15.0%)	1 (5.0%)	6 (30.0%)	2 (10.0%)
Number of fetuses with any anomalies		7 (4.5%)	3 (1.9%)	1 (0.6%)	7 (5.2%)	3 (2.1%)
Number of dams with variant fetuses		7 (35.0%)	9 (45.0%)	12 (60.0%)	17 (85.0%) FF	10 (50.0%)
Number of fetuses with any variations		10 (6.7%)	13 (9.5%)	19 (12.7%)	27 (18.7%) $$, S	16 (10.6%)
Variation						
Thymic remnant in neck		3 (3)	3 (3)	5 (5)	3 (3)	2 (2)
	Mean	1.9	2.0	3.3	2.1	1.3
	S.D.	4.7	5.0	5.9	5.2	4.1
Supernumerary coronary ostium	a)	1 (1)	0 (0)	1 (1)	6 (6)	2 (2)
	Mean b)	0.7	0.0	0.8	4.4 $, S	1.3
	S.D.	3.2	0.0	3.7	7.1	4.1
High coronary ostium		0 (0)	0 (0)	1 (1)	0 (0)	2 (2)
	Mean	0.0	0.0	0.6	0.0	1.7
	S.D.	0.0	0.0	2.8	0.0	5.4
Left umbilical artery		0 (0)	1 (1)	2 (2)	2 (2)	0 (0)
	Mean	0.0	0.8	1.3	1.3	0.0
	S.D.	0.0	3.7	3.8	4.1	0.0
Dilatation of renal pelvis		6 (4)	10 (8)	12 (7)	18 (13)	10 (6)
	Mean	4.1	7.5	7.9	12.4 $	6.2
	S.D.	8.8	10.2	12.3	11.5	11.4
Dilatation of ureter		0 (0)	2 (2)	0 (0)	4 (4)	0 (0)
	Mean	0.0	1.3	0.0	2.5 $	0.0
	S.D.	0.0	4.1	0.0	5.2	0.0
Convoluted ureter		0 (0)	2 (2)	1 (1)	1 (1)	0 (0)
	Mean	0.0	1.3	0.7	0.6	0.0
	S.D.	0.0	3.8	3.2	2.5	0.0

a):Number of anomalous or variant fetuses (number of dams with anomalous or variant fetuses)
b):Number of anomalous or variant fetuses / number of fetuses examined x 100(%), on litter basis
Significantly different from control : F,P<0.05; FF,P<0.01 (Fisher test), group 1 vs group 2, 3, 4, 5 (2 group comparison)
Significantly different from control : $,P<0.05; $$,P<0.01 (Wilcoxon test), group 1 vs group 2, 3, 4, 5 (2 group comparison)
Significantly different from control : S,P<0.05 (Shirley-Williams Test), group 2 vs groups 3, 4

Sponsor's Tabulated Summary

Effects of SYR-322, Administered Concurrently with AD-4833(HCl), on Embryo-Fetal Development in Rats

Animals		Crl:CD(SD), Females: 11 weeks old (Mating)				
Test article		Distilled water	AD-4833(HCl)	SYR-322/AD-4833(HCl)		
Group number		1	2	3	4	5
Dosage level (mg/kg/day)		0	40	30/40	100/40	30/20
Dosage volume (mL/kg)		10	10	10	10	10
Number of copulated females		20	20	20	20	20
Number of pregnant females		20	20	20	20	20
Number of deaths		0	0	0	0	0
Dams						
Clinical signs		—	—	—	—	—
Body weight gain		—	(Day 6-10) *↑ (Day 14-18) **↓ (Day 18-20) *↓	(Day 6-10) *↑ (Day 14-18) *↓ (Day 18-20) ↓	(Day 6-10) *↑ (Day 14-18) **↓ (Day 18-20) **↓	(Day 6-10) *↑
Food consumption (g/animal/day)		—	—	—	(Day 6- 8) W↓ #	—
		—	(Day 8-10) *↑	(Day 8-10) *↑	(Day 8-10)W↓ #	(Day 8-10) *↑
Necropsy findings		—	—	—	—	—
Number of corpora lutea		16.8±0.8	17.0±2.3	16.4±1.9	16.9±1.8	16.6±1.8
Number of implants		16.2±0.9	16.3±2.7	15.9±1.9	15.9±1.9	15.8±1.9
Placentae						
Gross abnormalities (%)	Fusion	0.00	0.00	0.00	0.00	0.72±3.20
Placental weight (g)	Male	0.44±0.05	0.54±0.09 ** #	0.55±0.06 ** #	0.55±0.08 ** #	0.51±0.09 ** #
	Female	0.43±0.05	0.55±0.09 ** #	0.54±0.06 ** #	0.56±0.07 ** #	0.53±0.07 ** #
Fetuses						
Post-implantation loss (%)		8.29±9.74	7.43±7.43	8.16±9.61	8.95±9.72	6.00±6.28
Number of dead embryos		1.4±1.6	1.3±1.3	1.4±1.8	1.5±1.7	1.0±1.1
Number of live fetuses		14.9±1.7	15.0±2.6	14.5±1.5	14.5±2.1	14.8±2.0
Body weight (g)	Male	3.41±0.23	3.32±0.27	3.30±0.24	3.23±0.21 * #	3.46±0.30
	Female	3.27±0.25	3.23±0.18	3.18±0.18	3.13±0.19 #	3.25±0.29
Sex ratio (Male/Female)		0.93 (143/154)	0.99 (149/151)	0.86 (134/156)	1.06 (149/140)	0.96 (145/151)

Day: Gestation day
—: No treatment-related effects
↑: Increased, ↓: Decreased
#: Adverse effects
*: P<0.05; **, P<0.01 (t test, Student or Aspin-Welch) group 1 vs group 2, 3, 4, or 5. (2 group comparison)
W: P<0.05 (Williams test) group 2 vs groups 3 or 4.

Animals		Crl:CD(SD), Females: 11 weeks old (Mating)				
Test article		Distilled water	AD-4833(HCl)	SYR-322/AD-4833(HCl)		
Group number		1	2	3	4	5
Dosage level (mg/kg/day)		0	40	30/40	100/40	30/20
Fetuses						
External anomalies (%)		0.0	0.0	0.0	0.0	0.0
Visceral anomalies (%)		4.5	1.9	0.6	5.2	2.1
Main type (%)	ST	0.6±2.8	0.0	0.0	0.0	0.0
	LALu	0.6±2.8	0.0	0.0	0.0	0.0
	VSD	3.9±7.4	1.9±4.7	0.6±2.8	5.2±9.9	2.1±7.0
	ASD	0.6±2.8	0.0	0.0	0.0	0.0
	AAS	0.6±2.8	0.0	0.0	0.0	0.0
	LALi	0.6±2.8	0.0	0.0	0.0	0.0
Visceral variations (%)		6.7	9.5	12.7	18.7 $$, S, #	10.6
Main type (%)	TRN	1.9±4.7	2.0±5.0	3.3±5.9	2.1±5.2	1.3±4.1
	SCO	0.7±3.2	0.0	0.8±3.7	4.4±7.1 $, S, #	1.3±4.1
	HCO	0.0	0.0	0.6±2.8	0.0	1.7±5.4
	LUA	0.0	0.8±3.7	1.3±3.8	1.3±4.1	0.0
	DRP	4.1±8.8	7.5±10.2	7.9±12.3	12.4±11.5 $, #	6.2±11.4
	DU	0.0	1.3±4.1	0.0	2.5±5.2 $, #	0.0
	CU	0.0	1.3±3.8	0.7±3.2	0.6±2.5	0.0
Skeletal anomalies (%)		0.0	/	/	0.0	/
Skeletal variations (%)		31.5	/	/	21.9	/
Main type (%)	FHD	4.9±11.1	/	/	0.8±3.7	/
	CFV	7.6±8.3	/	/	10.5±12.3	/
	OF7	0.6±2.8	/	/	0.0	/
	SCT	2.0±4.8	/	/	2.0±4.8	/
	CR	0.0	/	/	0.8±3.7	/
	SSR14	17.0±20.3	/	/	7.2±14.4	/
	RR13	2.0±6.3	/	/	0.0	/
	SS	1.3±4.1	/	/	2.1±5.0	/
	AS	0.6±2.8	/	/	0.0	/
Mean of ossified sacral and caudal vertebrae		7.81±0.48	/	/	7.63±0.44	/

/: Not examined, #: Adverse effects, S: P<0.05 (Shirley-Williams Test) group 2 vs groups 3 or 4.
$: P<0.05; $$, P<0.01 (Wilcoxon test) group 1 vs group 2, 3, 4 or 5. (2 group comparison)

Visceral; ST: Small thymus, LALu: Lobulation anomaly of lung, VSD: Ventricular septal defect, ASD: Atrial septal defect,
AAS: Aortic arch stenosis, LALi: Lobulation anomaly of liver, TRN: Thymic remnant in neck, SCO: Supernumerary coronary ostium,
HCO: High coronary ostium, LUA: Left umbilical artery, DRP: Dilatation of renal pelvis, DU: Dilatation of ureter, CU: Convoluted ureter

Skeletal; FHD: Foramen hypoglossi double, CFV: Closure of transverse foramen of one or more cervical vertebral arches,
OF7: Opening of transverse foramen of 7th cervical vertebral arches, SCT: Splitting of ossification centers of thoracic vertebral bodies,
CR: Cervical ribs, SSR14 Short supernumerary rib (14th), RR13: Reduced 13th ribs, SS: Splitting of sternebrae, AS: Asymmetry of sternebrae

Conclusion	From the above results, it is concluded that combined administration of SYR-322 and AD-4833(HCl) potentiate the effects of AD-4833 alone in terms of fetal growth and most of visceral variation, but did not induce embryo-fetal mortality or fetal anomalies indicating the teratogenicity.

2.6.7 TOXICOLOGY TABULATED SUMMARY

RAT (Combination Treatment)	NOAEL (mg/kg/day)	MULTIPLE OF MRHD	BASIS
Single dose TK (non-GLP) *0/3.6, 0/14.5 (pio only)* *30/0, 100/0 (alo. only)* *100/3.6, 30/14.5 (alo/pio)*	None (no toxicity endpoints)	Not applicable	Alogliptin, M-I, M-II – no co-treatment TK effect Pioglitazone, M-III, M-IV – no co-treatment TK effect Pio M-II ↑ 3-6x with alo co-treatment (6-12% vs. 2-3% of pio exposure)
4-Week (non-GLP) *0/14.5 (pio only)* *30/14.5, 100/14.5, 30/3.6 (alo/pio)*	None (range-finding)	Not applicable	No additive or synergistic effects Pio. toxicity → slight ↑ heart weight (10-20%)
13-Week (GLP) *0/0 (vehicle)* *0/14.5 (pio)* *30/14.5, 100/14.5, 30/3.6 (alo/pio)*	100 mg/kg (alo) 30/3.6 (alo/pio)	33X (alo) 6X/7X (alo/pio)	No additive or synergistic effects TK: sl. ↑ pio T_{max}, ↑ pio M-II Pio. tox. → sl. ↑ heart weight, cardiomyopathy, spleen extramedullary hematopoiesis, adipocyte hyper. (bone marrow, brown/white fat), adrenal cortical cell hypertrophy
Embryofetal development (Seg II) (GLP) *0/0 (vehicle)* *0/40 (pio)* *30/40, 100/40, 30/20 (alo/pio)*	Dams: < 30/20 (alo/pio) Fetus: < 40 pio; 30 mg/kg alo	Fetus: 6X (alo)	No teratogenic effects Dams: ↓ BW, BW gain, ↑ placental weights (16-30%) Fetus (pio): sl. ↓ BW, visceral variations Fetus (alo+pio): ↓ BW (4-5%), potentiated ↑ visceral variations

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/s/

David Carlson
6/8/2009 10:08:46 AM
PHARMACOLOGIST
Pharm/tox recommends approval

Todd Bourcier
6/8/2009 11:18:55 AM
PHARMACOLOGIST
I concur.

SUPERVISOR MEMO

Date:	08 June 2009
RE:	NDA 22-246
Sponsor:	Takeda Pharmaceuticals
Drug/Indication	Nesina (alogliptin, DPP4 inhibitor) + Actos (pioglitazone, TZD) Fixed Dose Combination Type 2 diabetes

Takeda pharmaceutical is seeking marketing approval for a fixed dose combination product of alogliptin + pioglitazone, proposed trade name (b) (4), as a treatment option for Type 2 diabetes. Alogliptin is not yet approved for marketing in the U.S. and is currently being reviewed by FDA under NDA 22-271. Pioglitazone (Actos) was approved in 1999 as a monotherapy for Type 2 diabetes under NDA 21-073. Takeda is the sponsor for both compounds.

Alogliptin is a member of the DPP4 inhibitor class of compounds whose primary mode of action consists of extending the half-life of the incretin GLP-1, thereby enhancing glucose-induced release of insulin from pancreatic beta cells. Pioglitazone is a thiazolidinedione agonist of PPARs gamma and alpha whose primary mode of action is improving insulin sensitivity in peripheral tissues. Promoting insulin release while also improving insulin action in peripheral tissues provides separate yet complementary mechanisms of action that are anticipated to better manage blood glucose in those patients not controlled by monotherapy alone.

Dr. David Carlson, the primary non-clinical reviewer, concludes that the pharmacology and toxicology data support approval of the alogliptin + pioglitazone combination product. *I concur with Dr. Carlson's assessment.*

Takeda proposes to use the same doses of alogliptin (12.5, 25mg) and pioglitazone (15, 30, 45mg) as those used (or to be used) for monotherapy. This yields six dose combinations, with the highest being 25mg alogliptin + 45mg pioglitazone. The once-a-day dose regimen is also the same for the monotherapies and the combination product.

Preclinical studies with the combination did not identify any new toxicity or any clinically significant change in the toxicity profile of alogliptin and pioglitazone. Toxicity of the combination was assessed in rats in a 3 month general toxicity study, an embryofetal development study, and a number of shorter duration dose-ranging studies.

As discussed in greater detail in Dr. Carlson's review, the primary preclinical findings of note include higher exposure to an active hydroxylated metabolite of pioglitazone (MII) and a higher incidence of fetal visceral variations with the combination.

Alogliptin increased generation of the active MII pioglitazone metabolite in rats by 2-3 fold, ultimately accounting for ~35% of exposure to parent. This apparent pharmacokinetic interaction in rats is of minimal clinical importance because MII is a minor metabolite in human subjects and would not contribute to the pharmacodynamic effect of pioglitazone even if modestly increased by alogliptin.

Alogliptin and pioglitazone are not teratogenic in rats when given alone or in combination. However, reduced fetal body weight and the incidence of some visceral variations (notably supernumerary coronary ostium, renal pelvis dilatation) observed with pioglitazone alone were exacerbated by high doses of alogliptin. This result does not appreciably alter the known reproductive toxicity profile of either compound, and the current pregnancy category of 'C' for pioglitazone is also appropriate for the combination product.

Non-clinical labeling issues to be resolved prior to an 'approval' action include revising language in sections on Pregnancy, Nursing Mothers, and Nonclinical Toxicology for aloglitpin.

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/s/

Todd Bourcier
6/8/2009 11:22:17 AM
PHARMACOLOGIST
pharm/tox TL memo

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

NDA Number: 22-426 **Applicant:** Takeda **Stamp Date:** September 22, 2008

Drug Name: Alogliptin + pioglitazone FDC **NDA/BLA Type:** 505(b)(1)

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		No data submitted in Nonclinical section. References original NDAs for alogliptin and pioglitazone. Sufficient 'Nonclinical Overview'.
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		Not applicable – see comment #1 above.
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		Not applicable – see comment #1 above.
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		Studies submitted in NDA 22-271 for alogliptin. Some combination toxicity studies were not reviewed in NDA 22-271 but they can be referenced for review in this NDA. No new carcinogenicity studies needed for FDC.
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).			Not applicable. Same drug substance formulations as alogliptin NDA and approved pioglitazone formulations.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		Studies submitted in NDA 22-271 for alogliptin. Some combination toxicity studies were not reviewed in NDA 22-271 but they can be referenced for review in this NDA.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		Have not completed alogliptin monotherapy labeling; no major issues in FDC label, but FDC labeling will likely need some pharmtox changes (e.g. carcinogenicity wording). Contains human exposure multiples based on total plasma exposure (AUC) comparisons.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		No new impurities identified in FDC tablets compared to either individual drug product.
11	Has the applicant addressed any abuse potential issues in the submission?			Not applicable.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable.

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.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

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/s/

David Carlson
10/21/2008 06:21:57 AM
PHARMACOLOGIST
OK to file

Todd Bourcier
10/21/2008 02:17:31 PM
PHARMACOLOGIST
Concur; NDA is filable for pharm/tox.